

Special Issue Reprint

Advances in Polymer Materials Based on Lignocellulosic Biomass

Edited by
Roland El Hage and Rodolphe Sonnier

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Advances in Polymer Materials Based on Lignocellulosic Biomass

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About the Editors

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Preface

The Reprint, “Advances in Polymer Materials Based on Lignocellulosic Biomass”, presents recent advances in sustainable polymer materials derived from renewable biomass resources. It highlights research on bio-based polymers, natural fiber composites, surface modification, flame retardancy, and functional materials for industrial and environmental applications. This Reprint aims to provide researchers and engineers with an updated overview of current developments in biomass valorization and sustainable polymer technologies while supporting the transition toward circular and environmentally friendly materials.

Roland El Hage and Rodolphe Sonnier

Guest Editors

Article

Evaluating UV Stability of *Miscanthus* × *giganteus* Particles via Radiografting of UV Absorbers

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Abstract: *Miscanthus* × *giganteus* particles possess excellent advantages in biodegradability and sustainability. However, their susceptibility to ultraviolet (UV) degradation limits wider outdoor applications. In the present work, electron beam (*e*-beam) radiation-induced grafting was used for the first time to attempt covalent grafting of UV absorbers onto miscanthus particles to address a major challenge in natural fiber stabilization. Two UV absorbers, 2-hydroxy-4-(methacryloyloxy) benzophenone (HMB) and 2-(4-benzoyl-3-hydroxyphenoxy) ethyl acrylate (BHEA), were explored using both pre-irradiation and simultaneous approaches. Pre-irradiation grafting did not achieve useful covalent fixation of HMB or BHEA, due in part to the premature decay of radicals at elevated temperatures and with solvent use, and the lignin-based quenching of radicals. Solvent-free mutual irradiation grafting failed due to immobility of the UV absorbers, while grafting of HMB in solvent failed due to radical-scavenging behavior. Grafting of BHEA was successfully achieved under solvent-based simultaneous irradiation, reaching up to 38 wt % DG in a butanone/2.5% H₂SO₄ system. This condition led to the improved UV stability of miscanthus particles, in which color change was reduced significantly after 1000 h of accelerated weathering; this was mainly linked to a beneficial pre-darkening effect which was induced by the presence of the acid. This work proposes a route of grafting strategy that aims to improve the photostability of miscanthus particles, paving the way for durable bio-based materials in outdoor composite applications.

Keywords: *Miscanthus* × *giganteus*; UV absorbers; radiation (*e*-beam)-induced grafting; accelerated weathering; photodegradation; colorimetric analysis

1. Introduction

UV aging enhancement in natural fibers is of vital importance in prolonging the lifespan and improving the functionality of materials that find utility in various applications, especially among biocomposites [1–4]. Among the different natural biomasses, *Miscanthus* × *giganteus* is a sustainable biomass grass of growing cultural and commercial importance. Even though it requires water sufficient to establish the deep roots of newly planted rhizomes [5,6], this noninvasive, sterile C4 crop composed of lignin, cellulose, hemicelluloses, extractives and inorganics [7–11] becomes more water-efficient once established, while also offering advantages in sustainability and biodegradability [12]. Its majority composition of cellulose (>50%) makes miscanthus a promising feedstock for biofuels, paper and fiber-composite materials [13]. It can be cultivated in most climatic

zones [14], growing over 3 m tall annually with minimal herbicide and nitrogen requirements. With a high productivity rate, *Miscanthus × giganteus* can produce up to 30 tons of dry matter per hectare. Additionally, pests and diseases have been found to have no significant impact on its cultivation [15]. It should be noted that miscanthus planting areas are still modest and have reached only about 10⁴ ha in the European Union. This situation is linked to establishment costs and market factors. Yet, policy incentives and its strong carbon-sequestration benefits are driving interest in upscaling miscanthus production [16].

However, lignocellulosic materials such as miscanthus or other natural plant fibers are vulnerable to ultraviolet (UV) light degradation, and this can present a significant obstacle. This consideration is essential when the materials are used outdoors, and they are exposed to sunlight, causing the fibers to be altered. In a recent work, we have investigated the UV artificial weathering of *Miscanthus × giganteus* [17]. That study demonstrated alterations in both color and chemical composition throughout the weathering process under ultraviolet (UV) light, along with a limiting effect attributed to the presence of extractives. The study highlights a browning effect occurring after UV weathering, which seems to be associated with structural changes, as confirmed by attenuated total reflectance–Fourier-transform infrared spectroscopy (ATR-FTIR). This was mainly related to an increase in carbonyl-absorbing bands around 1700 cm^{−1} and a decrease in aromatic skeletons near 1500 cm^{−1} in the FTIR spectra, due to the lignin presence (23 wt %) that impacted the colorimetry parameters. Moreover, like the other lignocellulosic materials, cellulose and hemicelluloses remain UV-transparent and degrade more slowly than the lignin fraction, which contains conjugated aromatic structures that absorb strongly in the UV region [17,18].

To resolve this issue, multiple approaches have been investigated in the literature, including those based on the grafting and adsorbing of photostabilizers (UV absorbers and radical scavengers) or even testing surface protection with coatings and chemical modifications of lignocellulosic resources [19,20]. UV absorbers act by absorbing energy and dissipating it as heat while radical scavengers react with the formed radicals, acting as antioxidants. Tragoonwichian and co-workers [21] modified cotton fabrics for improved UV protection based on admicellar polymerization. The authors synthesized 2-hydroxy-4-acryloyloxybenzophenone (HAB) by bonding 2,4-dihydroxybenzophenone to acryloyl chloride. The latter was polymerized on cotton using a sodium dodecylbenzene sulfonate as a surfactant by forming a superficial thin film. The study was evaluated by the authors by use of the Ultraviolet Protection Factor (UPF). The results showed a significant increase in terms of UPF value, from 4 to greater than 40, which highlighted a reduction of UV transmission and an increase in UV protection due to HAB grafting at concentrations above 1.2 mM. In a second work, Tragoonwichian and coworkers [22] copolymerized two UV-absorbing monomers, 2-[3-(2H-benzotriazol-2-yl)-4-hydroxyphenyl]ethyl methacrylate (BEM) and 2-hydroxy-4-acryloyloxybenzophenone (HAB), on cotton fabrics, utilizing admicellar polymerization. The results showed that the fabrics coated using a 1:1 monomer ratio, and with the same total monomer concentration of 3.0 mM for both UV absorbers, yielded the highest UV protection, with a UPF value of 97, in comparison to the 73 and 72 that were obtained from BEM and HAB alone, tested at an equal monomer concentration. An alternative approach was also investigated by the authors of [23]. The authors adopted two-step grafting routes. The first step was based on cotton surface modification using vinyltriethoxysilane to provide polymerizable vinyl groups. In the second step, an ultraviolet-absorbing monomer, 2-[3-(2H-benzotriazol-2-yl)-4-hydroxyphenyl]ethyl methacrylate (BEM), was then polymerized and successfully coated the treated fibers. The study highlighted a significant reduction of ultraviolet transmission, in which UPF values reached 40 and 77 after polymerization using BEM with concentrations of 2.0 mM and 10.0 mM, respectively, in comparison to a UPF of 4 before treatment; this resulted in excellent ultraviolet pro-

tection. Gorjanc and coworkers [24] examined the UV-protective properties of untreated and plasma-treated cotton fabric dyed with Cibaron Deep Red S-B and treated (or not) with Tinofast CEL UV absorber. The investigation showed that UV protection increased after UV-absorber treatment, as quantified by the UPF measurements. The UPF value of the fabrics was about 4 before treatment and increased to 68–74 after treatment. It was also shown that plasma treatment enhanced UV-absorber adsorption, which is linked to an increase in oxygen functional group content on the cotton surface. However, the UV protection seems to decrease after several washing cycles, in which physically attached UV-absorber molecules and dye molecules are removed. This clearly demonstrates the importance of covalent-bond grafting for enhanced UV durability, which is not achieved via surface adsorption only.

Natural fibers' durability under UV exposure is a critical barrier to their outdoor applications. Moreover, as discussed before, *Miscanthus × giganteus* is of great importance [13], but remains vulnerable to photodegradation, which alters its color and chemical structure. The use of surface coatings or the physical adsorption of UV absorbers lack long-term effectiveness due to weak physical interaction, as opposed to durable covalent bonds. Hence, radiation-induced grafting has recently gained prominence as an effective method used to covalently attach functional molecules to lignocellulosic fibers. This technique, applied as pre-irradiation or simultaneous irradiation, can lead to the generation of free radicals capable of initiating polymerization and covalent-bond formation [25]. Over the years, radiografting has been considered an effective way to modify lignocellulosic materials, and diverse properties such as flame retardancy [8,26], hydrophobicity [27,28], dye and pollutant adsorption [29], carbon dioxide (CO₂) sorbents [30] and antibacterial applications [31] have been investigated. However, there is a noticeable gap in the literature concerning UV absorbers grafting onto natural fibers, because the radical-scavenging nature of these UV absorbers complicates the process. To the best of our knowledge, no study has systematically evaluated the *e*-beam grafting of UV absorbers onto *miscanthus* particles.

In this work, we address this gap by investigating both pre-irradiation and simultaneous irradiation techniques used for grafting two different UV absorbers, 2-Hydroxy-4-(methacryloyloxy)benzophenone (HMB) and 2-(4-Benzoyl-3-hydroxyphenoxy) ethyl acrylate (BHEA), onto *Miscanthus × giganteus* particles while avoiding any use of harmful chemicals. These molecules can naturally hinder the formation and stability of the radicals necessary for covalent bonding. Therefore, in the present work, we optimized a variety of factors, including irradiation conditions, solvent systems and additives, to ensure effective grafting. We demonstrated that, while HMB resists grafting, BHEA can be effectively grafted under optimized conditions, leading to improved photostability. We also show that a solvent system and sulfuric acid addition not only increase grafting efficiency but also induce color-related and structural modifications that enhance long-term UV resistance. This efficient grafting and pre-darkening represent a novel strategy for stabilizing natural fibers. By filling this gap, this study aims to provide a novel and environmentally friendly solution, and one which aligns with green chemistry principles by avoiding any use of harmful chemicals, aiming to enhance the UV stability of natural fibers, thereby expanding their potential applications in the composites field and beyond.

2. Results and Discussion

An overview of the experimental pathway of the study is presented as a schematic representation in Figure 1. As shown, *Miscanthus* particles were subjected to pre-irradiation and simultaneous grafting, followed by the washing and drying of samples before further evaluation. Only the grafted samples were subsequently compressed into disks and

subjected to UV-weathering tests for stability assessment. Each grafting route and its outcome is analyzed and discussed in the following sections.

Raw Miscanthus Particles (500–800 μm)

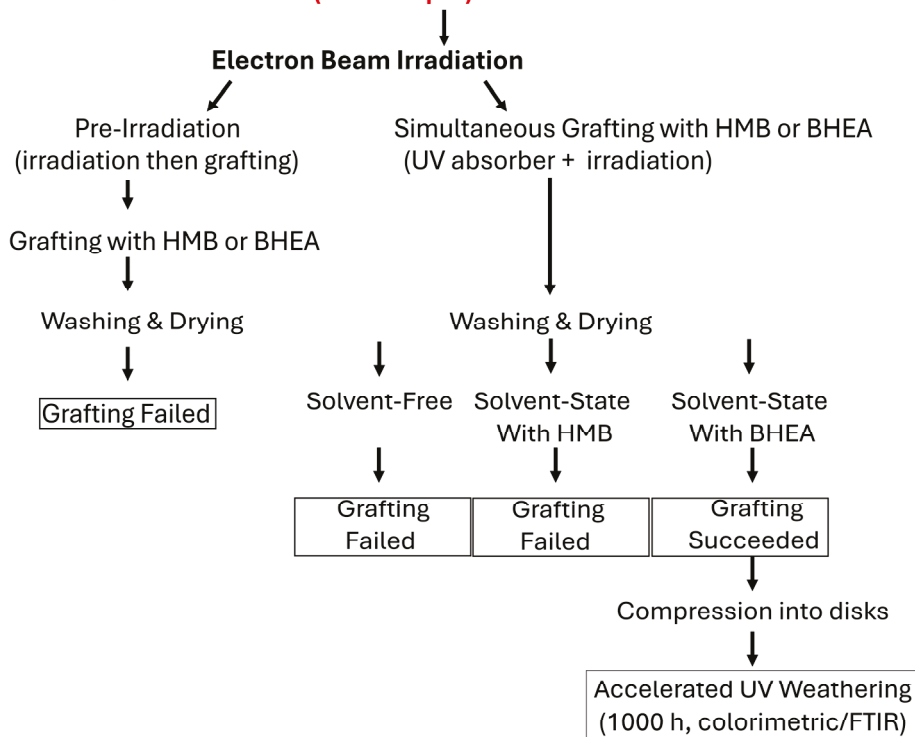


Figure 1. Schematic representation of the grafting routes.

2.1. Pre-Irradiation Grafting of HMB and BHEA UV Absorbers

Table 1a shows the results of experiments involving pre-irradiated Miscanthus particles under an air atmosphere. For the grafting step, HMB and BHEA UV absorbers were used in solutions degassed with argon as an inert gas, under various conditions. The most important parameters are the ratio of miscanthus to UV absorber, UV-absorber concentration, butanone/water ratio, ferrous ammonium sulfate $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ (Mohr's salt) presence, miscanthus/solution concentration, and radiation dose. Radiation generally induces the formation of free radicals throughout the material, which can create active sites and initiate radical copolymerization. The pre-irradiation was performed under an oxygen atmosphere, which forms free radicals capable of initiating the desired chemical reaction, but oxygen could also result in the deactivation of free radicals and the formation of unwanted oxygenated species, such as peroxides. Hence, in the grafting step, argon was used as an inert atmosphere to ensure that free radicals would remain active and free to start the desired grafting reaction, thereby optimizing the efficiency of the grafting process. It is clear from the DG results that no grafting occurred, despite the different UV-absorber ratios (HMB/BHEA), UV-absorber concentrations, and irradiation doses. In the ATR-FTIR spectra (Figure 2), an example of four samples is shown for HMB grafting (the same behavior was observed for BHEA “spectra not shown” and the different grafting conditions used): spectra are drawn for native miscanthus (non-grafted but pre-irradiated at a dose of 50 kGy), HMB molecule, pre-irradiated miscanthus particles after grafting with HMB but before washing (Table 1a-2) and pre-irradiated miscanthus particles after grafting with HMB and washing (Table 1a-2). The native miscanthus spectrum is dominated by broad O–H stretching near 3400 cm^{-1} , C–H stretching around 2900 cm^{-1} and characteristic carbohydrate ring vibrations in the $1000\text{--}1100\text{ cm}^{-1}$ region [17,32]. Moreover, in the $1700\text{--}1500\text{ cm}^{-1}$ region, miscanthus also has characteristic bands due to hemi-cell-

lulose, cellulose and lignin moieties. The unconjugated C=O stretching of acetyl or ester groups of cellulose and lignin are observed as a sharp band at the 1730–1740 cm^{-1} band. Aromatic C=C skeleton vibrations from lignin components are detected at the 1600 cm^{-1} and 1510 cm^{-1} bands. Since nitrogen content is minimal in miscanthus [33], no strong N–H or C–N features are detected.

Table 1. Experimental conditions and degree of grafting (DG, wt %), for pre-irradiation and simultaneous-irradiation routes: (a) pre-irradiation in air atmosphere followed by grafting in argon atmosphere (HMB or BHEA); (b) simultaneous solvent-free (HMB or BHEA); (c) Simultaneous in solvent state (HMB); and (d) simultaneous in solvent state (BEHA).

N ^o	Miscanthus/ HMB or BHEA (m/m)	UV Absorber (wt %)	Butanone/ Water (m/m)	Mohr's Salt/Miscanthus (mg/g)	Miscanthus/ Solvent (m/m)	Atmosphere	Dose (kGy)	DG (wt %)
(a) Pre-irradiation under air atmosphere with grafting in argon atmosphere (HMB or BHEA)								
1	1/1	5	80/20	-	1/20	Argon	50	None
2	1/1.5	8	90/20	27	1/20	Argon	50	None
3	1/1.5	8	95/5	27	1/20	Argon	50	None
4	1/1.5	8	90/10	27	1/20	Argon	50	None
5	1/1.5	8	80/20	27	1/20	Argon	50	None
6	1/1.5	8	70/30	27	1/20	Argon	50	None
7	1/1.5	8	90/10	27	1/20	Argon	100	None
(b) Simultaneous solvent-free (HMB or BHEA)								
8	1/1	-	-	-	-	Air	50	None
9	1/1	-	-	-	-	Air	100	None
(c) Simultaneous in solvent state (HMB)								
10	1/1	11	100	-	1/9	Air	50	None
11	1/1	6	100	-	1/18	Air	50	None
12	1/1	13	80/20	27	1/8	Air	100	None
13	1/1.5	15	80/20	-	1/10	Air	100	None
14	1/1	6	80/20	-	1/18	Argon	100	None
15	1/1	11	100	-	1/9	Argon	100	None
(d) Simultaneous in solvent state (BEHA)								
(1) Effect of water addition								
16	1/1	17	100	-	1/6	Air	100	3.3 ± 1
17	1/1	17	80/20	-	1/6	Air	100	35.2 ± 2
(2) Effect of BHEA concentration								
18	1/0.125	2	80/20	-	1/6	Air	50	None
19	1/0.25	5	80/20	-	1/6	Air	50	None
20	1/0.5	9	80/20	-	1/6	Air	50	3.2 ± 1
21	1/1	17	80/20	-	1/6	Air	50	21.7 ± 2
(3) Effect of irradiation dose								
22	1/1	17	80/20	-	1/6	Air	10	8.7 ± 1
23	1/1	17	80/20	-	1/6	Air	20	14.4 ± 1
24	1/1	17	80/20	-	1/6	Air	50	21.7 ± 2
25	1/1	17	80/20	-	1/6	Air	100	35.2 ± 2
(4) Effect of additives addition								
26	1/1	17	80/20	-	1/6	Air	100	35.2 ± 2
27	1/1	17	80/20 *	-	1/6	Air	100	36.6 ± 1
28	1/1	17	80/20 **	-	1/6	Air	100	38.4 ± 1

* 80/20: the water was replaced by 2.5 wt % aqueous urea solution. ** 80/20: the water was replaced by 2.5 wt % aqueous sulfuric acid solution (H_2SO_4).

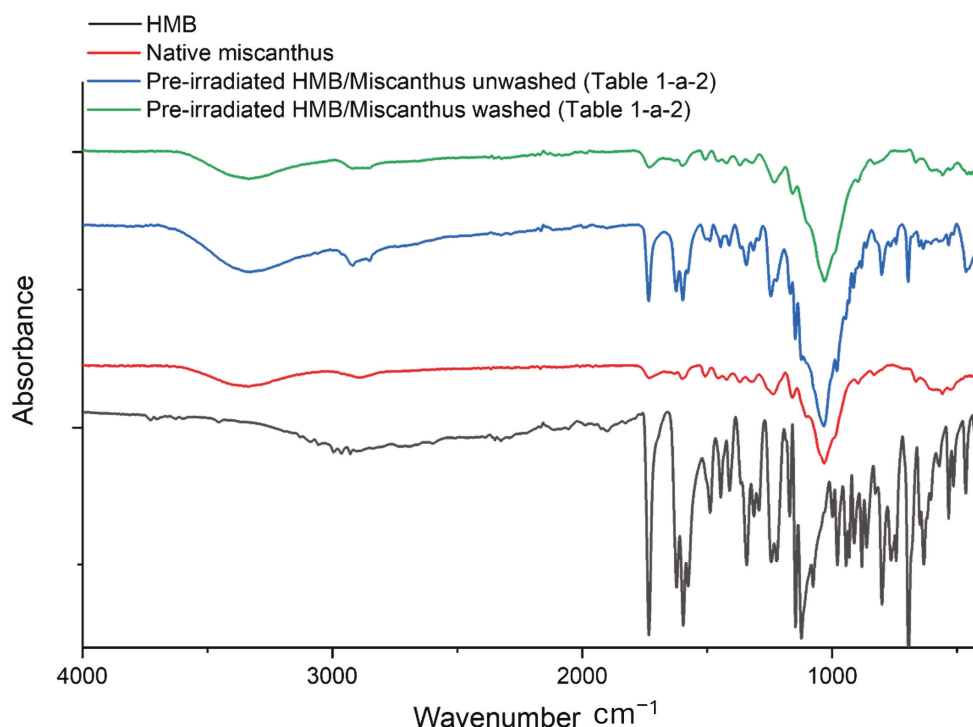


Figure 2. FTIR spectra of HMB, Native miscanthus, Unwashed pre-irradiated HMB/miscanthus and Washed pre-irradiated HMB/miscanthus.

In contrast, the spectrum of the aromatic ketone, HMB, displays distinct signals that can include a strong C=O stretch near 1700 cm^{-1} and aromatic-ring vibrations in the $1500\text{--}1600\text{ cm}^{-1}$ range. This is typical for conjugated benzophenone ketones [34]. Since no amine groups are present in HMB structure, hence no N–H or C–N bands are expected. It could be observed from the spectrum of pre-irradiated miscanthus particles after grafting reaction but before washing that additional bands were apparent in the $1700\text{--}1500\text{ cm}^{-1}$ region that match the UV absorber, indicating that HMB was deposited on the fiber surface. However, once the sample is washed, these HMB-related bands disappear, and the spectrum of the pre-irradiated miscanthus particles after grafting and washing closely resembles that of native miscanthus. This is evident when comparing the $1700\text{--}1500\text{ cm}^{-1}$ region, which agrees with the DG results and confirms that the HMB was not covalently grafted and then removed by the washing step.

This behavior could be linked to several factors, such as the ineffective activation of the particles, the chemical properties of HMB and BHEA and improper interaction between the active sites on the miscanthus particles and UV absorbers. It should be highlighted that the ratio of miscanthus to UV absorbers and the concentrations of the UV absorbers did not influence the grafting outcome. Additionally, all experiments with pre-irradiated miscanthus particles were performed at a dose of 50 kGy, except for one experiment conducted at 100 kGy. It is possible that the dose needs to be raised further to cross the threshold of the level needed for effective radical formation and subsequent grafting. A higher dose can also lead to fiber degradation and depolymerization [9]. Mohr's salt was added in the grafting process, with an aim to improve substrate activation and aid the grafting reaction through the dissociation of peroxides (formed during irradiation in an air atmosphere, as stated before). The level of Mohr's salt was fixed at a constant 27 mg/g of miscanthus particles across all experiments. Mohr's salt is utilized to dissociate peroxides present on the miscanthus particles after pre-irradiation [35–37]: $\text{ROOH} + \text{Fe}^{2+} \rightarrow \text{RO}^\bullet + \text{OH}^- + \text{Fe}^{3+}$. Nevertheless, the Mohr's salt addition was unsuccessful.

Additionally, the activation of grafting was conducted in an inert atmosphere, under a controlled argon atmosphere at 80 °C, which is typically used to suppress the oxidation of radicals (residual radicals from irradiation or radicals formed by peroxide decomposition due to the Mohr's salt). The controlled atmosphere would have favored grafting, yet the absence of grafting reveals that the inert atmosphere alone was not enough to initiate the desired reaction. Moreover, HMB and BHEA molecular structures would not easily be prone to radical grafting onto miscanthus particles. Even though the molecules are UV absorbers, they could be weakly reactive towards radical sites. The study of Brendle and coworkers [6], based on Electron Paramagnetic Resonance analyses, provides explanations for this observation by quantifying the presence of free radicals. Since pre-irradiation of the miscanthus particles was conducted under air, reactive radical species were generated, primarily peroxy radicals, within the fiber matrix. According to this study, radicals generated in lignocellulosic fibers, such as miscanthus, are largely concentrated in lignin-rich regions. EPR analysis [6] has demonstrated that lignin-based radicals exhibit lower thermal stability compared to those located in cellulose. It seems that at a grafting temperature of 80 °C, the radical species in miscanthus particles decay rapidly, with the majority of the peroxy radicals disappearing within the first 30 min. This rapid decay indicates that the radical concentration necessary to initiate grafting reactions may not have been present shortly after the start of the experiment. Also, active radicals are required to initiate polymerization, but it seems that their short lifetime at 80 °C prevented the grafting of HMB or BHEA on miscanthus particles.

Brendle and coworkers [8] also proved that the use of Mohr's salt as a reducing agent permits grafting by dissociation of peroxide species formed during irradiation in air, allowing the initiation of polymerization reaction on lignin-rich substrates, specifically, *Miscanthus × giganteus* stem fragments of dimethyl(methacryloyloxy)methyl phosphonate (MAPC1). Based on that finding, in the present study, once peroxides are dissociated by Mohr's salt, the resulting alkoxy radicals (RO•) can be considered the active species that could initiate the grafting and which could react with the acrylate or methacrylate groups of the grafting agents (HMB and BHEA). Despite that, no grafting occurred, regardless of this parameter, for these UV absorbers, which implies that the presence of Mohr's salt is not playing an important role in this grafting reaction.

In addition, the use of a butanone–water mixture as the solvent seems to have a significant role in the radical dynamics. In fact, the solvent system may influence radical stability on the miscanthus particle's surface. Grafting will be hindered if the solvent induces radical recombination or quenches radicals. Past research has determined that radical recombination and quenching reactions can be highly influenced by solvents, which consequently influence grafting efficiency directly [35]. According to the EPR results [8], water greatly influences the stability and availability of radicals in lignocellulosic fibers, particularly in miscanthus, due to its highly amorphous and lignin-rich nature compared to flax fibers. When water was added to pre-irradiated miscanthus particles, the radical concentration decreased drastically, keeping only 11% of the initial population of radicals (Figure 3). In this study, the water in the butanone-based solvent system would have caused extensive fiber swelling and increased molecular mobility, enabling water to interact with the radicals. The combination of thermal instability and the water's quenching of the radicals would have further reduced the radical population, rendering the particles essentially inactive for the initiation of graft polymerization. This quenching effect is consistent with the absence of grafting that was observed in the experiments.

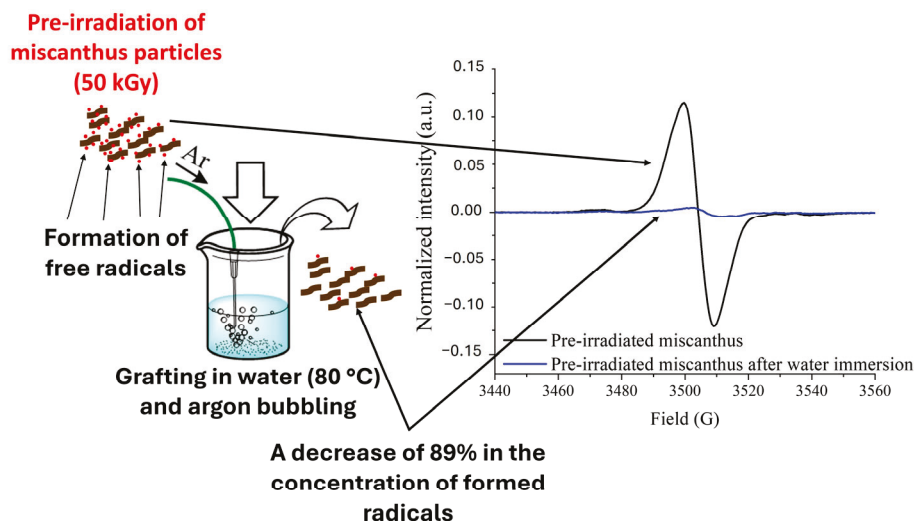


Figure 3. EPR spectra of native pre-irradiated miscanthus particles and post-grafting samples; adapted from Brendle et al. (2014) [8].

One of the most important parameters that control the outcome is the miscanthus particles' composition. It was clearly seen in Brendle and co-workers' [8] work, based on the EPR results, that radicals in lignin are more thermally unstable and more susceptible to interaction with the environmental factors, e.g., water or heat, relative to radicals located in cellulose. Miscanthus particles with high lignin content form radicals of lower stability at elevated temperatures; this was compared by the authors to processes involving cellulose-rich fibers like flax. With the lignin content in miscanthus, radicals formed during pre-irradiation would have been restricted primarily to the lignin matrix. Below 80 °C, these lignin-based radicals undergo decay quite easily, which further explains the rapid loss of radical species during the grafting process.

The absence of grafting in this part of this work can therefore be linked to a variety of factors, i.e., the high level of thermal decay of radicals below 80 °C; the use of a butanone–water solvent system, which led to a quenching effect; and the unstable nature of lignin-based radicals on miscanthus particles. Even though the argon atmosphere prevented oxygen quenching, it was unable to reverse thermal instability and water-induced radical loss.

2.2. Solvent-Free Simultaneous-Irradiation Grafting of HMB and BHEA UV Absorbers

The results, shown in Table 1b, of the simultaneous solvent-free grafting reactions of UV absorbers HMB and BHEA onto miscanthus particles via electron beam irradiation under 100 kGy in air showed no detectable grafting (DG% = None), pointing to the main challenges in this approach. FTIR analyses were also performed. The latter results are not presented, since no spectral modifications were detected between native and grafted miscanthus particles, a finding which agrees with the DG results. The failure of grafting can be attributed to the limited mobility of UV absorbers, which form solid residues between the fibers following solvent evaporation, as shown in Figure 4.

In solid-state conditions, the restricted mobility of the UV absorbers would have inhibited their reaction with reactive sites of the particles, a critical factor for successful grafting. In addition, irradiation in the air atmosphere may lead to the quenching by oxygen of the free radicals that initiate the grafting reaction. These results led us to conclude that the lack of observable grafting in the solvent-free simultaneous grafting of UV absorbers HMB and BHEA onto miscanthus particles highlights some of the fundamental difficulties, most significantly the limited mobility of the UV absorbers in their solid-state form.

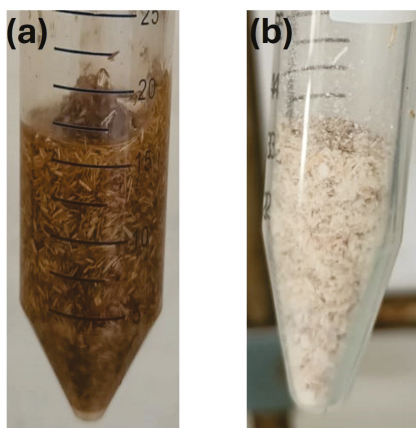


Figure 4. Photographs of miscanthus particles before and after solvent evaporation: (a) Particles in solution; (b) Particles with BHEA precipitation (white powder) after solvent removal.

In contrast, other studies in the literature [8,38] indicate successful examples of grafting where the monomer was in the liquid state, which suggests that mobility of the monomer is crucial in grafting effectiveness. For instance, Brendle and coworkers [26] demonstrated effective grafting of phosphonated monomers on flax fibers when exposed to electron beam irradiation, even with lower doses of irradiation like 10 kGy. The grafting obtained in the study can be attributed in large part to the liquid state of the monomers, which permits efficient interaction with the reactive sites of the samples. Similarly, Hajj and coworkers [38] explored radiation-induced grafting of vinyl phosphonic acid on flax fibers and achieved significant phosphorus grafting using liquid monomer solutions. This suggests that adequate monomer mobility is also required, in addition to factors relating to the radical's formation, to ensure that the reactive species can access the reactive sites of the particles. Hence, switching to liquid-phase monomers to evaluate the grafting effectiveness of the UV absorbers on miscanthus particles was explored in simultaneous irradiation.

2.3. Solvent-Based Simultaneous-Irradiation Grafting of HMB and BHEA UV Absorbers

In the experiments summarized in Table 1c, attempts were made to graft 2-Hydroxy-4-(methacryloyloxy) benzophenone (HMB) onto miscanthus particles via simultaneous irradiation under solvent-based conditions. Despite variations in solvent composition (butanone/water), atmosphere (air vs. argon), radiation dose (50–100 kGy) and the presence or absence of Mohr's salt, no detectable grafting yield was observed in any of the trials. Note that Mohr's salt was introduced to mitigate the formation of free polymer chains and to limit scenarios in which there is a definitive risk of homo-polymerization [39,40]. FTIR investigations were conducted, but the results are not given, since no spectral differences were detected between native and grafted miscanthus particles, as evidenced by the DG results. One possible explanation might be the resonance stabilization of the methacrylate moiety in the HMB structure (Figure 5), which may reduce the formation of the active radical sites needed for successful grafting. In addition, the benzophenone moiety of HMB can scavenge free radicals in certain instances. The HMB conjugated structure seems to introduce additional stereo and electronic barriers that may hinder efficient grafting [41]. This conjugation induces a delocalization of the radical electron density over the aromatic system and leads to a stabilized intermediate. This stabilizing action reduces the reactivity of the radical species and, therefore, the effective radical concentration available for initiation of grafting reactions [42]. The HMB methacrylate group, while reactive under many polymerization conditions, may be rendered less reactive with the resultant radicals of miscanthus particles, due to this intramolecular resonance. In addition, oxygen availability in an air atmosphere also possesses a proven inhibitive influ-

ence relative to radical polymerization [43,44]. Thus, in some experiments (Table 1c-14,15), argon was employed to avoid the inhibitory effect of oxygen. It must be noted that oxygen inhibition is known to be pronounced at lower doses and rates, which was not the case in our experiments [45]. The results indicate that this modification did not promote grafting. Additionally, the irradiation doses and solvent ratios used may not have been the ones most appropriate for grafting. Overall, these results suggest that further optimization is necessary to overcome the inhibitory factors and achieve grafting of HMB on miscanthus particles.

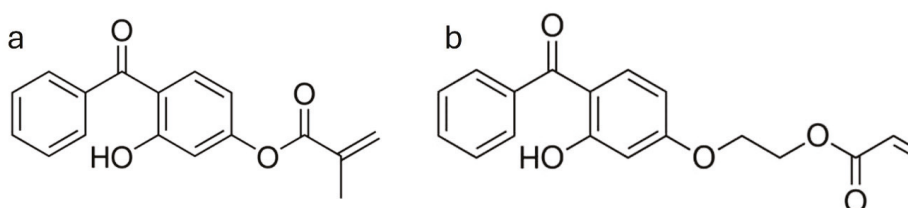


Figure 5. Chemical Structures of (a) 2-Hydroxy-4-(methacryloyloxy)benzophenone (HMB) and (b) 2-(4-Benzoyl-3-hydroxyphenoxy)ethyl Acrylate (BHEA).

To overcome these challenges, one final attempt was made with 2-(4-benzoyl-3-hydroxyphenoxy) ethyl acrylate (BHEA). Relative to HMB, BHEA bears an acrylate moiety and ought to exhibit a diminished resonance effect, considering the longer chain attached to the aromatic ring (Figure 5). This structural difference may reduce both electronic and steric hindrance to grafting [46], hence potentially increasing radical grafting efficiency in miscanthus particles. For that reason, in this section of the work, we studied systematically the parameters influencing the degree of grafting (DG%) of BHEA, based on the solvent-based radiation-induced process. This approach has proven effective, and the BHEA is grafted in varying quantities by the application of certain conditions, which highlights the effects of process parameters on grafting efficiency (Table 1d and Figure 6). The experiments were designed to clarify the roles of important parameters (i.e., the effects of introducing water into butanone, BHEA concentration, irradiation dose and the use of aqueous urea and sulfuric acid as supplementary additives). By comparing grafting efficiencies under these various conditions, we hoped to clarify how each of the parameters influences the graft polymerization. Qualitative Fourier-transform infrared spectroscopy (FTIR) characterization in ATR mode was investigated to evaluate the BHEA grafting by noting increased or additional characteristics evident in the carbonyl and aromatic bands (Figure 7).

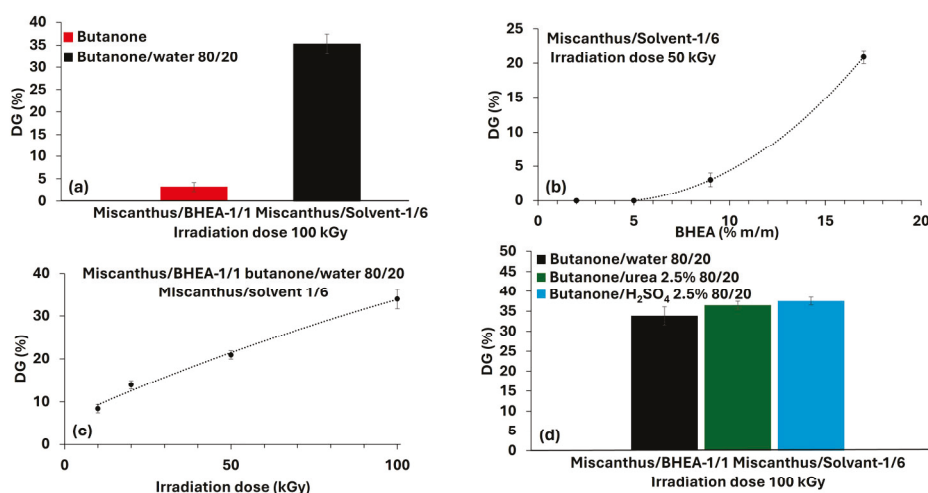


Figure 6. Effects of key parameters on degree of grafting (DG%): influence of (a) Solvent composition; (b) BHEA concentration; (c) Irradiation dose; and (d) Additives incorporation.

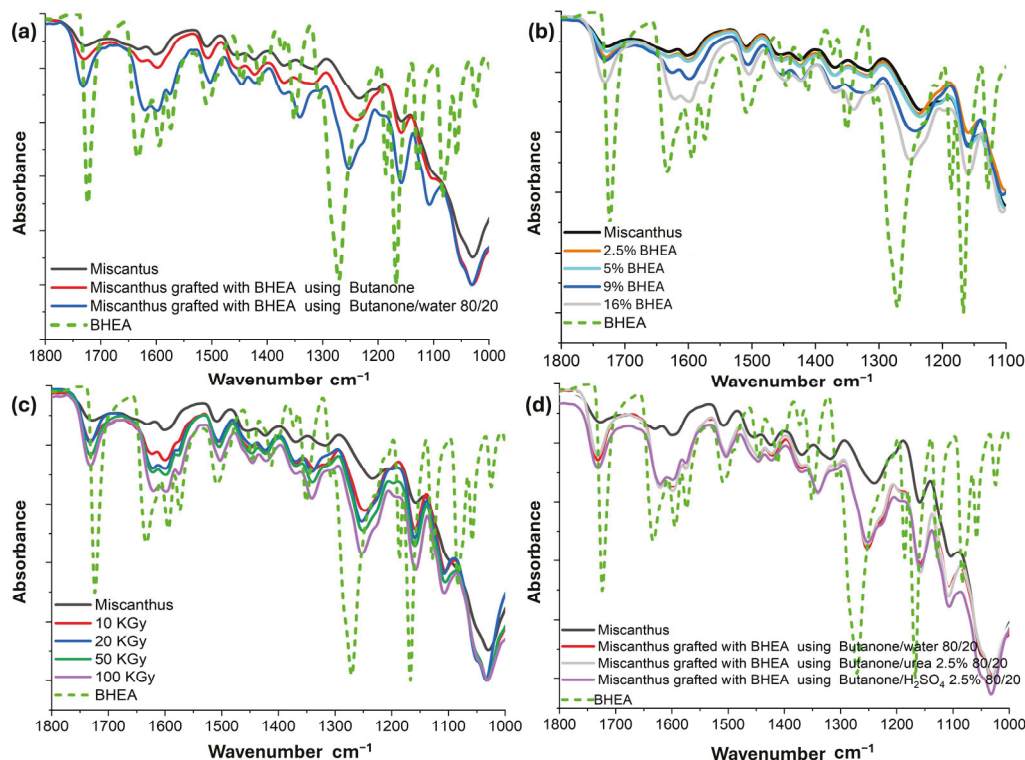


Figure 7. FTIR-ATR spectra of miscanthus particles grafted with 2-(4-benzoyl-3-hydroxyphenoxy) ethyl acrylate (BHEA) under various conditions: (a) Solvent effect (butanone vs. butanone/water); (b) Effect of BHEA concentration; (c) Effect of irradiation dose; and (d) Effects of additives (urea and H_2SO_4).

2.3.1. Effect of Water Addition

Grafting efficiency is influenced by several parameters, such as solvent composition, fiber swelling and reactive species generation. To study such effects, initial tests were conducted using butanone as the solvent, and then other assays were conducted using an 80/20 (*v/v*) butanone/water mixture. Various results were obtained for the two solvent systems (Figure 6a). When using butanone alone, the grafting efficiency remained low, with a grafting yield of only 3.3 ± 1 wt %. When water was added, a significant increase was obtained, reaching 35.2 ± 2 wt %. This observation was confirmed by the FTIR-ATR analysis, as shown in Figure 7a. In this figure the spectra of untreated Miscanthus (irradiated at 100 kGy but without BHEA), of miscanthus grafted with BHEA in pure butanone, of miscanthus grafted with BHEA in a butanone/water (80/20) mixture and of pure BHEA are compared. A key region of interest in FTIR for confirming BHEA grafting is the carbonyl-stretching vibration typically observed around $1720\text{--}1730\text{ cm}^{-1}$, which corresponds to the ester group of the acrylate moiety [26,47–49]. In addition, aromatic-ring vibrations from the benzoyl/phenolic ring often appear near 1600 cm^{-1} and can become more prominent as the amount of BHEA on the surface increases. For more precision, the pure BHEA spectrum displays strong bands at $1720\text{--}1730\text{ cm}^{-1}$ (ester $\text{C}=\text{O}$) and near 1600 cm^{-1} (aromatic ring), serving as the reference for identifying graft-related signals. Untreated miscanthus (irradiated at 100 kGy) shows the characteristic lignocellulosic bands, including the broad O–H stretching around 3400 cm^{-1} , C–H stretching near 2900 cm^{-1} and various C–O and C–C stretches in the $1050\text{--}1150\text{ cm}^{-1}$ region typical of polysaccharides [17,32,50]. Miscanthus grafted in butanone exhibits a moderate variation around $1720\text{--}1730\text{ cm}^{-1}$, suggesting the presence of ester groups from BHEA. Some slight enhancements in the aromatic region (around 1600 cm^{-1}) are also visible.

However, miscanthus grafted in butanone/water (80/20) exhibits a sharper band in $1720\text{--}1730\text{ cm}^{-1}$ and an intense aromatic band close to 1600 cm^{-1} , which suggests the higher grafting degree of BHEA [26,47–49]. Water is believed to facilitate fiber swelling, enhancing monomer penetration and improving grafting efficiency. These observations confirm that water mixed with butanone improves BHEA grafting, which agrees with the measured degree of grafting (DG). Two mechanisms seem to be behind this improvement. First, water could swell the miscanthus particles and make them more porous, so that the penetration of more monomers can be achieved. This swelling is evident, as observed in the comparative photographs in Figure 8, where the miscanthus particles treated with unmixed butanone remain compact, resulting in two phases with precipitation of fibers. Hence, only surface grafting can be promoted by less-polar solvents, since the monomer diffusion is slower. However, in the presence of a butanone/water mixture, the miscanthus particles swell immensely (Figure 8). Thus, the use of a good swelling solvent like water facilitates bulk grafting by promoting monomer diffusion through the compaction of the miscanthus particles [35]. Such structural alteration is associated with greater accessibility of the reactive sites to grafting. Second, water radiolysis during ionization generates highly active radical species such as hydroxyl radicals ($\text{HO}^\bullet$), hydrogen radicals ($\text{H}^\bullet$) and hydroperoxyl radicals ($\text{HO}_2^\bullet$), as well as hydrated electrons (aqueous e^-), among others [29,35,51]. These species enhance local active site density, resulting in more radical species on the miscanthus particles that can permit chain initiation at or near the surface. Thus, the addition of water not only provides greater accessibility to BHEA but might also maximize chemical pathways more suited to favoring grafting and lead to very high levels of improvement in grafting efficiency. Furthermore, water would generate more reactive radicals in comparison to butanone [52], promoting the grafting process even more.

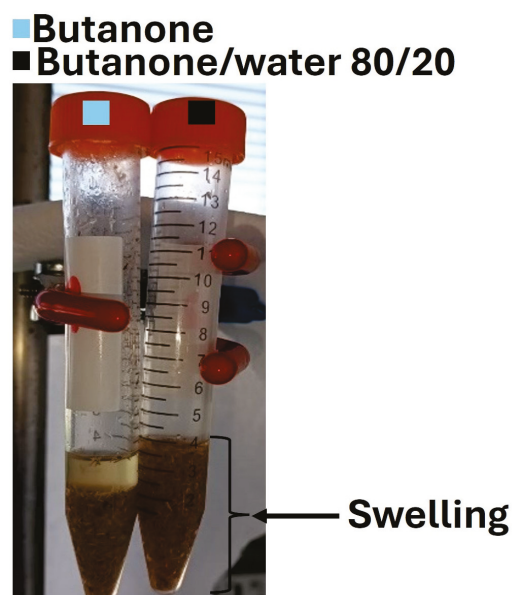


Figure 8. Photograph of miscanthus particles in unmixed butanone vs. butanone/water mixture: evidence of swelling.

2.3.2. Effect of BHEA Concentration

The impact of varying the BHEA concentration from 2 wt % to 17 wt % was also assessed at 50 kGy, as presented in Table 1d-(2) and Figure 6b. The results show that at very low monomer levels (2 and 5 wt %), grafting was essentially negligible. A moderate grafting of 3.2 ± 1 wt % was obtained at 9 wt % of BHEA, while 17 wt % of BHEA resulted in a high DG of 21.7 ± 2 wt %. FTIR-ATR analysis was also performed, to investigate

the effect of BHEA concentration on the grafting of miscanthus particles, as shown in Figure 7b. The spectra include miscanthus alone, samples irradiated at 50 kGy without BHEA and miscanthus grafted with varying BHEA concentrations (2 wt %, 5 wt %, 9 wt % and 17 wt %), alongside pure BHEA as a reference. At low concentrations (2 wt % and 5 wt %), the spectra are like that of native miscanthus, with negligible intensity in the ester carbonyl region $1720\text{--}1730\text{ cm}^{-1}$ [8,47–49]. This observation aligns with the degree of grafting (DG) values, which were undetectable at these concentrations. However, for 9 wt % BHEA, a slight increase in the carbonyl band and a weak enhancement around 1600 cm^{-1} suggest some degree of grafting, consistent with a measurable but limited DG. At the highest concentration (17 wt %), a significant intensification of the carbonyl band and more distinct aromatic signals near 1600 cm^{-1} confirm a higher grafting level, aligning with the previously reported DG values.

This is explained by the increased availability of monomers at higher concentrations, which resulted in a higher grafting rate [53]. Similar trends have been reported in the literature. For instance, Rasoul and coworkers [54] reported that increases in styrene concentration achieved using two other solvent systems, dichloromethane and benzene, in the grafted medium led to large increases in the extent of the grafting. This outcome was ascribed to the enhancement of monomer diffusivity within the polymer matrix, and the subsequent increase in opportunities for interaction with active sites. Similarly, in the research by Ishak and coworkers [55], the effect of the concentration of GMA, using a water/ethanol mixture, on the yield of grafting was systematically studied in flax textiles. Their findings indicate that it is possible to considerably increase the mass gain directly dependent on the rate of grafting by increasing the concentration of GMA in the grafting solution. For example, when flax fabrics were irradiated at 10 kGy using 1 mol/L GMA solution, the grafting rate was about 15 wt %. When the concentration of GMA was raised to 1.5 mol/L and 2 mol/L, the grafting rates were about 20 wt % and 30 wt %, respectively. These findings are in line with the reported behavior of BHEA in this study, for which the results show that increasing monomer concentration significantly improves grafting efficiency, indicting the necessity of optimizing the monomer concentrations in simultaneous radiation grafting processes.

2.3.3. Effect of Irradiation Dose

The effect of various irradiation doses on the degree of grafting (DG) of BHEA in miscanthus particles was studied. The amount of BHEA was kept constant at 17 wt %, a value already established as being optimal for the achievement of high grafting levels. The irradiation doses employed ranged from 10 to 100 kGy. As shown in Table 1d-(3) and Figure 6c, varying the irradiation dose led to a progressive rise in grafting: $8.7 \pm 1\text{ wt \%}$ at 10 kGy, $14.4 \pm 1\text{ wt \%}$ at 20 kGy, $21.7 \pm 2\text{ wt \%}$ at 50 kGy and $35.2 \pm 2\text{ wt \%}$ at 100 kGy. The results demonstrate an almost linear trend of increasing DG relative to higher irradiation doses, indicating that radiation dose is a critical parameter in the grafting process of BHEA. The FTIR-ATR spectra, as shown in Figure 7c, for particles irradiated under different irradiation doses display a parallel intensification of the key BHEA bands. It can be observed that progressive modifications of miscanthus spectrum are obtained with increases in the irradiation dose. While still qualitative, these spectral observations clearly substantiate the determination that higher doses produce more grafted polymer chains on the substrate. Importantly, the appearance and gradual intensification of the band near 1720 cm^{-1} , attributed to the ester C=O stretching of BHEA, strongly support successful grafting [8,47–49]. This band, barely visible at 10 kGy, becomes increasingly intense at higher doses (20, 50 and 100 kGy), confirming the progressive incorporation of BHEA as the degree of grafting (DG) rises, as quantified previously. Furthermore, the intensification

of aromatic C=C stretching bands at 1600 and 1510 cm^{-1} , not prominent in raw miscanthus but characteristic of BHEA, corroborates the grafting. These bands also increase in intensity with higher doses, in agreement with the finding of increased DG. Additionally, modifications are observed in the C–O–C and C–O bands within the 1250–1000 cm^{-1} regions, where both lignocellulosic components and ester groups of BHEA absorb. The increased complexity and intensification in this region suggest overlapping contributions from both grafted BHEA moieties and miscanthus particles. Hence, the increases in the intensity of the ester (C=O) and aromatic (C=C) bands directly reflect the amount of BHEA grafted, highlighting the role of irradiation in promoting the radical sites required for efficient graft polymerization [56].

The progressive rise in DG can be attributed to the formation of a greater number of radical sites on the miscanthus particles as the radiation dose increases. Higher doses of irradiation generate more free radicals, which then serve as active sites for the initiation of graft polymerization [35]. This dose–radical-formation relationship is known in the literature. For instance, in research investigating the radiation grafting of poly(butyl acrylate) on ethylene vinyl acetate copolymer, it was observed that an increase in the gamma irradiation dose from 10 to 50 kGy led to a concurrent increase in the grafting yield because of the higher availability of high-energy radiation for the formation of active sites for monomer grafting [56]. The same tendency has been reported elsewhere in the literature. Ishak and coworkers [55] investigated the radiation-induced grafting of glycidyl methacrylate on flax fabrics and reported that the grafting percentage increased with increased doses of irradiation. Also, Rasoul and co-workers [54] investigated the radiografting of styrene onto (poly(tetrafluoroethylene-co-ethylene) and reported the linear dependence of the radiation dose on grafting efficiency at an optimum level. These findings point out the importance of irradiation dose as a controlling factor, even for BHEA, in the context of miscanthus particles. Hence, the higher availability of radicals induced by higher doses allows for more efficient attachment of monomers, improving the overall grafting yield.

2.3.4. Effects of the Addition of Additives (Urea and H_2SO_4)

In the final set of trials (Figure 6d), the water portion in the 80/20 butanone/water system was replaced by either 2.5 wt % aqueous urea or 2.5 wt % aqueous H_2SO_4 . Both additives led to modest yet measurable gains in DG relative to the standard 80/20 mixture: 36.6 ± 1 wt % (urea) and 38.4 ± 1 wt % (H_2SO_4), versus 35.2 ± 2 wt % without additives. Urea can increase fiber swelling and solubilize some fiber components, thereby enhancing monomer penetration [57,58]. However, H_2SO_4 might trigger further radical-formation mechanisms, possibly through acid-catalyzed reactions in the fiber surface or through a modified radiolysis process. The presence of H^+ ions is crucial in inducing the conversion of solvated electrons to $\text{H}^\bullet$ radicals, which induce radical formation on the substrate through hydrogen atom abstraction [59–61]. Therefore, the addition of H_2SO_4 can slightly enhance grafting yields in some systems. Thus, in this study, both additives contribute to a limited increase in grafting yield. These small differences are not reflected in the FTIR spectra of treated samples (Figure 7d).

2.4. Accelerated UV Weathering of Compressed Miscanthus-Based Discs

The photostability of lignocellulosic materials is a complex phenomenon influenced by their intrinsic chemical composition, the presence of chromophoric groups and the modifications induced by grafting treatments [17,19,21–24]. In this study, the evolution in the color of miscanthus-based discs after 1000 h of UV weathering was assessed using four key parameters: total-color variation ΔE_{ab}^* , lightness (ΔL^*), chroma (ΔC_{ab}^*) and

hue (ΔH_{ab}^*). These parameters provide complementary information regarding the overall color change ΔE_{ab}^* . Figure 9 presents the results for the color change parameters, in diagram form. It should be noted that samples exhibiting detectable modifications in terms of effective and progressive grafting were retained for further UV weathering and characterization.

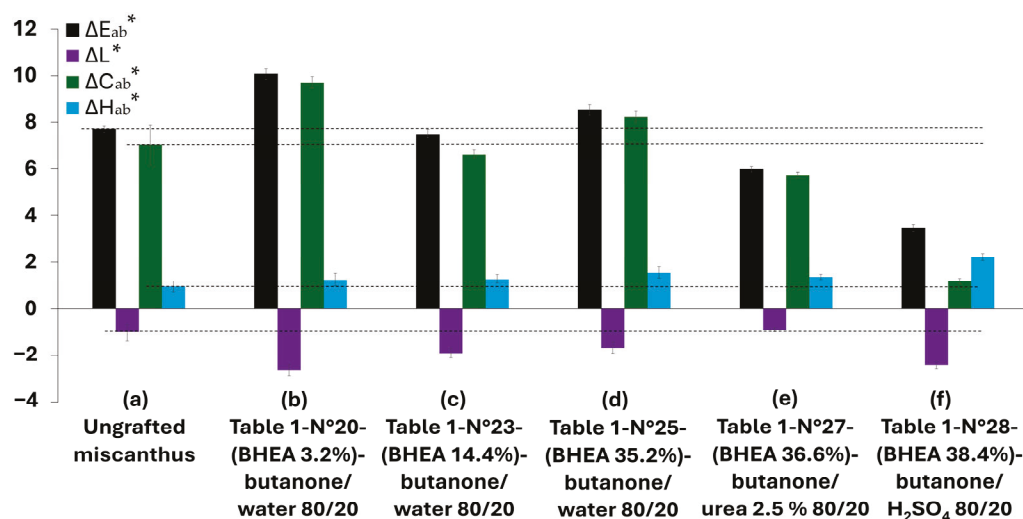


Figure 9. Color variation (ΔE_{ab}^*), lightness (ΔL^*), chroma (ΔC_{ab}^*) and hue (ΔH_{ab}^*) of raw miscanthus (a) ungrafted and miscanthus grafted with different concentrations of BHEA [(b) 3.2%, (c) 14.4 %, (d) 35.2%, (e) 36.6% and (f) 38.4%], after 1000 h of UV weathering. (Dashed curves are included as guidelines for following the evolution of each color component in the results.).

For the ungrafted miscanthus, UV exposure for 1000 h resulted in a moderate overall color change ($\Delta E_{ab}^* \approx 7.7$) that exceeded the threshold value of 2, the limit at which the human eye perceives a color difference, and surpassed 5, the threshold value at which colors are distinctly recognized as different [62]. The results show also a slight darkening ($\Delta L^* \approx -1.02$), a marked increase in chroma ($\Delta C_{ab}^* \approx 7$), and only minimal hue shift ($\Delta H_{ab}^* \approx 0.94$). These observations align with our previous work [17], in which, owing to the relatively high lignin content ($\approx 23\%$) of the samples, the development of chromophoric carbonyl and quinonoid structures under UV irradiation led primarily to browning and increased color saturation.

Grafting treatments using BHEA in various formulations were explored to evaluate the photostability of miscanthus-based discs. The results show that none of the treatments (Table 1, N° 20, 23, 25 and 27) provided a satisfactory improvement in UV photostability, as they all produced overall color changes (ΔE_{ab}^*) above the perceptibility threshold of 5, indicating that the treated miscanthus particles exhibited distinct color differences that could be clearly recognized by the human eye. For instance, miscanthus particles treated with a low BHEA level (3.2 wt % in a butanone/water system) showed a high color variation ($\Delta E_{ab}^* \approx 10.1$), stronger darkening ($\Delta L^* \approx -2.6$), a marked increase in chroma ($\Delta C_{ab}^* \approx 9.7$) and a modest hue shift ($\Delta H_{ab}^* \approx 1.3$). This would mean that limited grafting not only fails to protect the fibers, but may also add more reactive sites, which contribute more to photodegradation and lead to greater color changes. With the grafting level increased to 14.4 wt % in the same butanone/water system, the behavior of the samples began to resemble that of the ungrafted miscanthus particles, exhibiting a less pronounced total-color change ($\Delta E_{ab}^* \approx 7.5$) and chroma change ($\Delta C_{ab}^* \approx 6.5$), while darkening ($\Delta L^* \approx -2.5$) and hue shift ($\Delta H_{ab}^* \approx 1.3$) remained significant. This indicates that, while a higher grafting level may help to limit the formation of additional chromophoric groups, it does not effectively counteract the oxidative degradation that leads to color changes. With

35.2 wt % BHEA grafting, the total-color variation rebounded to around $\Delta E_{ab}^* \approx 9$, with persistent darkening ($\Delta L^* \approx -2.7$) and further increases in chroma ($\Delta C_{ab}^* \approx 8.5$) and hue ($\Delta H_{ab}^* \approx 1.6$). This suggests that an elevated grafting ratio within a water-based system can be responsible for the accumulation of secondary chromophore structures, ruining the potential benefits of surface modification. Similarly, increasing the grafting level to 36.6 wt % using a butanone/urea system provided only a partial decrease in color change ($\Delta E_{ab}^* \approx 6.0$), slight darkening ($\Delta L^* \approx -0.9$) and moderate increases in chroma ($\Delta C_{ab}^* \approx 5.7$) and hue ($\Delta H_{ab}^* \approx 1.4$). Although this modification resulted in a slightly lower level of total-color variation, the variation still exceeded the perceptibility threshold, reinforcing the determination that increasing the degree of grafting does not necessarily improve UV stability.

The most significant enhancement in UV stability was achieved using a formulation of 38.4 wt % BHEA with 2.5% sulfuric acid in a butanone system. This treatment yielded the lowest overall color change ($\Delta E_{ab}^* \approx 3.4$) and the smallest variation in chroma ($\Delta C_{ab}^* \approx 1.2$). Although this formulation exhibited a high degree of darkening ($\Delta L^* \approx -2.4$) and the highest hue ($\Delta H_{ab}^* \approx 2.2$), the overall perceptible color change was minimized. This could be directly linked to the color modification in the miscanthus fibers after treatment and before UV weathering, as can be clearly observed in Figure 10a compared to Figure 10c.

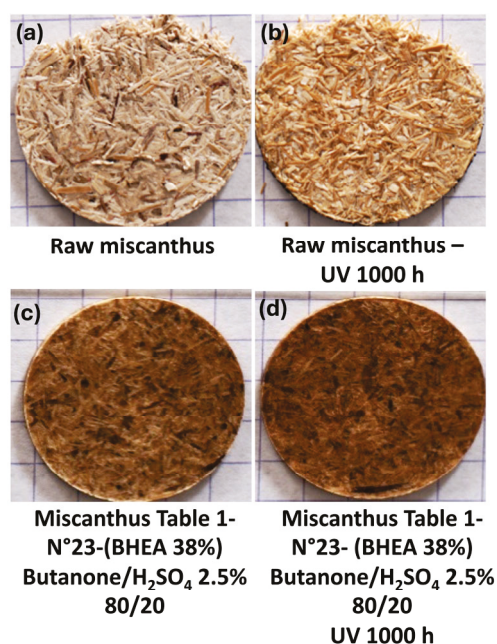


Figure 10. Photographs of raw miscanthus (a) before and (b) after 1000 h of weathering and miscanthus grafted with 38% of BHEA (Table 1, N° 23) discs, (c) before and (d) after 1000 h of weathering.

For aid of understanding, Figure 11 presents the color variation measurements for miscanthus particles grafted in acidic medium before exposure to weathering (Figure 10c). The calculation of the color parameters was made against untreated miscanthus fibers (Figure 10a). The results show a significant increase in overall color change ($\Delta E_{ab}^* \approx 16.5$). Such a high increase points out that the acid treatment plays an important part in changing the natural color of fibers even before their exposure to UV. The most remarkable observation is the strong decrease in lightness ($\Delta L^* \approx -14$), showing a darkening effect favored by the grafting reaction in acid medium. This tendency is coupled with chroma ($\Delta C_{ab}^* \approx 8.0$) and hue ($\Delta H_{ab}^* \approx 2.8$) changes that together are responsible for the perceptible color shift after grafting and before weathering. The darkening of the fibers before weathering is representative of a structural or chemical change in the lignocellulosic components, which can be a precursor to improved UV stability. This observation agrees with the work of

Shi and coworkers [63] on sulfuric acid treatment of wood; the work suggests that wood treatment with sulfuric acid could lead to a perceptible color shift in which the color turned to a dark chocolate-brown because of the acid degradation before UV exposure. Acidic treatment likely promotes conjugated chromophore development via glycosidic linkage cleavage and modification of aromatic-ring structures in lignin. This effect is in line with the dominant darkening effect ($\Delta L^* \approx -14$) found in this study and suggests that new chromophore structures enhance visible light absorption. Hence, the impact of this initial color modification becomes evident when examining the long-term weathering behavior, as shown in Figures 9 and 10. In the case of native (untreated) miscanthus, UV exposure over 1000 h led to a significant color change, with visible browning and particle decohesion, as confirmed by the microscopic observations in Figure 12b. As previously discussed, this browning is attributed to lignin degradation leading to the formation of chromophore carbonyl and quinoid structures, which are responsible for color saturation and an increase in darkening [17]. Contrarily, the miscanthus particles grafted with 38 wt % of BHEA in acidic medium exhibited a steady post-weathering color profile, as seen in Figures 9 and 10d.

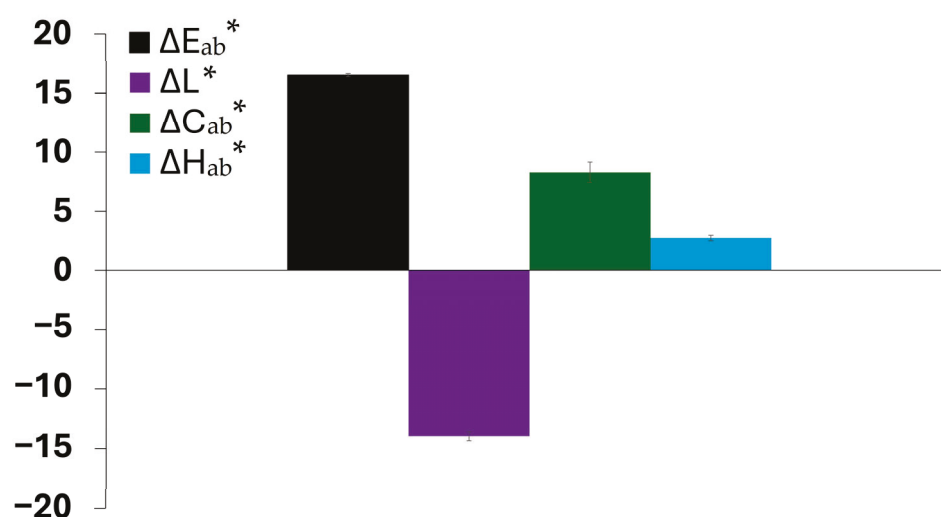


Figure 11. Total-color variation (ΔE_{ab}^*), lightness (ΔL^*), chroma (ΔC_{ab}^*) and hue (ΔH_{ab}^*) of miscanthus grafted with BHEA in butanone/2.5% H_2SO_4 80/20 system before weathering.

The pre-weathering darkening effect appears to have positively influenced the fiber's resistance to UV-induced color modification, as the overall post-weathering color change was minimized. Hence, BHEA-grafted miscanthus maintains a more intact fiber structure and a cohesive miscanthus-based disc with fewer visible defects and a more homogeneous color distribution, as shown by the microscopic observations in Figure 12d. This indicates that the chemical modification not only suppresses color alteration but also ensures the physical integrity of the particles, hindering excessive degradation. This suggests that acid-catalyzed grafting not only alters the color of the particles initially, but would also enhance resistance to photodegradation.

These findings underscore the significance of chemical modification methods in controlling miscanthus particles' photodegradation. While acid treatment alters the inherent color, it becomes a promising strategy that ensures increased color stability. Rather than having an inherent color that tends to depreciate with time, color darkening is more suitable for ensuring the increased UV stability of miscanthus particles through minimizing the perceived color change.

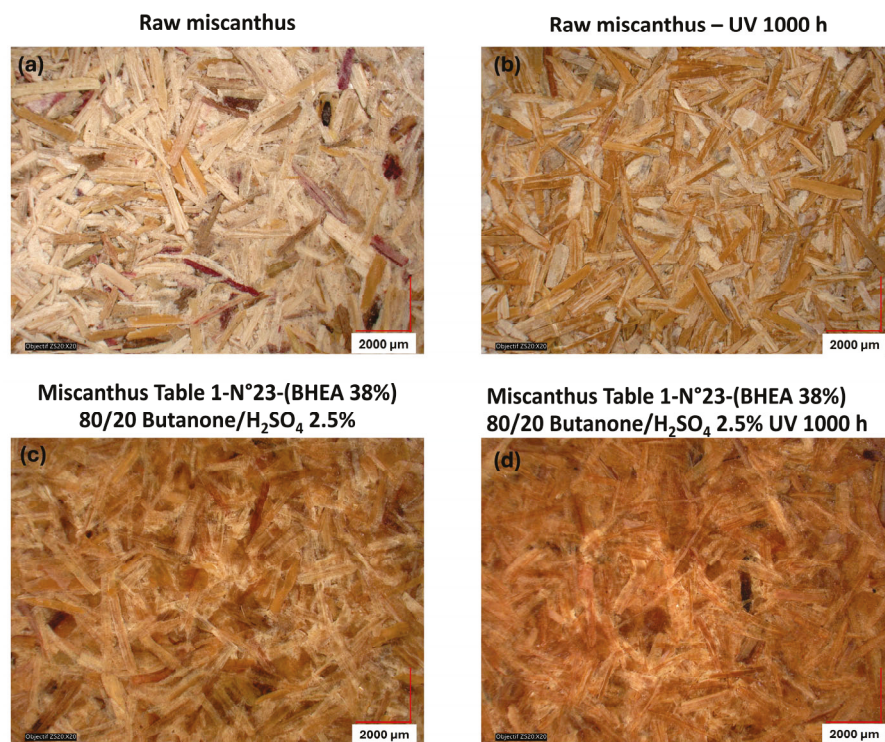


Figure 12. Microscopic observations of raw miscanthus discs (a) before and (b) after 1000 h of UV weathering and miscanthus grafted 38% of BHEA (c) before and (d) after 1000 h of UV weathering (Table 1, N° 23), before and after 1000 h of UV weathering.

3. Materials and Methods

3.1. Materials

Miscanthus raw materials were harvested in Normandy, France, and conditioned in sealed plastic bags, which were produced by Addiplast company (Saint-Pal-de-Mons, France). Miscanthus particles underwent a sieving process to remove any dusty components and retain fragments within the 500–800 µm size range. Ethanol (96% purity) was procured from PANREAC (Castellar del Vallès, Spain). Sulfuric acid (98% purity) was obtained from CHEM-LAB (Zedelgem, Belgium). Tetrahydrofuran (99.8% purity) and Butanone (99% purity) were purchased from Fisher Scientific (Illkirch, France). Mohr's salt Prolabo ((NH₄)₂Fe (SO₄)₂ · 6 H₂O) was supplied by VWR (Les Ulis, France). 2-Hydroxy-4-(methacryloyloxy)benzophenone (HMB, 99% purity, Figure 5a) and 2-(4-Benzoyl-3-hydroxyphenoxy)ethyl acrylate (BHEA, 97% purity, Figure 5b) UV absorbers were acquired from ABCR (Lyon, France).

3.2. Methods

3.2.1. General Procedure of Miscanthus Particle Modification by Pre-Irradiation Grafting

Miscanthus particles were irradiated at Ionisos SA (Chaumesnil, France), using a Linac electron beam (*e*-beam) delivering a dose rate of approximately 300 kGy/min and operating at 10 MeV and 34 kW. The target doses, measured by polystyrene calorimetry (uncertainty = 3.8%), were set at 50 kGy and 100 kGy, with the process carried out in air at room temperature. To prevent rises in the temperature of the irradiated samples, the 50 kGy and 100 kGy doses were delivered in two and four passes, respectively, each limited to 25 kGy per pass. The irradiated miscanthus particles were immediately stored at −18 °C and transported to the laboratory in refrigerated containers at the same temperature, to ensure the stability of the generated radical species. Throughout the study, the irradiated particles were stored in a freezer at −15 °C and removed only a few minutes before the

grafting tests. HMB and BHEA solutions were prepared in butanone, as this solvent offers the highest solubility for these molecules, with concentrations reaching up to 17 wt % in butanone. In contrast, the solubility of these molecules in water is significantly limited; hence, the water content in the butanone mixture was minimized. To achieve the grafting process, the HMB and BHEA prepared solutions were degassed by argon, as an inert gas, for 15 min in a container before the addition of the pre-irradiated miscanthus particles. In most of the assays, Mohr's salt was added to the aqueous part before bubbling. The conditions tested involved varying the following parameters: Miscanthus/BHEA or HMB mass ratios; butanone/water mass ratios; BHEA concentration; and miscanthus/solution mass ratios. Table 1a summarizes the different assays prepared in this part. After the pouring of the pre-irradiated miscanthus particles, degassing continued for an additional 2 min, and the centrifuge tubes were each tightly closed with a cap. The containers were then heated to 80 °C for 1 h. The treated miscanthus particles were recovered through filtration and washed 3 times with butanone and distilled water, each wash conducted at 40 °C for 10 min in an ultrasonic bath. The particles were allowed to dry in a fume hood for 24 h until weight stabilization. For the purpose of accuracy, each experiment was conducted in duplicate.

3.2.2. General Procedure of Miscanthus Particle Modification by Simultaneous-Irradiation Grafting

Simultaneous irradiation grafting was achieved via 2 routes. The first route is based on solvent-free grafting during irradiation and adapted from the work of Hajj et al. (2019) [38]. Hence, miscanthus particles were mixed with HMB dissolved in butanone; the particle/solution weight ratio was 1/1. After mixing, the particles were allowed to embed in the solution for 1 h. The mixture was then placed in an extractor hood to facilitate solvent evaporation. The dried miscanthus particles were irradiated with the *e*-beam at a radiation dose of 100 kGy, in air and at room temperature, by Ionisos SA (Chaumesnil, France). Once received, the particles were washed 3 times with 40 °C butanone in an ultrasonic bath to eliminate any unreacted monomers, oligomers, or polymers. The washed particles were then left in an extractor hood to dry until their weights stabilized. To ensure precision, the experiment was performed in triplicate. The various assays of this route are summarized in Table 1b. The second route involves grafting in a solvent state during irradiation, inspired by the method described by Ishak et al. (2024) [55]. HMB and BHEA were dissolved in butanone or an 80/20 butanone/water mixture, then combined with miscanthus particles in a centrifuge tube, which was immediately sealed to prevent solvent evaporation. The centrifuge tubes containing both the particles and the UV absorbers dissolved in the solvent were exposed to *e*-beam irradiation at different radiation doses, in air and at room temperature; this was realized by Ionisos SA (Chaumesnil, France). It is important to note that only the miscanthus/BHEA and miscanthus/solution ratios were assessed. Additionally, some tests were conducted replacing the water part with other additives, including sulfuric acid (2.5 wt % H₂SO₄) and urea (2.5 wt %). The irradiated samples were filtered; washed 3 times with butanone/water 80/20 and two times with butanone, at 40 °C in an ultrasonic bath; and then dried in an oven at 60 °C for 2 h and then left under the fume hood until weight stabilization. To ensure the validity of the results, each experiment was repeated in triplicate. An overview of the various assays of this route is presented in Table 1c,d.

3.2.3. Miscanthus Disc Preparation

Following the method outlined by El Hage and co-workers [17], binder-less compressed discs of miscanthus were prepared by measuring 500 mg of raw or modified particles. These particles were then compressed under a pressure of 30 kN for 2 min at

a temperature of 105 °C, utilizing a Prontopress-2 press (Struers A/S, Copenhagen, Denmark). The resulting discs, each with a diameter of 2.5 cm and a thickness of 2 mm, were allowed to cool to room temperature for 4 min before being recovered.

3.2.4. Accelerated UV Weathering

Two discs of each miscanthus sample were placed in a QUV/se accelerated weathering tester (Q-Lab, Cleveland, OH, USA). Aging was conducted, following the condition described by El Hage et al. (2024) [17], for a total of 1000 h. Repetitive 12-hour cycles were applied, consisting of 8 h of UV exposure at a wavelength of 340 nm, with an irradiance of 0.76 W/m² and a temperature of 60 °C, followed by 4 h of dark exposure at 40 °C. After a total of 1000 h of UV exposure, the samples were recovered for evaluation and analysis.

3.2.5. Spectrophotometer Analysis

Following the experimental procedure outlined by El Hage and coworkers [17], color measurements of the miscanthus compressed discs, both before and after weathering, were conducted using a polychromator-type spectroradiometer (Konica-Minolta CS-2000, Tokyo, Japan). This instrument enables precise light measurements, allowing for the determination of the spectral reflectance of colored surfaces. The CIE $L^*a^*b^*$ 1976 values were then calculated, along with chroma (C^*), which measures the intensity of a color, and hue angle (h^*), which determines a color's position on the color wheel. These parameters were determined by averaging three measurements taken for each area. The total-color difference ($\Delta E_{ab}^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$), the lightness (ΔL^*), the chroma (ΔC_{ab}^*) and the hue (ΔH_{ab}^*) were calculated and compared. Δa and Δb , which are parts of the calculation for ΔE_{ab}^* , denote the differences in the a^* and b^* components between two colors, reflecting shifts in the green–red and blue–yellow hues, respectively. An increase in ΔL^* indicates a lightening, while a decrease indicates a darkening of the color. ΔC_{ab}^* measures the change in chroma, with higher values indicating increased saturation. ΔH_{ab}^* represents the difference in hue angles between the colors.

3.2.6. Attenuated Total Reflectance–Fourier-Transform Infrared Spectroscopy (ATR-FTIR)

The surfaces of both native and grafted miscanthus particle discs were analyzed before and after grafting, using a Vertex 70 single-reflection diamond ATR-FTIR spectrometer (Bruker Corporation, Ettlingen, Germany). For each sample, baseline-corrected spectra were recorded at different exposure times, with 32 scans and a resolution of 4 cm^{−1}. FTIR signals and the corresponding assignments for *Miscanthus* × *giganteus* particles are compiled in Supporting Information S1.

3.2.7. Optical Microscope Observation

The surfaces of the raw and grafted miscanthus particle disks were observed using a fifth generation Keyence VHX-7000 digital microscope (Itasca, IL, USA). Images were captured with a high-performance VHX-7020 camera, utilizing the VH-ZST real zoom lens, at a standard overall magnification of 100x.

3.2.8. Degree of Grafting Measurements (DG wt %)

For all grafting procedures, the degree of grafting was assessed by weighing the particles with an Quintix124-1 Sartorius[®] analytical balance (Goettingen, Germany), with a precision of 10^{−4} g. Particle weights were recorded both before and after grafting. The dry mass of the particles was determined using a Sartorius[™] MA 160 infrared moisture analyzer (Goettingen, Germany). In this procedure, 0.2 g of particles are heated to 105 °C until completely dried. The dry matter percentage is automatically calculated by the infrared balance, accounting for the initial and final fiber masses. Each analysis was performed in

triplicate to ensure reproducibility, and the average value from these repetitions was used. An additional parameter was considered to account for the weight loss that could occur from the washing steps, due to the use of solvents that remove extractive compounds. To address this, identical experiments were conducted without UV absorbers while applying the same washing procedures. Consequently, the weight loss fraction (P) associated with these washing steps was estimated and included in the calculation to avoid any underestimation in the calculation of the grafting degree. Hence, the degree of grafting is calculated as follows in Equation (1):

$$\text{DG wt \%} = \left[100 \cdot \frac{(\text{final dry weight} - \text{starting dry weight} \times (1 - P))(\text{g})}{\text{starting dry weight} \times (1 - P)(\text{g})} \right] \quad (1)$$

4. Conclusions

This work highlights the need for UV-absorber grafting onto *Miscanthus × giganteus* particles to alleviate the inherent tendency of these particles towards photodegradation. The pre-irradiation grafting attempts with HMB and BHEA were failed attempts, mainly due to the rapid radical degradation under the tested conditions. Solvent-free simultaneous grafting was also unsuccessful as the solid-state UV absorbers lacked the mobility necessary for penetration of the particles and initiation of covalent bonding. Simultaneous irradiation of HMB within a solvent system was also unable to yield detectable grafting, likely due to the HMB's radical-scavenging and resonance stabilization ability, which inhibited polymerization. On the other hand, simultaneous irradiation under butanone/water conditions, and especially with BHEA, led to effective grafting and greatly improved the UV stability. Among the significant parameters influencing graft yield were solvent composition, BHEA concentration, irradiation dose and the incorporation of additives such as urea or sulfuric acid. Most notably, the highest grafting level (38 wt %) and photoprotection were achieved by grafting BHEA in a butanone medium containing 2.5% sulfuric acid. Although this formulation produced a minimized overall color change ($\Delta E_{ab}^* \approx 3.4$ after 1000 h of accelerated UV exposure), the enhanced photostability was not directly linked to the degree of grafting but rather to a pronounced initial darkening of the fibers induced by the sulfuric acid treatment prior to weathering. This preliminary color transition appears to alter the chromophore structure of the particles and consequently partially inhibit subsequent UV alteration. These findings validate successful miscanthus grafting through BHEA monomer and propose a complex inter-relationship between color stability, chemical modification and BHEA grafting. Hence, beyond grafting efficiency, the initial chemical treatment plays a crucial role in defining the long-term photostability of the material. Additional studies could be focused on the incorporation of these fibers in a polymeric matrix, and a scale-up of the process for industrial applications in the fiber-reinforced composites field, to evaluate their durability.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/molecules30173649/s1>, Supporting Information S1: FTIR signals and corresponding assignment for *Miscanthus × giganteus* particles in this study.

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References

1. Muthuraj, R.; Misra, M.; Mohanty, A.K. Biocomposite Consisting of Miscanthus Fiber and Biodegradable Binary Blend Matrix: Compatibilization and Performance Evaluation. *RSC Adv.* **2017**, *7*, 27538–27548. [CrossRef]
2. El Hage, R.; Khalaf, Y.; Lacoste, C.; Nakhil, M.; Lacroix, P.; Bergeret, A. A Flame Retarded Chitosan Binder for Insulating Miscanthus/Recycled Textile Fibers Reinforced Biocomposites. *J. Appl. Polym. Sci.* **2019**, *136*, 47306. [CrossRef]
3. Ichim, M.; Muresan, E.I.; Codau, E. Natural-Fiber-Reinforced Polymer Composites for Furniture Applications. *Polymers* **2024**, *16*, 3113. [CrossRef] [PubMed]
4. Shettigar, S.; Gowrishankar, M.C.; Shettar, M. Review on Aging Behavior and Durability Enhancement of Bamboo Fiber-Reinforced Polymer Composites. *Molecules* **2025**, *30*, 3062. [CrossRef]
5. Clifton-Brown, J.C.; Lewandowski, I. Water Use Efficiency and Biomass Partitioning of Three Different Miscanthus Genotypes with Limited and Unlimited Water Supply. *Ann. Bot.* **2000**, *86*, 191–200. [CrossRef]
6. Zub, H.W.; Brancourt-Hulmel, M. Agronomic and Physiological Performances of Different Species of Miscanthus, a Major Energy Crop. A Review. *Agron. Sustain. Dev.* **2010**, *30*, 201–214. [CrossRef]
7. El Hage, R.; Chrusciel, L.; Desharnais, L.; Brosse, N. Effect of Autohydrolysis of *Miscanthus* × *giganteus* on Lignin Structure and Organosolv Delignification. *Bioresour. Technol.* **2010**, *101*, 9321–9329. [CrossRef]
8. Brendlé, C.; El Hage, R.; Clément, J.L.; Rouif, S.; Sonnier, R.; Otazaghine, B. Radiation-Induced Graft Copolymerization of a Phosphonate Monomer onto Short Flax Fibers and *Miscanthus* × *giganteus* Stem Fragments: Influence of Substrate Composition. *Eur. Polym. J.* **2024**, *213*, 113094. [CrossRef]
9. Le Moigne, N.; Sonnier, R.; El Hage, R.; Rouif, S. Radiation-Induced Modifications in Natural Fibres and Their 812 Biocomposites: Opportunities for Controlled Physico-Chemical Modification Pathways? *Ind. Crops Prod.* **2017**, *109*, 199–213. [CrossRef]
10. Gismatulina, Y.A.; Budaeva, V.V.; Kortusov, A.N.; Kashcheyeva, E.I.; Gladysheva, E.K.; Mironova, G.F.; Skiba, E.A.; Shavyrkina, N.A.; Korchagina, A.A.; Zolotukhin, V.N.; et al. Evaluation of Chemical Composition of *Miscanthus* × *Giganteus* Raised in Different Climate Regions in Russia. *Plants* **2022**, *11*, 2791. [CrossRef]
11. Abdalla, M.; Hastings, A.; Campbell, G.; Chen, H.; Smith, P. Assessing the Sustainability of Miscanthus and Willow as Global Bioenergy Crops: Current and Future Climate Conditions (Part 1). *Agronomy* **2024**, *14*, 3020. [CrossRef]
12. Hodgson, E.M.; McCalmont, J.; Rowe, R.; Whitaker, J.; Holder, A.; Clifton-Brown, J.C.; Thornton, J.; Hastings, A.; Robson, P.R.H.; Webster, R.J.; et al. Upscaling Miscanthus Production in the United Kingdom: The Benefits, Challenges, and Trade-Offs. *GCB Bioenergy* **2024**, *16*, e13177. [CrossRef]
13. Shavyrkina, N.A.; Budaeva, V.V.; Skiba, E.A.; Gismatulina, Y.A.; Sakovich, G.V. Review of Current Prospects for Using Miscanthus-Based Polymers. *Polymers* **2023**, *15*, 3097. [CrossRef] [PubMed]
14. Kalinina, O.; Nunn, C.; Sanderson, R.; Hastings, A.F.S.; Van Der Weijde, T.; Özgüven, M.; Tarakanov, I.; Schüle, H.; Trindade, L.M.; Dolstra, O.; et al. Extending Miscanthus Cultivation with Novel Germplasm at Six Contrasting Sites. *Front. Plant Sci.* **2017**, *8*, 563. [CrossRef]
15. Venendaal, R.; Jorgensen, U.; Fosters, C.A. European Energy Crops: A Synthesis. *Biomass Bioenergy* **1997**, *13*, 147–185. [CrossRef]
16. Moll, L.; Wever, C.; Völkerling, G.; Pude, R. Increase of Miscanthus Cultivation with New Roles in Materials Production—A Review. *Agronomy* **2020**, *10*, 308. [CrossRef]
17. El Hage, R.; Carvalho Martins, R.; Brendlé, C.; Lafon-Pham, D.; Sonnier, R. Enhancing Insight into Photochemical Weathering of Flax and Miscanthus: Exploring Diverse Chemical Compositions and Composite Materials. *Molecules* **2024**, *29*, 3945. [CrossRef]
18. Andrady, A.L.; Heikkilä, A.M.; Pandey, K.K.; Bruckman, L.S.; White, C.C.; Zhu, M.; Zhu, L. Effects of UV Radiation on Natural and Synthetic Materials. *Photochem. Photobiol. Sci.* **2023**, *22*, 1177–1202. [CrossRef]
19. Jirouš-Rajković, V.; Miklečić, J. Enhancing Weathering Resistance of Wood—A Review. *Polymers* **2021**, *13*, 1980. [CrossRef]
20. Ares-Elejoste, P.; Pérez, A.; Gondra, K.; Seoane-Rivero, R. Development of New Lignin-Based Coatings with Ultraviolet Resistance for Biobased Composite Materials. *Polymers* **2024**, *16*, 2175. [CrossRef]
21. Tragoonwichian, S.; O'Rear, E.A.; Yanumet, N. Admicellar Polymerization of 2-Hydroxy-4-Acryloyloxybenzophenone: The Production of UV-Protective Cotton. *Colloids Surf. A Physicochem. Eng. Asp.* **2008**, *329*, 87–94. [CrossRef]

22. Tragoonwichian, S.; O'Rear, E.A.; Yanumet, N. Broad Ultraviolet Protection by Copolymerization of 2-[3-(2H-Benzotriazol-2-Yl)-4-Hydroxyphenyl]Ethyl Methacrylate and 2-Hydroxy-4-Acryloyloxybenzophenone on Cotton via Admicellar Polymerization. *J. Appl. Polym. Sci.* **2008**, *108*, 4004–4013. [CrossRef]
23. Tragoonwichian, S.; O'Rear, E.A.; Yanumet, N. Grafting Polymerization of 2-[3-(2H-Benzotriazol-2-Yl)-4-Hydroxyphenyl]Ethyl Methacrylate on Vinyltriethoxysilane-Treated Cotton for the Preparation of Ultraviolet-Protective Fabrics. *J. Appl. Polym. Sci.* **2009**, *114*, 62–69. [CrossRef]
24. Gorjanc, M.; Jazbec, K.; Mozetič, M.; Kert, M. UV Protective Properties of Cotton Fabric Treated with Plasma, UV Absorber, and Reactive Dye. *Fibers Polym.* **2014**, *15*, 2095–2104. [CrossRef]
25. Odian, G.; Chandler, H.W. Radiation-induced graft polymerization. In *Advances in Nuclear Science and Technology*; Academic Press: Cambridge, MA, USA, 1962; pp. 85–109. [CrossRef]
26. Brendlé, C.; El Hage, R.; Clément, J.L.; Rouif, S.; Sonnier, R.; Otazaghine, B. Flame Retardancy of Short Flax Fibers Modified by Radiation-Induced Grafting of Phosphonated Monomers: Comparison between Pre- and Simultaneous Irradiation Grafting. *Molecules* **2024**, *29*, 1176. [CrossRef] [PubMed]
27. Takács, E.; Wojnárovits, L.; Borsa, J.; Rácz, I. Hydrophilic/Hydrophobic Character of Grafted Cellulose. *Radiat. Phys. Chem.* **2010**, *79*, 467–470. [CrossRef]
28. Taibi, J.; Rouif, S.; Ameduri, B.; Sonnier, R.; Otazaghine, B. Radiation Induced Graft Polymerization of Fluorinated Monomers onto Flax Fabrics for the Control of Hydrophobic and Oleophobic Properties. *Polymer* **2023**, *281*, 126132. [CrossRef]
29. Wojnárovits, L.; Földváry, C.M.; Takács, E. Radiation-Induced Grafting of Cellulose for Adsorption of Hazardous Water Pollutants: A Review. *Radiat. Phys. Chem.* **2010**, *79*, 848–862. [CrossRef]
30. Fastow, E.; Cook, S.; Dean, P.; Ott, P.; Wilson, J.; Yoon, H.; Dietz, T.; Bateman, F.; Al-Sheikhly, M. Single-Step Synthesis of Atmospheric CO₂ Sorbents through Radiation-Induced Graft Polymerization on Commercial-Grade Fabrics. *Radiat. Res.* **2019**, *192*, 219. [CrossRef]
31. Goel, N.K.; Rao, M.S.; Kumar, V.; Bhardwaj, Y.K.; Chaudhari, C.V.; Dubey, K.A.; Sabharwal, S. Synthesis of Antibacterial Cotton Fabric by Radiation-Induced Grafting of [2-(Methacryloyloxy)Ethyl]Trimethylammonium Chloride (MAETC) onto Cotton. *Radiat. Phys. Chem.* **2009**, *78*, 399–406. [CrossRef]
32. Bergs, M.; Völkerling, G.; Kraska, T.; Pude, R.; Do, X.T.; Kusch, P.; Monakhova, Y.; Konow, C.; Schulze, M. *Miscanthus* × *giganteus* Stem Versus Leaf-Derived Lignins Differing in Monolignol Ratio and Linkage. *Int. J. Mol. Sci.* **2019**, *20*, 1200. [CrossRef] [PubMed]
33. Voča, N.; Leto, J.; Karažija, T.; Bilandžija, N.; Peter, A.; Kutnjak, H.; Šurić, J.; Poljak, M. Energy Properties and Biomass Yield of *Miscanthus* × *giganteus* Fertilized by Municipal Sewage Sludge. *Molecules* **2021**, *26*, 4371. [CrossRef] [PubMed]
34. Raghothaman, N.; Joseph, P.S. Vibrational Assignments and Normal Coordinate of 2-Hydroxy-4-Methoxybenzophenone. *Asian J. Chem.* **2011**, *23*, 1353–1356.
35. Ashfaq, A.; Clochard, M.-C.; Coqueret, X.; Dispenza, C.; Driscoll, M.S.; Ulański, P.; Al-Sheikhly, M. Polymerization Reactions and Modifications of Polymers by Ionizing Radiation. *Polymers* **2020**, *12*, 2877. [CrossRef]
36. Moane, S.; Raftery, D.P.; Smyth, M.R.; Leonard, R.G. Decomposition of Peroxides by Transition Metal Ions in Anaerobic Adhesive Cure Chemistry. *Int. J. Adhes. Adhes.* **1999**, *19*, 49–57. [CrossRef]
37. Kochi, J.K. Chemistry of Alkoxy Radicals: Cleavage Reactions. *Am. Chem. Soc.* **1962**, *84*, 1193–1197. [CrossRef]
38. Hajj, R.; El Hage, R.; Sonnier, R.; Otazaghine, B.; Gallard, B.; Rouif, S.; Nakhil, M.; Lopez-Cuesta, J.-M. Grafting of Phosphorus Flame Retardants on Flax Fabrics: Comparison between Two Routes. *Polym. Degrad. Stab.* **2018**, *147*, 25–34. [CrossRef]
39. El-Gendy, E.H. Modification of Cotton Fabrics via Radiation Graft Copolymerization with Acrylic Acid, Acrylonitrile and Their Mixtures. *Indian. J. Fibre Text. Res.* **2022**, *27*, 266–273.
40. Takács, E.; Mirzadeh, H.; Wojnárovits, L.; Borsa, J.; Mirzataheri, M.; Benke, N. Comparison of Simultaneous and Pre-Irradiation Grafting of N-Vinylpyrrolidone to Cotton-Cellulose. *Nucl. Instrum. Methods Phys. Res. B* **2007**, *265*, 217–220. [CrossRef]
41. Carbone, N.D.; Ene, M.; Lancaster, J.R.; Koberstein, J.T. Kinetics and Mechanisms of Radical-Based Branching/Cross-Linking Reactions in Preformed Polymers Induced by Benzophenone and Bis-Benzophenone Photoinitiators. *Macromolecules* **2013**, *46*, 5434–5444. [CrossRef]
42. Odian, G. *Principles of Polymerization*, 4th ed.; Wiley Interscience: Hoboken, NJ, USA, 2004.
43. Simič, R.; Mandal, J.; Zhang, K.; Spencer, N.D. Oxygen Inhibition of Free-Radical Polymerization Is the Dominant Mechanism behind the “Mold Effect” on Hydrogels. *Soft Matter* **2021**, *17*, 6394–6403. [CrossRef]
44. O'Brien, A.K.; Cramer, N.B.; Bowman, C.N. Oxygen Inhibition in Thiol–Acrylate Photopolymerizations. *J. Polym. Sci. A Polym. Chem.* **2006**, *44*, 2007–2014. [CrossRef]
45. Moad, G.; Solomon, D.H. *The Chemistry of Radical Polymerization*; Elsevier: Amsterdam, The Netherlands, 2005.
46. Yiğitoğlu, M.; Arslan, M. 4-Vinylpyridine and 2-Hydroxyethylmethacrylate Monomer Mixture Graft Copolymerization onto Poly(Ethylene Terephthalate) Fibers Using Benzoyl Peroxide. *Polym. Bull.* **2007**, *58*, 785–798. [CrossRef]
47. Mohammed, A.D.; Ahmed, A.J. Studies on Graft Copolymerization of Acrylic Acid onto Acetylated Cellulose from Maize Cob. *J. Turk. Chem. Soc. Sect. A Chem.* **2022**, *9*, 571–578. [CrossRef]

48. Rahmawati; Suhartini, M.; Budianto, E. Radiation Graft Copolymerization of Acrylic Acid onto Rice Straw Cellulose. *Macromol. Symp.* **2015**, *353*, 231–239.
49. Silverstein, R.M.; Webster, F.X.; Kiemle, D.J. *Spectrometric Identification of Organic Compounds*, 7th ed.; Wiley: Hoboken, NJ, USA, 2005.
50. Liu, C.F.; Xu, F.; Sun, J.X.; Ren, J.L.; Curling, S.; Sun, R.C.; Fowler, P.; Baird, M.S. Physicochemical Characterization of Cellulose from Perennial Ryegrass Leaves (*Lolium Perenne*). *Carbohydr. Res.* **2006**, *341*, 2677–2687. [CrossRef]
51. Henglein, A. J. W. T. Spinks. R. J. Woods: *An Introduction to Radiation Chemistry, Third Edition*, John-Wiley and Sons, Inc., New York, Toronto 1990. ISBN 0-471-61403-3. 574 Seiten, Preis: DM 91, 45.; Wiley-VCH: Weinheim, Germany, 1991.
52. Henderson, M.A. Ethyl Radical Ejection during Photodecomposition of Butanone on TiO₂(1 1 0). *Surf. Sci.* **2008**, *602*, 3188–3193. [CrossRef]
53. Bhattacharya, A.; Misra, B.N. Grafting: A Versatile Means to Modify Polymers: Techniques, Factors and Applications. *Prog. Polym. Sci.* **2004**, *29*, 767–814. [CrossRef]
54. Cardona, F.; George, G.A.; Hill, D.J.T.; Rasoul, F.; Maeji, J. Copolymers Obtained by the Radiation-Induced Grafting of Styrene onto Poly(Tetrafluoroethylene-Co-Perfluoropropylvinyl Ether) Substrates. 1. Preparation and Structural Investigation. *Macromolecules* **2002**, *35*, 355–364. [CrossRef]
55. Ishak, A.; Longuet, C.; Otazaghine, B.; Sonnier, R. Enhancing Flame Retardancy of Flax Fabrics through Phytic Acid Grafting. *ACS Appl. Polym. Mater.* **2024**, *6*, 5642–5652. [CrossRef]
56. Siddiq, A.; Ghobashy, M.M.; El-Adasy, A.-B.A.A.M.; Ashmawy, A.M. Gamma Radiation-Induced Grafting of Poly(Butyl Acrylate) onto Ethylene Vinyl Acetate Copolymer for Improved Crude Oil Flowability. *Sci. Rep.* **2024**, *14*, 8863. [CrossRef]
57. Moussa, M.; El Hage, R.; Sonnier, R.; Chrusciel, L.; Ziegler-Devin, I.; Brosse, N. Toward the Cottonization of Hemp Fibers by Steam Explosion. *Flame-Retard. Fibers. Ind. Crops Prod.* **2020**, *151*, 112242. [CrossRef]
58. Antoun, K.; Ayadi, M.; El Hage, R.; Nakhil, M.; Sonnier, R.; Gardiennet, C.; Le Moigne, N.; Besserer, A.; Brosse, N. Renewable Phosphorous-Based Flame Retardant for Lignocellulosic Fibers. *Ind. Crops Prod.* **2022**, *186*, 115265. [CrossRef]
59. Garnett, J.L.; Jankiewicz, S.V.; Sangster, D.F. Effect of Mineral Acid on Polymer Produced during Radiation-induced Grafting of Styrene Monomer. *J. Polym. Sci. Polym. Lett. Ed.* **1982**, *20*, 171–175. [CrossRef]
60. Ang, C.H.; Garnett, J.L.; Jankiewicz, S.V.; Sangster, D. *Acid Effect in UV- and Radiation-Induced Grafting of Styrene to Cellulose*; ACS Publication: Washington, DC, USA, 1982; pp. 141–154.
61. Rawat, S.; Misra, N.; Shelkar, S.A.; Kumar, V. Tailoring Acid Free-Paper Based Analytical Devices (Af-PADs) via Radiation Assisted Modification of Cellulose Paper. *Carbohydr. Polym.* **2023**, *317*, 121116. [CrossRef]
62. Mokrzycki, W.; Tatol, M. Color Difference Delta E-A Survey Colour Difference ΔE-A Survey. *Mach. Graph. Vis. Vision.* **2011**, *20*, 383–411.
63. Shi, J.; Lu, Y.; Zhang, Y.; Cai, L.; Shi, S.Q. Effect of Thermal Treatment with Water, H₂SO₄ and NaOH Aqueous Solution on Color, Cell Wall and Chemical Structure of Poplar Wood. *Sci. Rep.* **2018**, *8*, 17735. [CrossRef] [PubMed]

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Article

Hydrophobic Coating of Paperboard Using Oak Wood-Derived Lignin Nanoparticles and Chitosan Composites

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Abstract: This study explores the potential application of lignin nanoparticles and chitosan–lignin nanoparticles (CLNs) as hydrophobic barrier coatings for paperboard. The lignin nanoparticles were initially prepared using a mixed solvent of ethanol and acetone. Their characteristics were examined via scanning electron microscopy (SEM) and dynamic light scattering, which revealed particle sizes in the range of 180–400 nm. The results indicated that the coatings with pure lignin nanoparticles failed to impart hydrophobicity to the paperboard, whereas the CLN coatings significantly enhanced hydrophobicity and reduced water absorption. The water contact angle increased from 109° to over 128° after the first CLN coating, remained at 127° with the second and third coating layers, and was maintained at 119° with four layers. Multilayer coatings were applied to improve barrier performance; however, no further enhancement in hydrophobicity was observed. The CLN-coated paper exhibited a significantly improved surface smoothness, as confirmed by SEM. The results indicate that a single-layer CLN coating is effective for imparting water-barrier properties to paperboard. In contrast, the coating with pure lignin nanoparticles resulted in cracked surfaces and inconsistent coating thicknesses.

Keywords: lignin; nanoparticles; chitosan; composites; green coating; water barrier

1. Introduction

Lignin is the second most abundant bio-based organic polymer on Earth, after cellulose [1]. Lignin is typically obtained as a byproduct of cellulose production in the pulp and paper industry and is extracted from both wood and non-wood plants. While cellulose is commonly used in the production of pulp and paper, derivatives, textiles, and other products, most extracted lignin is treated as waste and used as fuel [2]. Lignin has long been considered a low-value-added product. Millions of tons of lignin are generated annually as waste from the kraft pulp industry [3–5]. Approximately 1–2% of lignin is converted into high-value-added products, whereas more than 90% is either burned for energy or simply discarded [5,6]. One of the major challenges in the utilization of lignin is its structural complexity. Lignin contains various phenolic groups and has a highly irregular and cross-linked structure that hinders its practical application. Additionally, different pretreatment methods and biomass sources result in variations in lignin structure, further complicating its industrial utilization [7].

Lignin can be extracted from lignocellulosic biomass through various methods, including alkaline, hydrothermal, and organosolv methods [8]. Alkaline solutions (e.g., NaOH,

KOH, NH_4OH) cleave ether and ester linkages in lignin–carbohydrate complexes, enhancing lignin solubility [9]. Hydrothermal treatment uses high-temperature water to hydrolyze the bond of lignin polymer [10]. In addition, organosolv extraction employs organic solvents with acid catalysts to break lignin linkages and separate it from other components [11]. Although acid treatments, such as those with H_2SO_4 , HCl, and HNO_3 , are primarily used for carbohydrate hydrolysis, they may also cause partial lignin depolymerization under severe conditions [12]. The aforementioned lignin extraction methods are known to generate various lignins with different molecular weights, functional groups, and structural characteristics, which significantly influence the chemical reactivity and compatibility of lignin in downstream applications, particularly in the formation of lignin nanoparticles or polymer composites [13]. Therefore, the lignin extraction method is a critical step in determining the physical properties of biomass–polymer composite materials.

Zhao et al. [5] reported that lignin possesses multiple functional groups that form complex hydrogen-bonding networks, which can hinder its miscibility with polymer matrices and lead to interfacial compatibility issues in composite materials. Moreover, lignin exhibits a high degree of polydispersity in its molecular weight, which contributes to its heterogeneity, limiting its processability and reproducibility in advanced material applications [14]. However, lignin continues to attract interest because of its diverse functional groups, which can be modified for the development of fine chemicals and advanced materials. The 90% of lignin that is currently underutilized represents a significant resource for value-added applications.

Lignin has also attracted considerable attention owing to its diverse functionalities, including antioxidant activity, ultraviolet (UV)-shielding capability, nanocarrier formation, dye adsorption, microfluidic-device integration, surface coating, additive functionality, drug delivery, and antimicrobial properties [3,4,15–17]. Machado et al. [3] reported that lignin-based nanocarriers possess broad functionalities, including nutrient retention and the sustained release of herbicides and fungicides. Lignin has also been combined with chitosan for wastewater treatment and food packaging applications [16]. Yu et al. [17] reported the application of lignin/ TiO_2 composites for UV shielding in sunscreen products. The incorporation of lignin into TiO_2 increased the sunscreen's sun protection factor by approximately 30–60% compared with formulations containing only nano- TiO_2 . Recent studies on lignin valorization have explored its use as a precursor for carbon fibers and adhesives and as a sustainable alternative to petroleum-based chemicals [1,18].

The conversion of lignin into lignin nanoparticles is a research trend that explores the possibilities and potential future applications of lignin [19]. There are many methods for nanoparticle synthesis. Typically, lignin extracted from wood and non-wood materials is converted into lignin nanoparticles using solvents such as ethanol, methanol, acetone, ethylene glycol, and tetrahydrofuran [5]. Lignin nanoparticles offer improved dispersibility and reactivity compared with bulk lignin, making them suitable for nanocomposites, drug delivery, and functional coatings [20].

Chitosan is a bio-based material derived from chitin extracted from shrimp, lobster, and crab shells [21]. It is used for various applications, including wastewater treatment, dye removal, biomedical devices, and drug delivery [22–24]. Other applications of chitosan are food packaging, tissue engineering, scaffolding, and composite reinforcement, as reported elsewhere [25–28]. Chitosan has been combined with cellulose nanofibers, silver nanoparticles, and polypyrrole to improve the hydrophobicity of coated materials [29,30]. It has considerable potential for diverse applications in the environmental, biomedical, and composite fields [31]. Furthermore, the biocompatibility and biodegradability of chitosan have led to its approval for certain biomedical and food-related applications, highlighting its safety and versatility. However, the potential applications of chitosan require further

investigation. In this study, chitosan–lignin composite nanoparticles (CLNs) were produced and used as a coating material on paperboard, and their effects on the hydrophobic properties of the paperboard were evaluated. In addition, lignin was investigated as a standalone coating material for paperboard because of its potential as a high-value-added product. The coating performance and hydrophobic properties of paperboard coated with lignin nanoparticles and CLNs were systematically investigated.

2. Results and Discussion

2.1. Lignin Nanoparticle Fabrication and Morphology

Lignin nanoparticles (LNPs) were fabricated using an ethanol/acetone solvent system. The fabricated lignin was dried in a vacuum oven at 45 °C for several days. The dried lignin was ground into a fine powder using a mortar and pestle and stored in sealed vials until use. The dried lignin powder was immersed overnight in ethanol/acetone under continuous stirring. The collected lignin nanoparticles were then used as a coating material.

Scanning electron microscopy (SEM) images indicated that the lignin nanoparticles had a spherical shape and uniform size. Figures 1 and 2 show the particle size distribution determined via dynamic light scattering (DLS) and SEM images, respectively, of the LNPs.

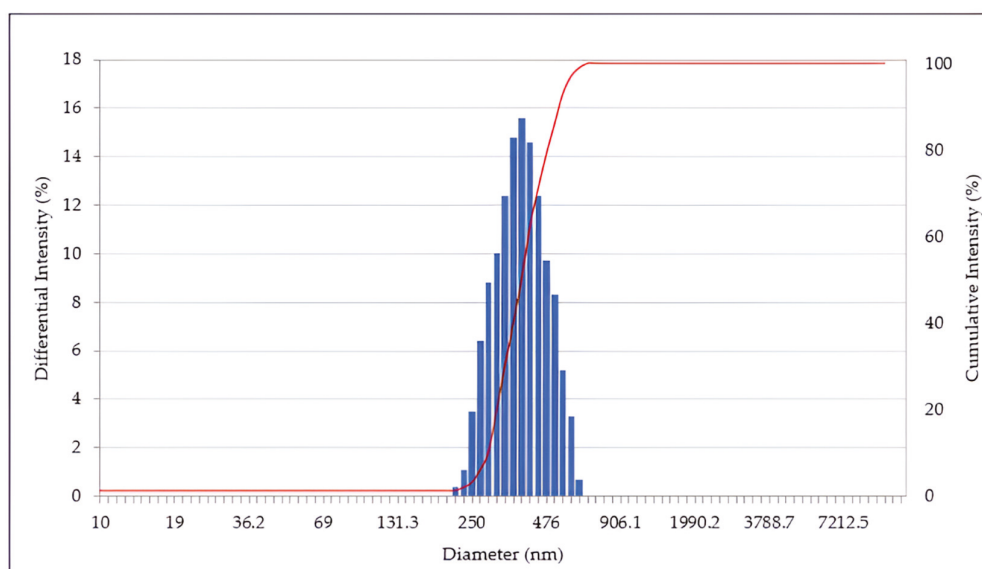


Figure 1. Size distribution of lignin nanoparticles analyzed via DLS. The blue columns indicate the differential intensity distribution, while the red line indicates the cumulative intensity distribution.

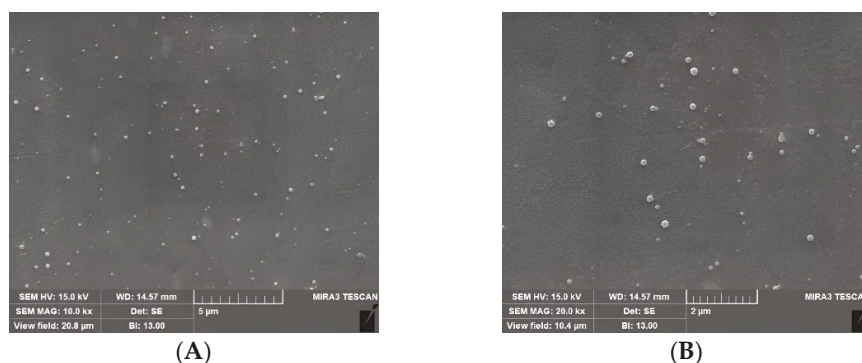


Figure 2. Lignin nanoparticles observed via SEM at magnifications corresponding to (A) 5 µm and (B) 2 µm scale bars.

2.2. DLS Spectrophotometer

The lignin nanoparticles were first analyzed using DLS, and the lignin particle size was found to be approximately 200–600 nm. The size distribution of the lignin nanoparticles obtained via DLS analysis is shown in Figure 1. The data analysis was performed at least four times to determine the particle size. The DLS analysis provided an overview of the lignin nanoparticle size distribution after fabrication. The largest cumulative number of lignin nanoparticles fell within the range of 300–400 nm, comprising approximately 70% of all nanoparticles. The remaining 30% of the nanoparticles were distributed in other size ranges: 15% were smaller than 300 nm, and another 15% were in the range of 500–650 nm. An example of a DLS intensity-based size distribution is shown in Figure 1.

2.3. SEM Analyzer

SEM was used to investigate the morphology of the lignin nanoparticles. The SEM images revealed the nanoparticles' spherical shape. Furthermore, they confirmed that the size range of the nanoparticles was 200–400 nm, which was consistent with the DLS analysis results. No significant aggregation or clustering of nanoparticles was observed, suggesting effective stabilization during the fabrication process. Figure 2 shows the size and morphology of the fabricated lignin nanoparticles as observed via SEM.

2.4. Coatings of Lignin Nanoparticles and CLNs for Paper Products

In this study, lignin nanoparticles and CLNs were investigated for their applicability as coating materials. Coatings with different basic weights were compared. Figures 3 and 4 show SEM images of the surface and cross-section of the paperboard coated with CLNs.

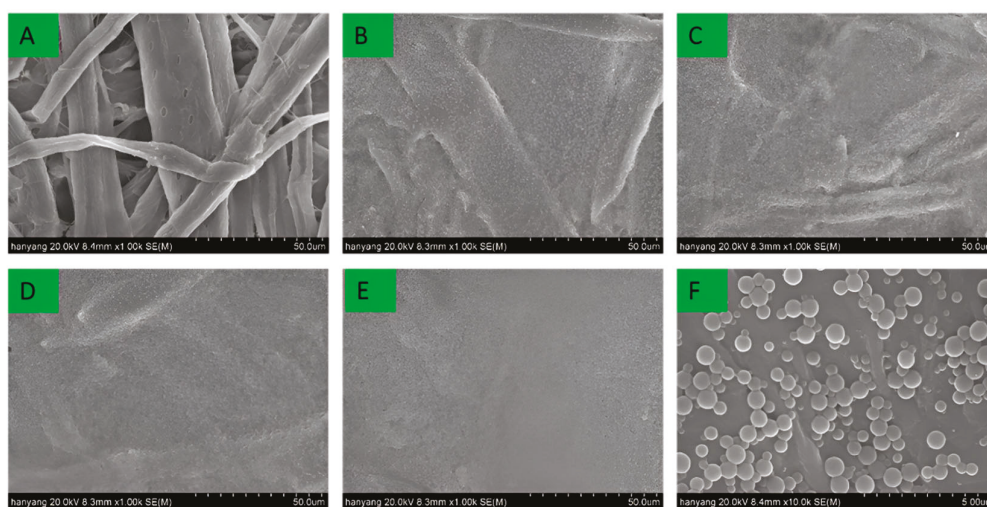


Figure 3. (A) Pristine paper substrate; (B–E) paper coated with chitosan–lignin nanoparticles in 1-, 2-, 3-, and 4-layer coatings, respectively (scale bar: 50 μ m); (F) high-magnification image of the 1-layer chitosan–lignin nanoparticle coating (scale bar: 5 μ m).

The uncoated paperboard exhibited a highly textured surface with numerous voids, significant porosity, and a clearly defined cellulose fiber morphology. However, the surface of the paperboard became smoother with an increasing number of CLN coating layers. A single layer of CLNs formed a thin and discontinuous film on the paperboard surface (Figure 3B). After four CLN coating layers, almost no pores or cellulose fiber morphology was observed. Furthermore, no cracks or broken areas were observed on the surfaces of the coated paperboards. When coated with four layers, the paperboard exhibited a smooth surface, with a markedly different structure from that of the uncoated paperboard (Figure 3A). In contrast, with one to three coating layers, traces of the original cellulose

fiber morphology remained partially visible. The lignin nanoparticles exhibited a spherical shape in SEM images, with particle sizes ranging from 200 to 500 nm, as confirmed by DLS and SEM. However, as shown in Figure 3F, the lignin nanoparticles in the coated paperboard formed aggregated clusters rather than maintaining individual dispersions. Figure 4 compares the cross-section of the one-layer and four-layer CLN coatings on the paperboard surface. The one-layer coating formed a thin and incomplete film, whereas the four-layer coating resulted in a significantly smoother and more continuous surface. This demonstrates that increasing the number of CLN layers improves the surface uniformity and coating quality.

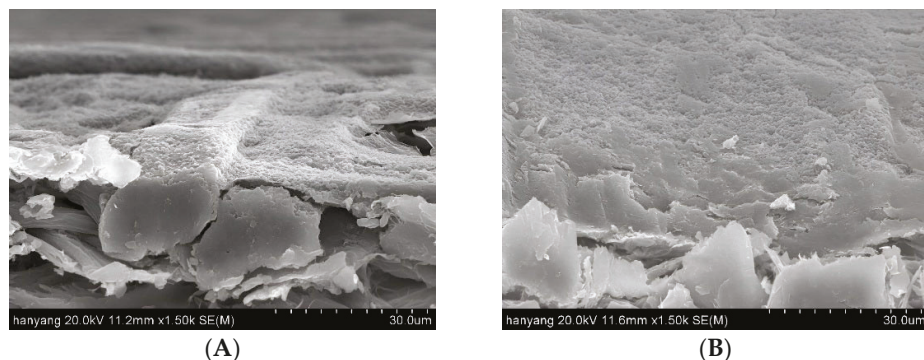


Figure 4. Cross-sectional SEM images of CLN-coated paperboard with (A) 1-layer and (B) 4-layer coatings (scale bar: 30 μm).

Figures 5 and 6 show the effect of the pure lignin nanoparticle coating on paperboard. In the second method, only lignin nanoparticles were used to coat the paperboard surface. The pure lignin coating exhibited poor coating uniformity and a noncontinuous layer, in contrast to the uniform coating achieved with CLNs. The pure lignin nanoparticle coating exhibited cracks, along with poor continuity between coated regions. After three to four coating layers were applied, the cellulose fibers were no longer visible; however, cracks in the coating became more apparent. This indicated that the lignin nanoparticles were not well bonded or attached to the paper substrate and could easily be removed. In both the surface and cross-sectional views, the samples coated with pure lignin nanoparticles exhibited a rougher texture than did those coated with CLNs. These results demonstrate that the combination of CLNs provides better coating adhesion and surface smoothness on paper than pure lignin nanoparticle coatings. Figure 6 shows the cross-sectional differences between the one-layer and four-layer coatings using pure LNs. While the four-layer coating provided relatively improved surface coverage compared to the one-layer coating, the surface remained non-uniform and less smooth than that of the CLN-coated sample.

2.5. Water Contact Angles of Pure Paper and Coated Paper

Figures 7 and 8 show the water contact angles (WCAs) of the pure paperboard, paperboard coated with lignin nanoparticles, and CLN-coated paperboard with different numbers of coating layers. The pure paperboard exhibited a WCA of approximately 109° , indicating its inherent hydrophobicity. However, coatings of lignin nanoparticles and CLNs affected the surface hydrophobicity. The CLN coating significantly enhanced the hydrophobicity of the paperboard. The WCA increased from 109° for the pure paperboard to 128° and 127° with one to three CLN coating layers, but it decreased to 119° with four layers. These results suggest that single-layer CLN coatings have sufficient hydrophobicity for practical application. Increasing the number of coating layers did not significantly improve the hydrophobic coating performance. Further studies are needed to investigate the effects of the coating parameters and optimize the CLN coating process.

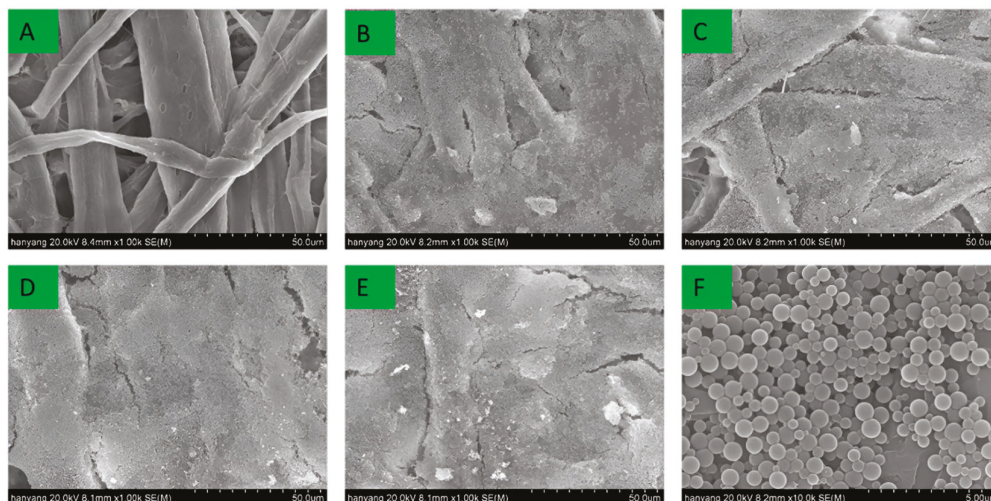


Figure 5. Surface morphology of (A) the uncoated paperboard substrate and (B–E) paperboard coated with lignin nanoparticles in 1-, 2-, 3-, and 4-layer coatings, respectively (scale bar: 50 µm); (F) high-magnification SEM image of 1-layer spray-coated lignin nanoparticles (scale bar: 5 µm).

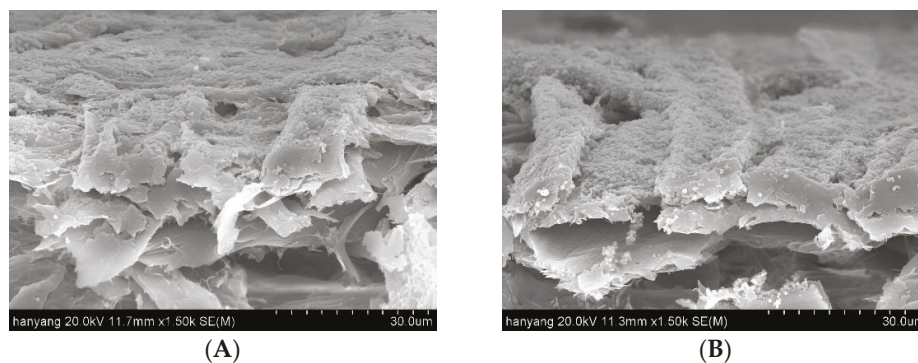


Figure 6. Cross-sectional SEM images of lignin nanoparticle-coated paperboard: (A) 1-layer and (B) 4-layer coatings (scale bar: 30 µm).

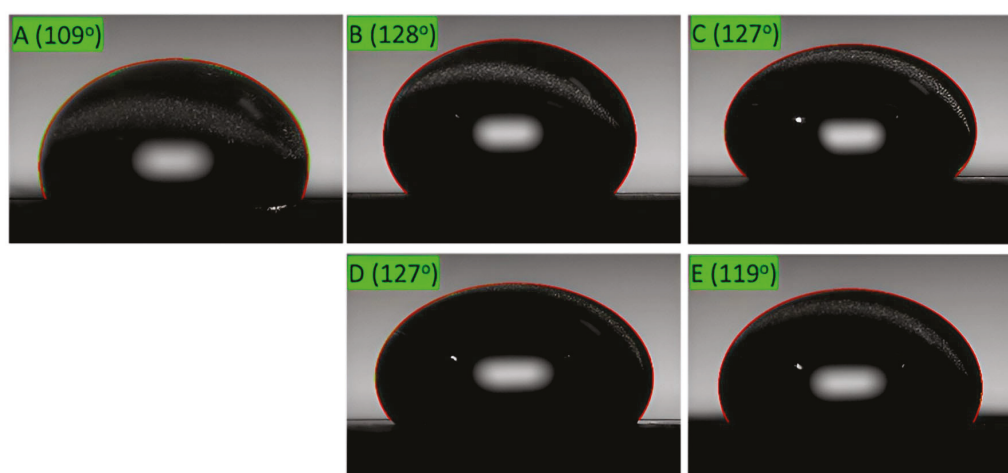


Figure 7. Surface morphology of (A) bare paperboard and paperboard coated with CLNs: (B) 1 layer; (C) 2 layers; (D) 3 layers; (E) 4 layers.

As shown in Figure 8, paperboard coated with pure lignin nanoparticles exhibited lower hydrophobicity than did paperboard coated with CLNs. The bare paperboard exhibited a WCA of 109°, which decreased as the number of pure lignin coating layers increased. The WCA decreased from 109° to 27° after four coating layers were applied. This suggests

that pure lignin nanoparticles are ineffective at providing hydrophobicity. This indicates that pure lignin nanoparticles are not ideal for hydrophobic coating applications. Additionally, the surface coated with pure lignin nanoparticles exhibited cracks and discontinuous coverage, in contrast to the uniform film observed on the CLN-coated sample. The coated layer was easily detached from the paperboard after drying. This phenomenon was evident in the Cobb test. A visible water ring remained after each Cobb test regardless of whether one or more layers of lignin nanoparticles were applied. These results raise the question of which factors contribute to the superior hydrophobicity of CLN coatings. As discussed earlier, the CLN-coated paperboard exhibited a significant improvement, with the WCA increasing from 109° to 128° .

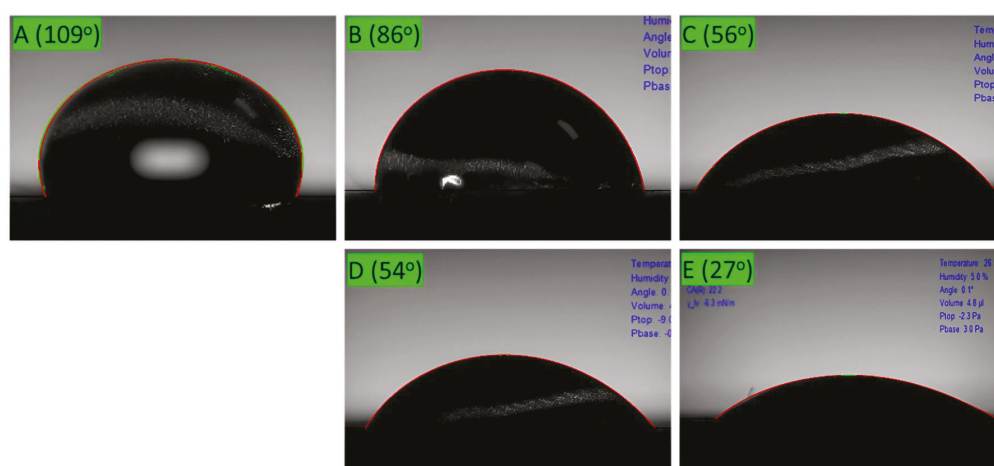


Figure 8. Surface morphology of (A) bare paperboard and lignin-coated paperboard with (B) 1 layer, (C) 2 layers, (D) 3 layers, and (E) 4 layers.

The Cobb₆₀ test was performed to evaluate the hydrophobicity of coated paperboard samples, and the results are shown in Figure 9. Both LN- and CLN-coated paperboards exhibited lower Cobb₆₀ values (52.2–54.5 g/m² and 45.0–50.4 g/m², respectively) than did the uncoated paperboard (58.6 g/m²), indicating enhanced hydrophobicity. For LN coatings, the Cobb₆₀ values decreased from 54.5 g/m² for the single-layered sample to 52.2 g/m² for the four-layered sample as the number of coating layers increased. In contrast, the single-layer CLN coating exhibited a significantly decreased Cobb₆₀ value (45.0 g/m²), then the value gradually increased again (47.9–49.1 g/m²). It is speculated that the single-layer CLN coating forms a relatively uniform and stable film, resulting in a notably reduced Cobb₆₀ value. As the number of CLN layers increases, the effect of chitosan's intrinsic hydrophilicity may become more pronounced, leading to a gradual increase in Cobb₆₀ values. Although chitosan has both hydrophilic (hydroxyl group) and hydrophobic (acetyl group) sites, it has far more hydrophilic than hydrophobic sites; therefore, it is known to be a hydrophilic polymer.

2.6. Chemical Compositions of Chitosan, Lignin, and CLNs

The chemical structures of chitosan, lignin, and the CLNs used for coating applications were investigated via FT-IR spectroscopy (Figure 10). A broad peak in the range of 3200–3450 cm^{−1} was assigned to OH stretching vibrations. Chitosan exhibited a sharper and more intense peak at approximately 3430 cm^{−1}, whereas lignin and CLNs exhibited broader and less intense peaks in the range of 3300–3450 cm^{−1}. The FT-IR spectrum also showed band shifts for chitosan at 2850, 2937, and 2970 cm^{−1} upon the formation of CLNs. These bands shifted toward the corresponding peaks of pure lignin with slightly lower intensities, whereas the broad O-H peak remained nearly unchanged. The peaks at 2937 and 2970 cm^{−1} were

assigned to C-H stretching vibrations. The peak at approximately 1460 cm^{-1} was assigned to the C-H stretching of the aromatic skeleton, whereas the peak at approximately 1423 cm^{-1} corresponded to bending in the phenolic group [16,32].

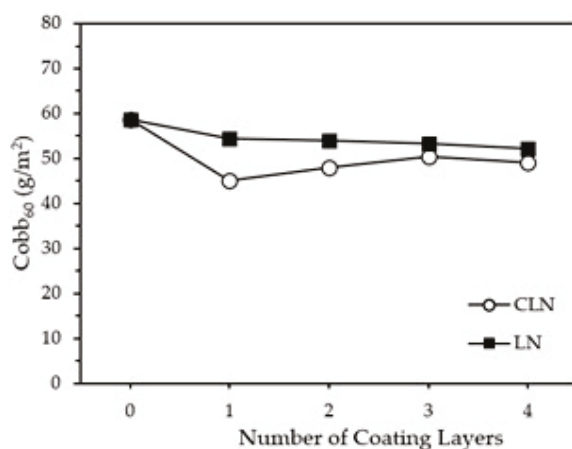


Figure 9. Cobb₆₀ values of uncoated paperboard and paperboards coated with CLNs and LNs.

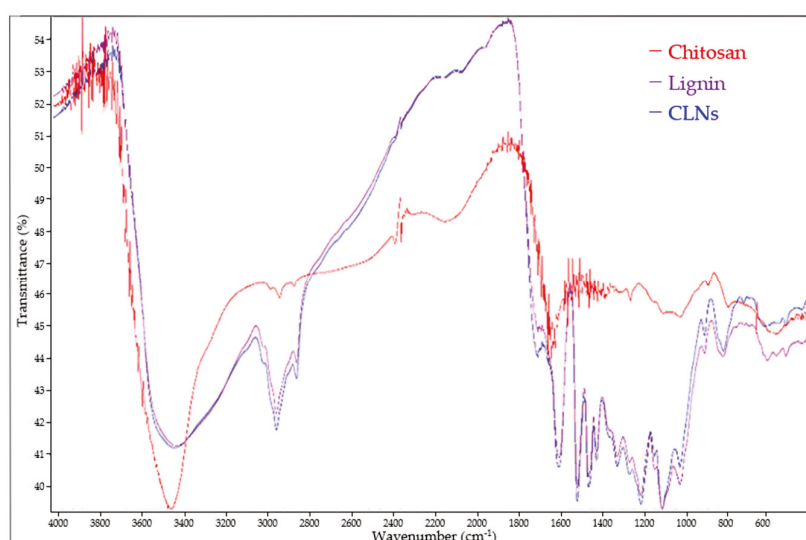


Figure 10. Fourier transform infrared (FT-IR) spectra of lignin nanoparticles, chitosan, and CLNs.

3. Materials and Methods

3.1. Materials

3.1.1. Feedstock

Oak wood was used in this study. It was cultivated and collected in Seoul, South Korea. After harvesting, it was air-dried at ambient temperature ($25\text{ }^{\circ}\text{C}$) and then mechanically ground and passed through a sieve to obtain particles ranging from 500 to $2000\text{ }\mu\text{m}$. The initial chemical composition, summarized in Table 1, was analyzed following the standard Laboratory Analytical Procedures (LAPs) (NREL/TP-510-42618 and NREL/TP-510-42622) [33,34].

3.1.2. Chemicals

Low-molecular-weight chitosan was purchased from Sigma-Aldrich Inc. (Cat. No. 448869, Lot No. BCCD0403, St. Louis, MO, USA). Sodium hydroxide (NaOH; 97%, Cat. No. 367176, Sigma-Aldrich, St. Louis, MO, USA) was used for the biomass pretreatment. Sulfuric acid (H_2SO_4 ; 95–98%, ACS Reagent, Cat. No. 258105, Sigma-Aldrich, St. Louis, MO,

USA), ethanol (ethanol, $\geq 99.9\%$, Cat. No. 4023–4110, Daejung Chemicals & Metals Co., Ltd., Siheung, Republic of Korea), and acetone ($\geq 99.5\%$, Cat. No. 1009–4410, Daejung Chemicals & Metals Co., Ltd., Siheung, Republic of Korea) were used without further purification.

Table 1. Solid-composition analysis of oak wood.

Component	Composition [%]
Glucan	38.6 ± 1.6
Xylan	6.2 ± 0.1
Galactan	trace
Arabinan	2.3 ± 0.2
Mannan	12.6 ± 1.5
ASL	5.6 ± 0.5
AIL	27.5 ± 1.2
Ash	0.6 ± 0.1
Total	93.4

Notes: All weight percentages were calculated using the oven-dry weight biomass. The data in the table represent the mean ($n = 3$) $\pm$ standard deviation. ASL: acid-soluble lignin, AIL: acid-insoluble lignin.

3.2. Lignin Extraction and Purification

Lignin was isolated from oak wood via treatment with 5.0% sodium hydroxide at 180 °C for 2 h. The resulting black liquor was filtered through glass filters to eliminate the remaining cellulose fibers and larger particles. Sulfuric acid was added to the black liquor to adjust the pH to approximately 2.5, inducing lignin precipitation. The precipitated solution was collected after settling overnight. The precipitate was washed multiple times via centrifugation at 10,000 rpm for 10–20 min using a centrifuge (Combi 514R, Hanil Scientific Inc., Gimpo, Republic of Korea) until the solution reached a pH of approximately 6. After washing, the lignin was dried at 45 °C in an oven for several days until it reached a constant weight, ground into a fine powder, and stored in a glass container until use. Lignin nanoparticles were synthesized using a solvent-exchange technique. The morphology, chemical structure, and coating performance of both the raw lignin and the synthesized nanoparticles were analyzed. Coating layers composed of either lignin nanoparticles alone or CLNs were prepared and applied as one to four sequential layers.

3.3. Lignin Nanoparticle Fabrication

The ground lignin particles were dissolved in an ethanol/acetone solvent overnight at room temperature. Subsequently, deionized (DI) water was added for approximately 15 min, and the volume of water required to dissolve the lignin solvent was 1:5 or 1:6 for obtaining the lignin nanoparticles. In brief, each batch of lignin nanoparticles was prepared by dissolving 2.0 g of the extracted lignin in 95% ethanol solvent under magnetic stirring. The samples were mixed at 200–300 RPM overnight. After that the LNs were precipitated overnight or collected by centrifugation. The suspension was then transferred to an evaporation dryer to evaporate the ethanol at 45 °C for 72 h until a constant weight was reached. The lignin nanoparticles were stored in a refrigerator until further use. The remaining lignin after ethanol treatment was further treated with acetone. In this step, 20 mL of acetone was added to the undissolved lignin and mixed at 200–300 RPM for approximately one hour. After that, DI was added and was stirred overnight. The LNs treated with acetone solvent were collected by centrifugation, and the acetone was removed using a rotary evaporator (RV 8 S99, IKA, Staufen, Germany) equipped with a heating bath (HB 10 S99).

3.4. Coating Method

Coating was performed using an autofilm applicator (DA-QP100, Dong Ah Trade Corp, Seoul, Republic of Korea). Lignin nanoparticles and chitosan were weighed for a 95:5 combination. Defined amounts of CLNs with 3% consistency were used for one-face coating application. The coating was applied using a bar coater (RDS26, Dong Ah Trade Corp, Seoul, Republic of Korea) at a speed of 50 mm/s. For comparison, the same coating procedure was also conducted using lignin nanoparticles alone, without chitosan. The coating weight depended on the number of layers, ranging from 1.67 g/m² for a single layer to 6.67 g/m² for four layers. The coated samples were dried in an oven at 105 °C for 10–15 min after the deposition of each layer, and this process was continued until the final layer was applied. After the coating process, the sample was kept in the oven dryer at 105 °C for approximately 3 h before being used for the Cobb₆₀ or WCA test.

3.5. Lignin Morphology

The particle size was analyzed via DLS (Photol, Otsuka Electronics Co., Ltd., Osaka, Japan). Measurements were performed at 25 °C using DI water as a diluent. The refractive index and dynamic viscosity of the medium were measured as 1.3328 and 0.9 mPa·s, respectively. More than four replicates of the lignin nanoparticles were used. Thus, the lignin nanoparticle size distribution was determined.

SEM (S-900, Hitachi Co., Tokyo, Japan) was used to evaluate the morphology of the lignin nanoparticles. A diluted solution of lignin nanoparticles was prepared, and a drop of the diluted solution was placed on Ag tape attached to the SEM holder. After drying, the samples were analyzed using SEM.

3.6. FT-IR Analysis of CLNs

The FT-IR spectra of chitosan, lignin, and CLNs were measured using a FT-IR spectrometer (Nicolet IS10, Thermo Scientific, Waltham, MA, USA) equipped with an attenuated total reflectance accessory. Each sample was analyzed in the wavenumber range of 650–4000 cm^{−1} to identify the presence of characteristic functional groups and observe possible changes in chemical structure resulting from nanoparticle formation. To ensure reliable measurements, each spectrum was collected by averaging multiple scans per sample.

3.7. Hydrophobicity of Lignin Nanoparticles and Lignin/Chitosan Composite

The hydrophobicity of the lignin and lignin nanoparticle-coated paper was evaluated by measuring their WCAs. The WCAs were measured using an optical contact-angle analyzer (SDL200TEZD, FEMTOFAB Co., Ltd., Pohang, Republic of Korea). For the WCA analysis, a 5 µL drop of deionized water was deposited on the surface of the paper or coated paper, and the contact angle was measured shortly after droplet placement. The measurement angles were obtained by averaging the contact angle values measured at 10 different locations on each sample. This procedure helped minimize variations due to surface irregularities and ensured consistent evaluation across different samples.

4. Conclusions

Lignin was extracted from oak wood using NaOH and converted into lignin nanoparticles using an ethanol/acetone solvent. The lignin nanoparticles had sizes of 200–400 nm, as determined via DLS and SEM. They were combined with chitosan to prepare CLNs, which were applied to paper as a hydrophobic coating. The paper coated with lignin nanoparticles alone did not exhibit hydrophobic properties. In comparison, the CLNs exhibited superior hydrophobic properties, along with substantially improved hydrophobicity, with a WCA of 128° compared to 109° for pure paperboard. One to three layers of coated CLNs would

be a good solution, as the hydrophobicity decreased with additional coating cycles. In this study, the detailed mechanism and composition optimization of the CLNs were not investigated. These are important topics for future work. In future research, we plan to study methods to increase the biomass content of coating materials and improve properties such as hydrophobicity, compatibility with polymers, and gas barrier properties. Based on this material, we will also focus on finding methods to utilize CLNs for packaging material applications.

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References

1. Thakur, V.K.; Thakur, M.K.; Raghavan, P.; Kessler, M.R. Progress in green polymer composites from lignin for multifunctional applications: A review. *ACS Sustain. Chem. Eng.* **2014**, *2*, 1072–1092. [CrossRef]
2. Ragauskas, A.J.; Beckham, G.T.; Biddy, M.J.; Chandra, R.; Chen, F.; Davis, M.F.; Davison, B.H.; Dixon, R.A.; Gilna, P.; Keller, M.; et al. Lignin valorization: Improving lignin processing in the biorefinery. *Science* **2014**, *344*, 1246843. [CrossRef]
3. Machado, T.O.; Beckers, S.J.; Fischer, J.; Müller, B.; Sayer, C.; de Araújo, P.H.H.; Landfester, K.; Wurm, F.R. Bio-based lignin nanocarriers loaded with fungicides as a versatile platform for drug delivery in plants. *Biomacromolecules* **2020**, *21*, 2755–2763. [CrossRef] [PubMed]
4. Wang, G.; Pang, T.; Chen, S.; Sui, W.; Si, C.; Ni, Y. Fabrication of lignin nanospheres by emulsification in a binary γ -valerolactone/glycerol system and their application as a bifunctional reducer and carrier for Pd nanoparticles with enhanced catalytic activity. *Green Chem.* **2020**, *22*, 8594–8603. [CrossRef]
5. Zhao, W.; Simmons, B.; Singh, S.; Ragauskas, A.; Cheng, G. From lignin association to nano-/micro-particle preparation: Extracting higher value of lignin. *Green Chem.* **2016**, *18*, 5693–5700. [CrossRef]
6. Pouteau, C.; Dole, P.; Cathala, B.; Averous, L.; Boquillon, N. Antioxidant properties of lignin in polypropylene. *Polym. Degrad. Stab.* **2003**, *81*, 9–18. [CrossRef]
7. Schutyser, W.; Renders, T.; Van den Bosch, S.; Koelewijn, S.-F.; Beckham, G.T.; Sels, B.F. Chemicals from lignin: An interplay of lignocellulose fractionation, depolymerisation, and upgrading. *Chem. Soc. Rev.* **2018**, *47*, 852–908. [CrossRef]
8. Taherzadeh, M.J.; Karimi, K. Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: A review. *Int. J. Mol. Sci.* **2008**, *9*, 1621–1651. [CrossRef]
9. Kim, J.S.; Lee, Y.; Kim, T.H. A review on alkaline pretreatment technology for bioconversion of lignocellulosic biomass. *Bioresour. Technol.* **2016**, *199*, 42–48. [CrossRef]
10. Garrote, G.; Dominguez, H.; Parajó, J. Hydrothermal processing of lignocellulosic materials. *Holz Als Roh-Und Werkst.* **1999**, *57*, 191–202. [CrossRef]
11. Park, Y.C.; Kim, T.H.; Kim, J.S. Effect of organosolv pretreatment on mechanically pretreated biomass by use of concentrated ethanol as the solvent. *Biotechnol. Bioprocess Eng.* **2017**, *22*, 431–439. [CrossRef]

12. Mosier, N.; Wyman, C.; Dale, B.; Elander, R.; Lee, Y.Y.; Holtzaple, M.; Ladisch, M. Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresour. Technol.* **2005**, *96*, 673–686. [CrossRef] [PubMed]
13. Upton, B.M.; Kasko, A.M. Strategies for the conversion of lignin to high-value polymeric materials: Review and perspective. *Chem. Rev.* **2016**, *116*, 2275–2306. [CrossRef] [PubMed]
14. Laurichesse, S.; Avérous, L. Chemical modification of lignins: Towards biobased polymers. *Prog. Polym. Sci.* **2014**, *39*, 1266–1290. [CrossRef]
15. Dong, X.; Dong, M.; Lu, Y.; Turley, A.; Jin, T.; Wu, C. Antimicrobial and antioxidant activities of lignin from residue of corn stover to ethanol production. *Ind. Crops Prod.* **2011**, *34*, 1629–1634. [CrossRef]
16. Sohni, S.; Hashim, R.; Nidaullah, H.; Lamaming, J.; Sulaiman, O. Chitosan/nano-lignin based composite as a new sorbent for enhanced removal of dye pollution from aqueous solutions. *Int. J. Biol. Macromol.* **2019**, *132*, 1304–1317. [CrossRef]
17. Yu, J.; Li, L.; Qian, Y.; Lou, H.; Yang, D.; Qiu, X. Facile and green preparation of high UV-blocking lignin/titanium dioxide nanocomposites for developing natural sunscreens. *Ind. Eng. Chem. Res.* **2018**, *57*, 15740–15748. [CrossRef]
18. Kai, D.; Tan, M.J.; Chee, P.L.; Chua, Y.K.; Yap, Y.L.; Loh, X.J. Towards lignin-based functional materials in a sustainable world. *Green Chem.* **2016**, *18*, 1175–1200. [CrossRef]
19. Zhang, Z.; Terrasson, V.; Guénin, E. Lignin nanoparticles and their nanocomposites. *Nanomaterials* **2021**, *11*, 1336. [CrossRef]
20. Qian, Y.; Qiu, X.; Zhu, S. Lignin: A nature-inspired sun blocker for broad-spectrum sunscreens. *Green Chem.* **2015**, *17*, 320–324. [CrossRef]
21. Kumar, M.N.R. A review of chitin and chitosan applications. *React. Funct. Polym.* **2000**, *46*, 1–27. [CrossRef]
22. Abo-Elseoud, W.S.; Hassan, M.L.; Sabaa, M.W.; Basha, M.; Hassan, E.A.; Fadel, S.M. Chitosan nanoparticles/cellulose nanocrystals nanocomposites as a carrier system for the controlled release of repaglinide. *Int. J. Biol. Macromol.* **2018**, *111*, 604–613. [CrossRef] [PubMed]
23. Akhavan-Kharazian, N.; Izadi-Vasafi, H. Preparation and characterization of chitosan/gelatin/nanocrystalline cellulose/calcium peroxide films for potential wound dressing applications. *Int. J. Biol. Macromol.* **2019**, *133*, 881–891. [CrossRef]
24. Poonguzhali, R.; Basha, S.K.; Kumari, V.S. Synthesis and characterization of chitosan-PVP-nanocellulose composites for in-vitro wound dressing application. *Int. J. Biol. Macromol.* **2017**, *105*, 111–120. [CrossRef] [PubMed]
25. Eulálio, H.Y.C.; Vieira, M.; Fideles, T.B.; Tomás, H.; Silva, S.M.L.; Peniche, C.A.; Fook, M.V.L. Physicochemical properties and cell viability of shrimp chitosan films as affected by film casting solvents. I-potential use as wound dressing. *Materials* **2020**, *13*, 5005. [CrossRef] [PubMed]
26. Hai, L.; Choi, E.S.; Zhai, L.; Panicker, P.S.; Kim, J. Green nanocomposite made with chitin and bamboo nanofibers and its mechanical, thermal and biodegradable properties for food packaging. *Int. J. Biol. Macromol.* **2020**, *144*, 491–499. [CrossRef]
27. Hai, L.V.; Zhai, L.; Kim, H.C.; Panicker, P.S.; Pham, D.H.; Kim, J. Chitosan nanofiber and cellulose nanofiber blended composite applicable for active food packaging. *Nanomaterials* **2020**, *10*, 1752. [CrossRef]
28. Maturavongsadit, P.; Paravyan, G.; Shrivastava, R.; Benhabbour, S.R. Thermo-/pH-responsive chitosan-cellulose nanocrystals based hydrogel with tunable mechanical properties for tissue regeneration applications. *Materialia* **2020**, *12*, 100681. [CrossRef]
29. Gao, Q.; Lei, M.; Zhou, K.; Liu, X.; Wang, S.; Li, H. Preparation of a microfibrillated cellulose/chitosan/polypyrrole film for Active Food Packaging. *Prog. Org. Coat.* **2020**, *149*, 105907. [CrossRef]
30. Salari, M.; Khiabani, M.S.; Mokarram, R.R.; Ghanbarzadeh, B.; Kafil, H.S. Development and evaluation of chitosan based active nanocomposite films containing bacterial cellulose nanocrystals and silver nanoparticles. *Food Hydrocoll.* **2018**, *84*, 414–423. [CrossRef]
31. Cai, Z.; Chen, P.; Jin, H.-J.; Kim, J. The effect of chitosan content on the crystallinity, thermal stability, and mechanical properties of bacterial cellulose—Chitosan composites. *Proc. Inst. Mech. Eng. Part C J. Mech. Eng. Sci.* **2009**, *223*, 2225–2230. [CrossRef]
32. Tagliaro, I.; Seccia, S.; Pellegrini, B.; Bertini, S.; Antonini, C. Chitosan-based coatings with tunable transparency and super-hydrophobicity: A solvent-free and fluorine-free approach by stearyl derivatization. *Carbohydr. Polym.* **2023**, *302*, 120424. [CrossRef]
33. NREL/TP-510-42618; Determination of Structural Carbohydrates and Lignin in Biomass. National Renewable Energy Laboratory: Golden, CO, USA, 2012.
34. NREL/TP-510-42622; Determination of Ash in Biomass. National Renewable Energy Laboratory: Golden, CO, USA, 2005.

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Article

Insight into the Mechanism for the Emergence of Thermally Stable Reflection Colors from Cholesteric Liquid Crystals of Etherified Ethyl Cellulose Derivatives and Methacrylic Acid

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Abstract: Ethyl cellulose (EC) and its derivatives are known to exhibit the cholesteric liquid crystal (CLC) phase with visible light reflection in a lyotropic manner after adding appropriate solvents. Generally, the reflection peak of conventional CLCs is easily wavelength shifted by temperature. However, our previous study showed that the reflection wavelength can be maintained even after heating for the lyotropic CLCs of completely pentyl-etherified EC derivatives with methacrylic acid (MAA). However, the emergence of thermally stable reflection colors still remains obscure in the mechanism at the mesoscopic scale. In this study, we evaluated the temperature dependence of the reflection wavelength for the lyotropic CLCs of a series of completely etherified EC derivatives possessing different alkoxy chains by addition of MAA. It was found that butyl- or pentyl-etherified EC derivatives are suitable for preparation of the lyotropic CLCs with visible Bragg reflection, whereas visible light reflection cannot be observed for the other mixtures of propyl- and hexyl-etherified EC derivatives with MAA. Furthermore, it turned out that lyotropic CLCs of pentyl-etherified EC derivatives with MAA show the smallest temperature dependence of their reflection wavelength. Based on the results of ultra-small-angle X-ray scattering (USAXS) and small-angle X-ray scattering (SAXS) measurements of CLC films, we presumed that the emergence of thermally stable reflection colors from the lyotropic CLCs of pentyl-etherified EC derivatives with MAA arises from their phase separation at the mesoscopic scale by changing the temperature.

Keywords: ethyl cellulose; cholesteric liquid crystal; Bragg reflection; small-angle X-ray scattering; phase separation

1. Introduction

The cholesteric liquid crystal (CLC) phase is generally composed of nematic liquid crystal layers of calamitic liquid crystal molecules with intrinsic molecular chirality, that is, optical activity; however, their orientational directors continuously rotate in a periodic manner at the macroscopic scale so as to self-organize the helical molecular assemblages with left- or right-handed sense by the helical twisting power of chiral molecules [1]. These molecular assemblages give rise to the periodic modulation in refractive index in the CLC medium, thereby leading to the emergence of light reflection at a specific wavelength through the light interference. This light reflection phenomenon by CLCs can be regarded as a kind of Bragg reflection. The reflection peak wavelength (λ) is numerically expressed as the following equation:

$$\lambda = n \cdot p \quad (1)$$

where n and p mean the average refractive index and the helical pitch length of CLC, respectively [2]. When the helical pitch length of the CLC medium is equally matched to several hundred nanometers, the light reflection phenomenon can be visualized as the reflection colors of blue, green and red. Due to the liquid crystallinity, the helical pitch length of CLC exhibits sensitivity to variations in temperature [3], electric field [4], magnetic field [5], mechanical stress [6], and photo-irradiation [7]. Such tunability of the CLC helical pitch length by the external stimuli allows for the on-demand control of reflection peak wavelength, enabling the technological applications to reflective color displays [8], full-color recording media [9], and so forth. Furthermore, the research on mirrorless lasing from CLCs has also attracted considerable interest from the perspective of photonic crystals [10–13].

However, as mentioned above, the CLC helical pitch length is very vulnerable to subtle temperature fluctuation due to the fluidity of liquid crystallinity. Eventually, the reflection peak wavelength is readily shifted in wide wavelength ranges by changing slight temperature [14–16]. In order to keep the reflection peak wavelength, specific temperature-controller is needed in the experiment. Moreover, conventional CLC materials are predominantly synthesized from finite petroleum resources, raising the natural resource issues for the future generation.

To address these emergency issues, we paid attention to cellulose derivatives. Cellulose and its derivatives have gained considerable interest as biomass resources, arising from the safety, biocompatibility, and abundance. This is because cellulose is a major component of plants. In addition, another unique property of cellulose and its derivatives is the appearance of CLC phase by dissolving or suspending them in appropriate solvents [17,18]. The mesophase is dependent on the concentration of cellulose derivatives, which is called the lyotropic liquid crystal phase. Taking account of the outstanding features, numerous studies have been conducted aiming for their application to functional materials of cellulose derivatives that can contribute to the realization of a sustainable society.

Ethyl cellulose (EC), as depicted in Figure 1A, is one of representative cellulose derivatives. In general, EC is prepared by reacting natural cellulose with ethylene oxide. Due to its safety, EC is nowadays available in not only the medical realm such as pharmaceutical agents [19], but also industrial realm such as components in optoelectronic devices [20]. More interestingly, EC has been reported as biodegradable materials for the contribution toward the circular economy [21].

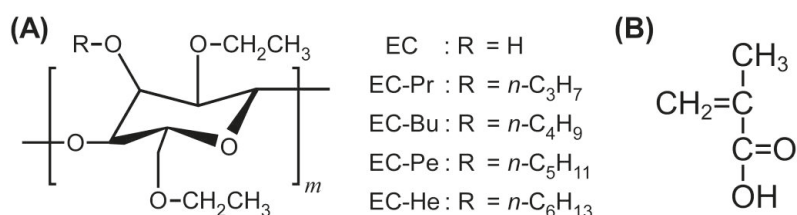


Figure 1. (A) Chemical structures of ethyl cellulose (EC) and its derivatives. (B) Chemical structure of methacrylic acid (MAA).

EC is known to exhibit the lyotropic CLC phase with visible light reflection [22–25]. For instance, the solutions of pristine EC in organic solvents such as chloroform [23,24] or acrylic acid [26] exhibit the lyotropic CLC phase. As another precedent, esterified EC derivatives exhibit the lyotropic CLC phase when dissolved in solvents at appropriate concentrations [23–25]. The mechanism of the Bragg reflection color change in CLCs from EC or its derivatives depending on their concentration can be explained by the difference in the helical pitch length of lyotropic CLC, corresponding to p in Equation (1). Therefore, the reflection peak wavelength of lyotropic CLCs is significantly dependent on the concentra-

tions of EC or its derivatives [26]. In our previous report, we succeeded that the reflection peak wavelength of lyotropic CLCs of completely pentyl-etherified EC derivatives with methacrylic acid (MAA; Figure 1B) can be maintained irrespective of temperature in the range between 30 °C and 110 °C [27]. This phenomenon is quite interesting. This is because CLCs generally exhibit the temperature-induced shift of reflection peak in the wide wavelength range as the thermotropic behavior. Therefore, the mechanism for the emergence of thermally stable Bragg reflection colors from the lyotropic CLCs of EC derivatives and MAA still remains unclear at the mesoscopic scale. In addition, the temperature dependences of lyotropic CLCs from EC derivatives substituted with other alkoxy groups have not been reported yet even though it may play a crucial role to the reflection peak wavelength of CLCs similar to the case of etherified derivatives of hydroxypropyl cellulose [28].

In this study, we evaluated the temperature dependence of reflection peak wavelength of lyotropic CLCs from the etherified EC derivatives and MAA. As presented in Figure 1A, a series of etherified EC derivatives possessing propyloxy (EC-Pr), butyloxy (EC-Bu), pentyloxy (EC-Pe), or hexyloxy groups (EC-He) were prepared in order to unravel the effect of their alkoxy side chain length of etherified EC derivatives on the light reflection properties. Although visible light reflection could not be observed for the mixtures of EC-Pr and EC-He with MAA, it turned out that EC-Bu and EC-Pe are suitable for the preparation of lyotropic CLCs with visible Bragg reflection. Furthermore, lyotropic CLCs of EC-Bu with MAA exhibited the relatively independence of reflection peak wavelength upon the changing the temperature, as compared to those from EC-Pe and MAA, suggesting that EC-Pe is a prime candidate to prepare the lyotropic CLC with a thermally stable reflection color. Ultra-small-angle X-ray scattering (USAXS) and small-angle X-ray scattering (SAXS) measurements of the cured CLC films revealed that the appearance of spherical scatterers by elevating the temperature, arising from the thermally induced phase separation between EC derivatives and MAA at the mesoscopic scale. Based on these experimental results, we presumed that the emergence of thermally stable reflection colors from lyotropic CLCs of EC-Pe with MAA can be ascribed to their constant CLC helical pitch length induced by the phase separation at the mesoscopic scale by heating treatment.

2. Results and Discussion

2.1. Characterization of EC Derivatives

Four types of completely etherified EC derivatives such as EC-Pr, EC-Bu, EC-Pe, and EC-He were prepared by the Williamson ether synthesis according to our previously reported procedure in order to elucidate the side chain effect of etherified EC derivatives on the light reflection properties of lyotropic CLCs with MAA (Figure 1A) [27]. All of the EC derivatives were obtained as white powder, and did not exhibit thermotropic liquid crystallinity on their own unlike etherified hydroxypropyl cellulose derivatives [28].

The etherified EC derivatives were characterized by measuring both Fourier transform infrared (FT-IR) and nuclear magnetic resonance (NMR) spectra to confirm that hydroxy groups of pristine EC are completely substituted with alkoxy groups. Figure S1A in the Supplementary Materials shows the comparison of FT-IR spectra between pristine EC and etherified EC derivatives. As compared these spectra, the pristine EC showed an intense peak from the O-H stretching vibration of the hydroxy groups in a broad wavenumber range from 3000 cm^{-1} to 3600 cm^{-1} . In contrast, the intense peaks around 3000–3600 cm^{-1} were not observed for the completely etherified EC derivatives so as to be broad bands with weak intensities. The experimental results imply that the hydroxy groups of pristine EC are completely etherified regardless of the difference in the chain length of alkoxy groups. Figure S1B in the Supplementary Materials represents the ^1H -NMR spectra of etherified EC derivatives in CDCl_3 . From the ^1H -NMR spectra, the DS values, that is, the degree of

substitution with alkoxy groups, are quantitatively estimated according to the following equation [27]:

$$DS = \frac{A(7 + 5MS)}{3W - (2x + 1)A} \quad (2)$$

where A is the integrated value of the signal peak “a” assigned to the protons of the terminal methyl group, W is the sum of the integrated values of all protons in EC derivatives, and x is the number of carbon atoms in alkoxy groups. For instance, x corresponds to 3 in the case of EC-Pr. As will be mentioned in Section 3.1, we adopted the MS value of 2.50 from the ^1H -NMR spectrum of pristine EC. By applying the experimental results to Equation (2), the DS values of all etherified EC derivatives were estimated to be 0.50, which also assured that the hydroxy groups of pristine EC are completely etherified for EC-Pr, EC-Bu, EC-Pe, and EC-He.

2.2. Reflection Properties of Lyotropic CLCs of EC Derivatives with MAA

When the lyotropic CLCs of EC-Pr, EC-Bu, EC-Pe, and EC-He with MAA were prepared, the light reflection properties were found to be different behavior as the alkoxy side chains. Although we found that EC-Bu and EC-Pe are suitable for the preparation of lyotropic CLCs with visible Bragg reflection, the reflection color was not observed for the lyotropic CLCs of EC-Pr with MAA at the concentration of 35–60 wt%. As measuring the transmission spectra of the lyotropic CLCs, no peak was detected in the visible wavelength region of 400–800 nm, which was the detectable wavelength range of the spectroscopy used in this study. As observed using polarized optical microscope (POM) under cross-Nicols, the optical anisotropy appeared in the lyotropic CLCs of EC-Pr with MAA. It is plausible that the lyotropic CLC of EC-Pr with MAA might reflect ultraviolet light or infrared light. Similarly, the lyotropic CLCs of EC-He with MAA at the concentration of 60–75 wt% showed no reflection color even though they showed optical anisotropy by the POM observation. From the previous report on the thermotropic CLCs of etherified derivatives of hydroxypropyl cellulose, the reflection peak wavelength red-shifts with the increase in the chain length of alkoxy groups substituted to the hydroxy groups of hydroxypropyl cellulose [28]. Therefore, it can be assumed that the lyotropic CLCs of EC-Pr and EC-He dissolved in MAA reflect ultraviolet and infrared light, respectively.

To investigate the temperature dependence of light reflection properties, six types of lyotropic CLCs were prepared from EC-Bu or EC-Pe. The preparation conditions and sample definition of lyotropic CLCs from EC-Bu or EC-Pe are summarized in Table 1. Figure 2 shows the transmission spectra of lyotropic CLCs of EC-Bu or EC-Pe, which were measured at 30 °C. When EC-Bu was completely dissolved in MAA at the concentration range of 59.2–61.5 wt%, the mixtures of EC-Bu and MAA showed reflection colors as blue, green, and red by the formation of the lyotropic CLC phase (Figure 2A). The reflection wavelength continuously shifted to the longer wavelength side as diluting the concentration of EC-Bu in MAA, arising from the increase in the helical pitch length of CLC. Similar light reflection properties were observed for the lyotropic CLCs of EC-Pe with MAA at 67.4–69.4 wt% (Figure 2B). The reflection color also changed to blue, green, and red in the descending order of the concentration of EC-Pe by the red-shift of reflection peak wavelength. As described in the Introduction part, the reflection wavelength of lyotropic CLCs of EC-Pe with MAA are unlikely to be changed upon the heating process. Since the detectable range of our transmission spectral measurement system is 400–800 nm, it is essential to prepare the lyotropic CLCs with visible Bragg reflection for the fair comparison of their reflection wavelength upon the heating process. From this reason, we focused on the lyotropic CLCs of EC-Bu or EC-Pe with MAA to explore the temperature-dependence of their reflection peak wavelength.

Table 1. Sample definition and preparation condition of lyotropic CLCs and their light reflection properties.

Sample	EC Derivatives	Polymer Conc. (wt%)	Reflection Wavelength at 30 °C (nm)	$\lambda_{\text{shift}}/10\text{ }^{\circ}\text{C}$ (nm) ^a
1	EC-Bu	61.5	413	−3.0
2	EC-Bu	60.5	541	11
3	EC-Bu	59.2	640	15
4	EC-Pe	69.4	436	−1.4
5	EC-Pe	68.3	529	1.1
6	EC-Pe	67.4	632	5.9

^a Average of reflection peak wavelength shift upon elevating the temperature range of 10 °C.

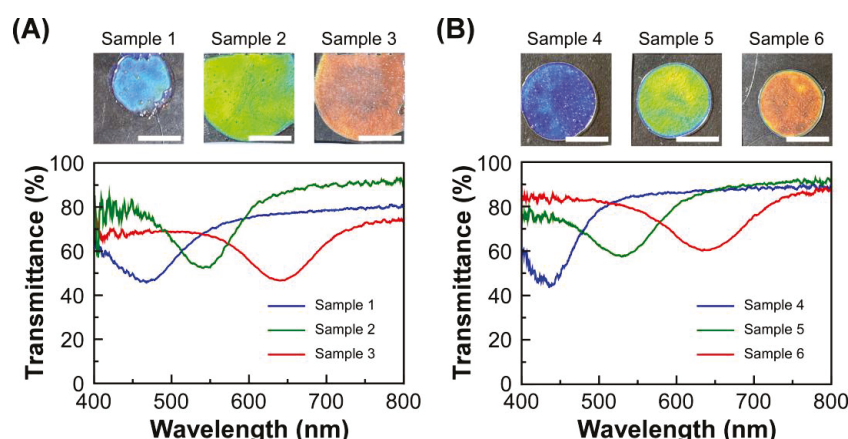


Figure 2. Changes in transmission spectra of cells of lyotropic CLCs of EC-Bu or EC-Pe with MAA as a function of the concentration of EC derivatives. The measurement temperature was set at 30 °C. Insets show the reflection images of CLC cells, and the white scale bars denote 5.0 mm. (A) EC-Bu in MAA, Samples 1–3; (B) EC-Pe in MAA, Samples 4–6.

2.3. Temperature Dependence of the Reflection Properties of the Lyotropic CLCs from EC Derivatives and MAA

The transmission spectrum measurements were conducted for the lyotropic CLCs of EC-Bu or EC-Pe with MAA upon heating the process from 30 °C to 110 °C. The isotropic phase transition temperature was found to be ~150 °C for all samples. Furthermore, the result suggested that the lyotropic CLCs undergo no structural deterioration upon heating, as evidenced by the negligible change in their transmission spectra after the measurements. As an example, Figure 3 shows the changes in transmission spectrum of Sample 4, which corresponds to the lyotropic CLC of EC-Pe in MAA at the EC-Pe concentration of 69.4 wt%, as a function of the temperature upon the heating process. The reflection peak wavelength red shifted upon heating from 30 °C up to 90 °C. However, interestingly, the reflection peak wavelength blue shifted as elevating the temperature over 90 °C. At this time, the baseline of the transmission spectrum became lower than those measured below 90 °C. For instance, the light transmittance at longer wavelength of 800 nm drastically decreased from ~90% to ~65% by rising the temperature from 90 °C to 110 °C. Although it is obvious that the helical molecular assemblage of lyotropic CLC is maintained because the reflection peak can be clearly observed even in this temperature range, the decrease in light transmittance upon elevating the temperature strongly suggested the structural change in the CLC helical molecular assemblage at the mesoscopic scale. Such tendencies were also observed for transmission spectra of the other lyotropic CLCs upon the heating process (Supplementary Materials, Figure S2). The plausible mechanism for the changes in the CLC helical molecular assemblage will be discussed in the proceeding section.

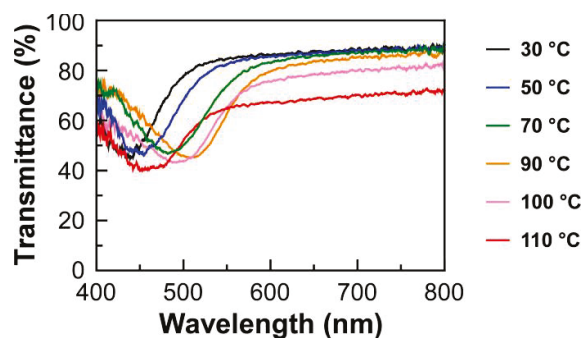


Figure 3. Changes in transmission spectrum of Sample 4 upon the heating process from 30 °C to 110 °C. The transmission spectral measurements were conducted at the temperature intervals of 10 °C. In this figure, only the transmission spectra at 30, 50, 70, 90, 100, and 110 °C are shown for reader's clarity.

To quantitatively analyze the changes in transmission spectra of lyotropic CLCs by changing the temperature, the reflection wavelength and light transmittance at 800 nm of each CLC were plotted against the temperature (Figure 4). The left panels of Figure 4 show the plots of the reflection wavelength of lyotropic CLCs of EC-Bu or EC-Pe exhibiting a blue reflection color (Figure 4A), green (Figure 4B), and red (Figure 4C), upon heating from 30 °C in a stepwise manner. As shown in Figure 4A, although the reflection wavelength of Sample 1 and Sample 4 continuously shifted to the longer wavelength side, it began to shift to the shorter wavelength side with increasing the temperature from 90 °C to 110 °C. However, it should be noted that the reflection color can be recognized as blue by naked eyes because of the relatively small wavelength shift of reflection peak. Therefore, it was indicated that EC derivatives that can make lyotropic CLCs with thermally stable reflection colors is not only limited to EC-Pe but also to EC-Bu. In fact, the reflection wavelength of Sample 2 and Sample 5 was maintained especially in the temperature range of 30–90 °C. Although the reflection wavelength slightly blue shifted when heated above 90 °C, the reflection color change is hardly recognized by naked eyes (Figure 4B). In the same manner, Sample 3 and Sample 6 showed exactly same tendency except that the blue-shift of reflection wavelength began at 70 °C, which was much lower than Sample 2 and Sample 5 (Figure 4C). Among the lyotropic CLCs exhibiting blue and green colors, Sample 3 and Sample 6 displayed a relatively large shift of reflection peak wavelength. This can be ascribed to the increase in the mobility of CLC arising from the decrease in the concentration of etherified EC derivatives when preparing lyotropic CLCs. To quantitatively discuss the shift of reflection wavelength of the lyotropic CLCs, the following equation was adopted to calculate the average wavelength shift of the reflection peak at the temperature range of 10 °C ($\lambda_{\text{shift}}/10\text{ °C}$).

$$\lambda_{\text{shift}}/10\text{ °C} = \{(\lambda_{110\text{ °C}} - \lambda_{30\text{ °C}})/(110\text{ °C} - 30\text{ °C})\} \times 10\text{ °C} \quad (3)$$

where $\lambda_{30\text{ °C}}$ and $\lambda_{110\text{ °C}}$ are the reflection wavelength of lyotropic CLC at 30 °C and 110 °C, respectively [27]. The $\lambda_{\text{shift}}/10\text{ °C}$ values are summarized in the fifth column of Table 1. Because the $\lambda_{\text{shift}}/10\text{ °C}$ values for lyotropic CLCs of EC-Bu with MAA (Samples 1–3) were larger than those of EC-Pe with MAA (Samples 4–6), it was indicated that EC-Pe is much more adequate to prepare lyotropic CLC with a thermally stable reflection color.

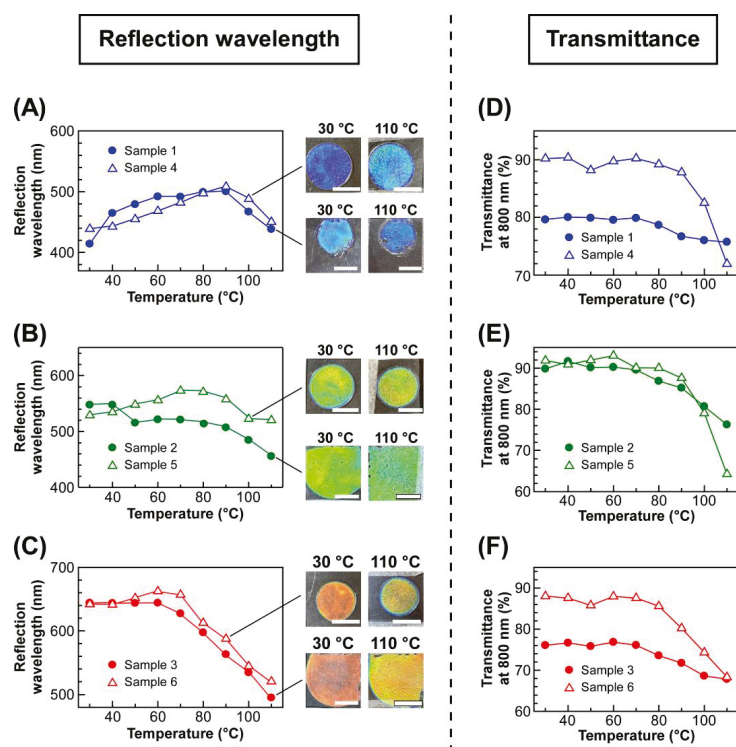


Figure 4. (Left panels) Changes in reflection peak wavelengths of lyotropic CLCs of EC-Bu or EC-Pe with MAA upon heating. (A) Lyotropic CLCs exhibiting a red reflection color, Samples 1 and 4. (B) Lyotropic CLCs exhibiting a green reflection color, Samples 2 and 5. (C) Lyotropic CLCs exhibiting a red reflection color, Samples 3 and 6. Insets represent the reflection images of CLCs, and the white scale bars are 5 mm. (Right panels) The change in light transmittances at 800 nm of the lyotropic CLC of EC-Bu or EC-Pe with MAA. (D) Lyotropic CLCs exhibiting a red reflection color, Samples 1 and 4. (E) Lyotropic CLCs exhibiting a green reflection color, Samples 2 and 5. (F) Lyotropic CLCs exhibiting a red reflection color, Samples 3 and 6.

As mentioned in the Section 2.2, the light transmittance of lyotropic CLCs decreased at elevated temperature. The right panels of Figure 4 show the changes in light transmittances at 800 nm for the lyotropic CLCs of EC-Bu or EC-Pe with MAA, exhibiting a blue reflection color (Figure 4D), green (Figure 4E), and red (Figure 4F), upon heating from 30 °C. The light transmittance of Sample 4 drastically decreased from ~90% to ~70% when the temperature exceeded 90 °C (Figure 4D, closed circles). Notably, this temperature agreed well with the temperature at which the reflection wavelength became maximum (Figure 4A, closed circles). Although the decrease in light transmittance was not clearly observed for Sample 1, the light transmittance at 90 °C was lower than that at 70 °C. These results indicated the temperature range can be divided into two regions; one is 30–90 °C, where the red-shift of reflection wavelength is observed, and the transmittance is maintained. The other is 90–110 °C, where the reflection wavelength blue-shifts and a decrease in light transmittance is observed. Similarly, the light transmittance of Samples 2 and 5 decreased above 90 °C, where the reflection wavelength began to blue-shift (Figure 4E). Furthermore, Sample 3 and Sample 6 also exhibited a similar tendency except that the inflection points of the plots shifted to 70 °C (Figure 4F). These results strongly implied that internal structure of the lyotropic CLCs of etherified EC derivatives with MAA drastically changes throughout the temperature range of 30–110 °C. Therefore, the thermally stable reflection color of the lyotropic CLCs could not be ascribed to the stability of the internal structure upon heating.

2.4. USAXS and SAXS Measurements of UV-Cured Film from Lyotropic CLCs

To investigate the mechanism for the emergence of thermally stable Bragg reflection colors of CLCs from etherified EC derivatives and MAA, we conducted USAXS and SAXS measurements using the synchrotron radiation. This is because SAXS measurements have been widely used for the mesoscopic structure analysis of polymer-dispersed liquid crystals [29], and phase separation behavior of polymer solutions [30,31]. Based on these precedents, we presumed that the difference in scattering length density between EC derivatives and MAA is large enough to obtain clear SAXS patterns.

However, when conducting SAXS measurements, another concern about the evaporation of MAA from the lyotropic CLC was raised. In general, the reflection peak wavelength of lyotropic CLCs are drastically affected by the evaporation of solvent, resulting in difference in the internal structure of CLCs. To solve this problem, we conducted USAXS and SAXS measurements at room temperature for CLC films prepared by irradiation with ultraviolet (UV) light at 365 nm to the lyotropic CLCs of EC derivatives with MAA at 30 °C, 70 °C, or 110 °C. These solid-state CLC films might maintain their internal helical molecular assemblages of lyotropic CLCs at each curing temperature because of the progress of photopolymerization of MAA and subsequent cross-linking via hydrogen bonds. This is also evident from the fact that the reflection peak wavelengths of lyotropic CLCs and the films prepared by UV-irradiation are almost identical.

Figure 5 shows the intensity profiles of UV-cured CLC films exhibiting a blue reflection color, which were prepared by using EC-Bu (Figure 5A) and EC-Pe (Figure 5B). These profiles were obtained by calculating the absolute intensity by using the standard of glassy carbon and plotting against the scattering vector (q). A shoulder-like peak was observed at $q = \sim 0.03 \text{ nm}^{-1}$ in the profiles of the CLC film from Sample 1 cured at 30 °C (Figure 5A, black circles). A similar shoulder-like peak was also observed at almost the same q value for the intensity profiles of the film from Sample 1 cured at 70 °C (Figure 5A, blue circles). Interestingly, this peak became apparent and shifted to $q = \sim 0.10 \text{ nm}^{-1}$ in the profile for the CLC film cured at 110 °C (Figure 5A, red circles). Furthermore, the intensity became proportional to q^{-4} in the q range of $0.10\text{--}0.50 \text{ nm}^{-1}$ and below 0.02 nm^{-1} , suggesting the appearance of spherical scatterers. The size of spherical scatterers (d) can be approximated by the following equation:

$$d = \frac{2\pi}{q_s} \quad (4)$$

where q_s is the scattering vector at the shoulder-like peak. Therefore, it can be assumed that spherical scatterers with diameters of $\sim 120 \text{ nm}$ are formed in Sample 1 as cured at 110 °C. It should be noted that these scatterers are aggregated because the Guinier region was not clearly observed and the intensity continuously increased with a decrease in q , suggesting the existence of extremely large scatterers that cannot be detected by the USAXS measurements, similar to the intensity profiles of composite materials consisting of polymeric matrices with aggregated inorganic fillers [32]. Such changes in USAXS-SAXS intensity profiles were also observed for the CLC films from EC-Pe. As shown in Figure 5B, the intensity profiles of the CLC films of Sample 4 cured at 30 °C and 70 °C. However, a shoulder-like peak appeared at $q = \sim 0.15 \text{ nm}^{-1}$ for the CLC film cured at 110 °C. Therefore, it was found that the scatterers with a size of $\sim 80 \text{ nm}$ exist in the CLC film. Furthermore, the emergence of a shoulder-like peak in USAXS-SAXS intensity profiles upon increasing the temperature were also observed for the CLC films fabricated from Samples 2, 3, 5, and 6 (Supplementary Materials, Figure S3). Although the USAXS-SAXS intensity profiles were differed by curing temperatures and the reflection wavelength of the films due to changes in scattering length density difference or in the volume fraction of the scatterer, each profile had two regions where the intensity became proportional to

q^{-4} . Therefore, it was indicated that the formation of aggregated spherical scatters upon increasing the temperature is irrelevant to the chemical structure of EC derivatives or the concentration of lyotropic CLCs with MAA. By considering the fact that X-ray is scattered by differences in electron density of the samples, we presumed that the internal helical molecular structure of lyotropic CLCs of EC derivatives with MAA is greatly transformed by changing the temperature.

This hypothesis can be also supported by the POM observation of lyotropic CLCs (Supplementary Materials, Figure S4). As observing the POM images of lyotropic CLCs of EC-Bu or EC-Pe with MAA exhibiting blue reflection colors at 30 °C, 70 °C, and 110 °C, all of the POM images were bright under cross-Nicols. Therefore, the lyotropic CLCs showed optical anisotropy arising from the liquid crystallinity [26]. However, the POM images of EC-Bu with MAA taken at 110 °C (Supplementary Materials, Figure S4C) and EC-Pe with MAA at 110 °C (Supplementary Materials, Figure S4F) showed a different morphology with spherical domains, suggesting the drastic changes in CLC helical molecular assemblages [33]. The size of spherical domains was estimated to be $\sim 20\ \mu\text{m}$ for the lyotropic CLC of EC-Bu with MAA, and $\sim 30\ \mu\text{m}$ for that of EC-Pe with MAA. Therefore, the lack of Guinier region in the USAXS-SAXS intensity profiles is reasonable because the size of scatterers, that is, domains observed by the POM, is extremely large to be measured by the USAXS.

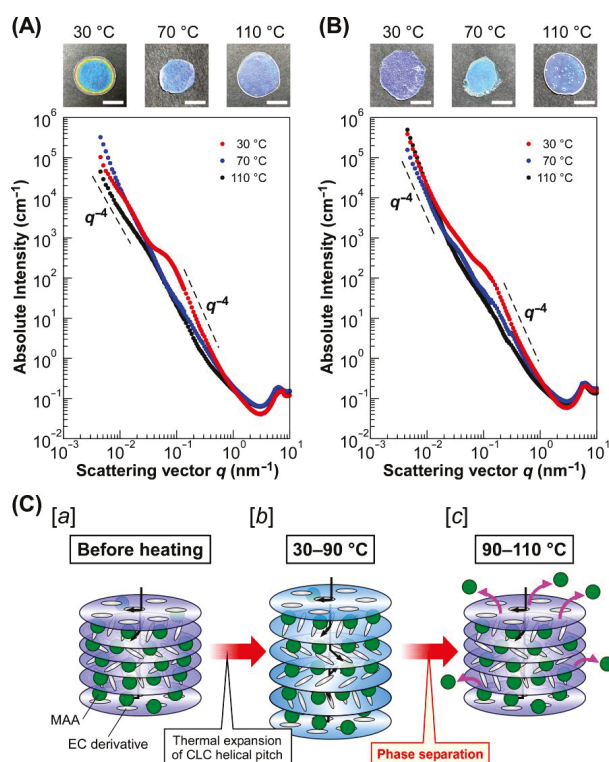


Figure 5. USAXS-SAXS intensity profiles of UV-cured films exhibiting blue reflection colors and plausible mechanism for the emergence of thermally stable Bragg reflection colors from lyotropic CLC of EC derivatives with MAA. (A,B) USAXS-SAXS intensity profiles of the solid-state CLC films from Sample 1 (A) and Sample 4 (B), which were cured with UV light at 30 °C (black circles), 70 °C (blue circles), and 110 °C (red circles). The insets represent the reflection images of UV-cured CLC films, and the white scale bars denote 5 mm. (C) Schematic illustration of structural changes in CLC from EC derivatives and MAA upon the heating process. [a] CLC structure before heating; [b] the CLC helical molecular assemblage at 30–90 °C, where reflection wavelength slightly red-shifts by thermal expansion of CLC helical pitch; [c] the CLC helical molecular assemblage at 90–110 °C, where MAA molecules expelled from the CLC helical molecular assemblage so as to induce the thermally induced phase separation at the mesoscopic scale.

2.5. Plausible Mechanism for the Emergence of Thermally Stable Bragg Reflection Colors from Lyotropic CLC of EC Derivatives and MAA

Based on the results mentioned in Sections 2.3 and 2.4, we propose herein a mechanism for the emergence of thermally stable Bragg reflection colors from lyotropic CLCs of completely etherified EC derivatives with MAA. After heating the lyotropic CLCs of completely etherified EC derivatives with MAA, the CLC helical pitch length tends to expand by the thermodynamically induced increase in the mobility of CLC media (Figure 5C, image *b*). This is evident from the experimental fact that the red-shift of the reflection peak wavelength was observed from 30 °C to ~90 °C (Samples 1, 2, 4, and 5) or ~70 °C (Samples 3 and 6). At this stage, the absence of the change in the USAXS-SAXS profiles at 30 °C and 70 °C suggests that no significant structural change happens in the lyotropic CLCs. However, the phase separation was induced upon rising the temperature, resulting in the expulsion of MAA from the CLC helical molecular assemblage (Figure 5C, image *c*). This may be ascribed to the increase in the hydrophobicity of the EC derivatives arising from the complete etherification or increase in entropy because of the enhancement of the mobility of EC derivatives by their liquid crystallinity [34]. Therefore, the reflection color of the lyotropic CLCs was unchanged because of the preservation of CLC helical pitch length by the expulsion of MAA from the CLC helical molecular assemblage.

Furthermore, the decrease in the transmittance upon heating can be attributed to the light scattering from the aggregated scatterers of MAA with a size of 40–100 nm, as confirmed from the USAXS-SAXS intensity profiles.

3. Materials and Methods

3.1. Materials

EC was purchased from the Tokyo Chemical Industry (Tokyo, Japan). The viscosity of 5 wt% solutions of pristine ECs in the mixed solvent of toluene and ethanol (volume ratio 8:2) is 9–11 mPa·s according to the datasheet of the manufacturer. The number average molecular weight (M_n) and weight average molecular weight (M_w) of pristine EC were determined to be 2.58×10^5 and 7.62×10^4 by size-exclusion chromatography measurements in our previous work [27]. The molar amount of chemically combined ethylene oxide per anhydroglucose unit, that is, the molecular substitution (MS), was found to be 2.50 by the ^1H -NMR spectrum measurement of pristine EC in CDCl_3 . Therefore, the average molecular weight per anhydroglucose monomer unit could be calculated to be 232. Before usage for the Williamson ether synthesis mentioned in the proceeding section, pristine EC was thoroughly dried under vacuum at room temperature for over 24 h.

Dehydrated *N,N*-dimethylacetamide (DMAc) as solvent for etherification of EC is obtained from the Fujifilm Wako Pure Chemical Co., Inc. (Tokyo, Japan). 1-Bromopropane (PrBr), 1-bromobutane (BuBr), 1-bromopentane (PeBr), and 1-bromohexane (HeBr) used for the synthesis of etherified EC derivatives, MAA used as a solvent of lyotropic CLCs and 2-hydroxy-2-methylpropiophenone (HMPP) used as a photoinitiator were purchased from the Tokyo Chemical Industry (Tokyo, Japan). Sodium hydroxide (NaOH) and potassium iodide (KI), used as the catalysts of the Williamson ether synthesis, were acquired from the Fujifilm Wako Pure Chemical Co., Inc. (Tokyo, Japan). All reagents are except EC were used as received.

3.2. Synthesis and Characterization of the Completely Etherified EC Derivatives with Alkoxy Side Chains

The completely etherified EC derivatives were prepared by the Williamson ether synthesis as reported in our previous study [27]. Here the synthesis and characterization of

propyl-etherified EC derivatives are described as an example (Supplementary Materials, Table S1, Sample Code: EC-Pr).

In a 100 mL round-bottomed flask, 3.00 g of EC was completely dissolved in 24.0 mL of dehydrated DMAc. After that, 4.01 mL of PrBr was added to the solution (5.00 eq. to hydroxy groups of EC). After stirring for 30 min at 65 °C, 2.40 g of powdered NaOH (0.10 g/mL for reaction solvents) and 0.27 g of KI (5.00 mol% to PrBr) were added. This reaction mixture was continuously stirred at 65 °C for 48 h. Subsequently, the reaction mixture was then purified via two rounds of centrifugation at 1.0×10^4 rpm for 5 min to remove any sediment such as sodium bromide. Then, the supernatant was dialyzed against an equivolume mixture of methanol and water for 3 h, and the dialysis was prolonged for additional 48 h in an equivolume mixture of tetrahydrofuran and methanol by using a Visking dialysis tube with pore size of ~5 nm and a molecular weight cutoff between 1.2×10^4 and 1.4×10^4 . The product was obtained through evaporation at 35 °C in vacuum for ~30 min and was finally dried for a few days to obtain purified EC-Pe.

The characterization of the EC derivatives was conducted by FT-IR spectroscopy equipped with an attenuated total reflection unit (FT-IR4700 and ATR PRO ONE, JASCO, Tokyo, Japan) and ^1H -NMR spectroscopy (JNM-ECZ400S, JEOL, Tokyo, Japan) in line with our previous report [27].

3.3. Fabrication Procedure of Lyotropic CLC Cells

The EC derivatives were completely dissolved in MAA using a planetary centrifugal mixer (AR-100, THINKY, Tokyo, Japan). The lyotropic CLC mixture was sandwiched between two glass substrates. The cell gap was adjusted using polytetrafluoroethylene film spacers with a thickness of 200 μm . The edge of each cell was sealed with epoxy resin to prevent the evaporation of MAA upon the heating process.

3.4. Optical Measurement of Lyotropic CLC Cells

The transmission spectrum of visible light in the lyotropic CLC cells was measured on a compact charge-coupled (CCD) spectrometer (USB2000+, Ocean Optics, Orlando, FL, USA) equipped with an optical fiber. The CLC cell was illuminated with white light from a tungsten halogen light source (HL2000, Ocean Optics, Orlando, FL, USA) from the surface normal. The collinearly transmitted light from the CLC cell was focused through two pieces of achromatic doublet lenses, and it was collected into the entrance of an optical fiber connected with the CCD spectrometer. The temperature of the CLC cell was precisely controlled using a hot-stage system for the optical microscope (HS82 and HS1, Mettler Toledo, Columbus, OH, USA). Polarized optical microscope images were taken with a CCD camera (EO-5012, Edmund, Barrington, NJ, USA) equipped on the microscope (IX71, Olympus, Tokyo, Japan).

3.5. Preparation of UV-Cured Films from Lyotropic CLCs

To the lyotropic CLC mixtures of EC derivatives with MAA, a photoinitiator of HMPP was added at ~1.0 wt%. Subsequently, this CLC mixture was sandwiched between a pair of glass substrates. The cell gap was adjusted using polytetrafluoroethylene film spacers with a thickness of 500 μm . The cured films were prepared by irradiation of UV light at 365 nm from a compact light source equipped with light-emitting diodes (CL-H1-365-9-1, Asahi Spectra, Tokyo, Japan). The light intensity was set to be 80 mW cm^{-2} by using a photodiode sensor (PD300-UV, Ophir, Jerusalem, Israel). The temperature of the CLC cell was precisely controlled to be 30 °C, 70 °C, or 110 °C by using a temperature-controllable hot-stage system for the optical microscope (HS82 and HS1, Mettler Toledo, Columbus, OH, USA).

3.6. USAXS and SAXS Measurements of UV-Cured Films

USAXS and SAXS measurements were carried out at the BL19B2 beamline at the SPring-8 (Hyogo, Japan) using a photon counting detector (PILATUS 2M, Dectris, Baden, Switzerland) at a camera length of ~41 m or ~1 m. The X-ray was irradiated from the surface normal of CLC film at an energy of 18 keV for 60 s. The USAXS and SAXS intensities were corrected by transmission and subtraction of background scattering from air blank. The absolute intensity was calculated based on that of glassy carbon. In addition, the SAXS measurements were also conducted using a laboratory SAXS equipment (SAXSpace, Anton Paar, Graz, Austria) to connect the intensity profiles measured at the SPring-8.

4. Conclusions

In this study, we prepared the completely etherified EC derivatives possessing propyl, butyl, pentyl, or hexyl ether groups through the Williamson ether synthesis in order to investigate the dependence of the alkoxy side chain length on the light reflection properties of lyotropic CLCs with MAA. Butyl- or pentyl-etherified EC derivatives showed the lyotropic CLC phase with visible Bragg reflection when dissolved in MAA. Furthermore, it was found that the lyotropic CLCs of completely pentyl-etherified EC derivatives and MAA exhibited the most stable reflection color upon heating, regardless of the difference in their reflection wavelength. The USAXS and SAXS measurements of the polymerized films of lyotropic CLCs of etherified EC derivatives and MAA revealed that spherical scatterers are formed upon heating, indicating the phase separation between EC derivatives and MAA at the mesoscopic scale. Therefore, it is plausible that MAA molecules are excluded from lyotropic CLC structure so as to maintain the helical pitch length, corresponding to the reflection peak wavelength, by changing the temperature. This report provides a promising guideline to control the temperature dependence of the reflection peak wavelength of lyotropic CLCs, which is important from both scientific and technological aspects in the fabrication of environmentally and human-friendly photonic devices from cellulose derivatives.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/molecules30132839/s1>, Figure S1: Characterization of EC derivatives. Comparison of FT-IR spectra between pristine EC and etherified EC derivatives. ¹H-NMR spectra of EC derivatives in CDCl₃; Figure S2: Changes in transmission spectra of lyotropic CLCs upon the heating process from 30 °C to 110 °C. The measurements were conducted at 10 °C intervals. In the figures, the spectra at 30, 50, 70, 90, 100, and 110 °C are shown for reader's clarity; Figure S3: USAXS-SAXS intensity profiles of UV-cured CLC films exhibiting green and red reflection colors from the lyotropic CLCs of EC derivatives with MAA; Table S1. Synthesis conditions of etherified EC derivatives; Figure S4: Polarized optical microphotographs of lyotropic CLCs of EC-Bu or EC-Pe with MAA exhibiting blue reflection colors under cross-Nicols.

Author Contributions: Conceptualization, S.F.; investigation, W.K.; data curation, W.K. and N.I.; writing—original draft preparation, W.K.; writing—review and editing, N.I. and S.F.; supervision, S.F.; project administration, S.F.; funding acquisition, N.I. and S.F. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

CLC	Cholesteric liquid crystal
EC	Ethyl cellulose
MAA	Methacrylic acid
FT	Fourier transform
IR	Infrared
NMR	Nuclear magnetic resonance
UV	Ultraviolet
USAXS	Ultra-small-angle X-ray scattering
SAXS	Small-angle X-ray scattering
POM	Polarized optical microscope

References

1. Coles, H. Chiral Nematics: Physical Properties and Applications. In *Handbook of Liquid Crystals*; Demus, D., Goodby, J., Gray, G.W., Spiess, H.-W., Vill, V., Eds.; Wiley-VCH: Weinheim, Germany, 1998; Volume 2A, pp. 335–409.
2. De Vries, H. Rotatory Power and Other Optical Properties of Certain Liquid Crystals. *Acta Crystallogr.* **1951**, *4*, 219–226. [CrossRef]
3. Ferguson, J.L. Liquid Crystals in Nondestructive Testing. *Appl. Opt.* **1968**, *7*, 1729–1738. [CrossRef] [PubMed]
4. Kahn, F.J. Electric-Field-Induced Color Changes and Pitch Dilation in Cholesteric Liquid Crystals. *Phys. Rev. Lett.* **1970**, *24*, 209–212. [CrossRef]
5. Dreher, R. Remarks on the Distortion of a Cholesteric Structure by a Magnetic Field. *Solid. State Commun.* **1973**, *13*, 1571–1574. [CrossRef]
6. Lelidis, I.; Barbero, G.; Alexe-Ionescu, A.L. Cholesteric Pitch Transitions Induced by Mechanical Strain. *Phys. Rev. E* **2013**, *87*, 022503. [CrossRef]
7. Sackmann, E. Photochemically Induced Reversible Color Changes in Cholesteric Liquid Crystals. *J. Am. Chem. Soc.* **1971**, *93*, 7088–7090. [CrossRef]
8. Hikmet, R.A.M.; Kemperman, H. Electrically Switchable Mirrors and Optical Components Made from Liquid-Crystal Gels. *Nature* **1998**, *392*, 476–479. [CrossRef]
9. Tamaoki, N.; Parfenov, A.V.; Masaki, A.; Matsuda, H. Rewritable Full-Color Recording on a Thin Solid Film of a Cholesteric Low-Molecular-Weight Compound. *Adv. Mater.* **1997**, *9*, 1102–1104. [CrossRef]
10. Finkelmann, H.; Kim, S.T.; Munoz, A.; Palffy-Muhoray, P.; Taheri, B. Tunable Mirrorless Lasing in Cholesteric Liquid Crystalline Elastomers. *Adv. Mater.* **2001**, *13*, 1069–1072. [CrossRef]
11. Hwang, J.; Song, M.H.; Park, B.; Nishimura, S.; Toyooka, T.; Wu, J.W.; Takanishi, Y.; Ishikawa, K.; Takezoe, H. Electro-Tunable Optical Diode Based on Photonic Bandgap Liquid-Crystal Heterojunctions. *Nat. Mater.* **2005**, *4*, 383–387. [CrossRef]
12. Ozaki, M.; Matsuhisa, Y.; Yoshida, H.; Ozaki, R.; Fujii, A. Photonic Crystals Based on Chiral Liquid Crystal. *Phys. Status Solidi Appl. Mater. Sci.* **2007**, *204*, 3777–3789. [CrossRef]
13. Furumi, S.; Tamaoki, N. Glass-Forming Cholesteric Liquid Crystal Oligomers for New Tunable Solid-State Laser. *Adv. Mater.* **2010**, *22*, 886–891. [CrossRef] [PubMed]
14. Natarajan, L.V.; Wofford, J.M.; Tondiglia, V.P.; Sutherland, R.L.; Koerner, H.; Vaia, R.A.; Bunning, T.J. Electro-Thermal Tuning in a Negative Dielectric Cholesteric Liquid Crystal Material. *J. Appl. Phys.* **2008**, *103*, 093107. [CrossRef]
15. Tzeng, S.Y.T.; Chen, C.N.; Tzeng, Y. Thermal Tuning Band Gap in Cholesteric Liquid Crystals. *Liq. Cryst.* **2010**, *37*, 1221–1224. [CrossRef]
16. Rogers, J.A.; Franklin, D.; Ueltschi, T.; Carlini, A.; Yao, S.; Reeder, J.; Richards, B.; Van Duyne, R.P. Bioresorbable Microdroplet Lasers as Injectable Systems for Transient Thermal Sensing and Modulation. *ACS Nano* **2021**, *15*, 2327–2339.

17. Charlet, G.; Gray, D. Chiroptical Filters from Aqueous (Hydroxypropyl) Cellulose Liquid Crystals. *J. Appl. Polym. Sci.* **1989**, *37*, 2517–2527. [CrossRef]
18. Revol, J.-F.; Bradford, H.; Giasson, J.; Marchessault, R.H.; Gray, D.G. Helicoidal Self-Ordering of Cellulose Microfibrils in Aqueous Suspension. *Int. J. Biol. Macromol.* **1992**, *14*, 170–172. [CrossRef]
19. Wasilewska, K.; Winnicka, K. Ethylcellulose—A Pharmaceutical Excipient with Multidirectional Application in Drug Dosage Forms Development. *Materials* **2019**, *12*, 3386. [CrossRef]
20. Tsuji, R.; Tanaka, K.; Oishi, K.; Shioki, T.; Satone, H.; Ito, S. Role and Function of Polymer Binder Thickeners in Carbon Pastes for Multiporous-Layered-Electrode Perovskite Solar Cells. *Chem. Mater.* **2023**, *35*, 8574–8589. [CrossRef]
21. Palmieri, E.; Cancelliere, R.; Maita, F.; Micheli, L.; Maiolo, L. An Ethyl Cellulose Novel Biodegradable Flexible Substrate Material for Sustainable Screen-Printing. *RSC Adv.* **2024**, *14*, 18103. [CrossRef]
22. Zugenmaier, P.; Haurand, P. Structural and Rheological Investigations on the Lyotropic, Liquid-Crystalline System: O-Ethylcellulose-Acetic Acid-Dichloroacetic Acid. *Carbohydr. Res.* **1987**, *160*, 369–380. [CrossRef]
23. Guo, J.X.; Gray, D.G. Chiroptical Behavior of (Acetyl)(Ethyl)Cellulose Liquid Crystalline Solutions in Chloroform. *Macromolecules* **1989**, *22*, 2086–2090. [CrossRef]
24. Guo, J.X.; Gray, D.G.; Gray, D.G. Preparation and Liquid-Crystalline Properties of (Acetyl)(Ethyl)Cellulose. *Macromolecules* **1989**, *22*, 2082–2086. [CrossRef]
25. Guo, J.X.; Gray, D.G. Effect of Degree of Acetylation and Solvent on the Chiroptical Properties of Lyotropic (Acetyl) (Ethyl) Cellulose Solutions. *J. Polym. Sci. Part B Polym. Phys.* **1994**, *32*, 2529–2537. [CrossRef]
26. Nishio, Y.; Fujiki, Y. Liquid-Crystalline Characteristics of Cellulose Derivatives: Binary and Ternary Mixtures of Ethyl Cellulose, Hydroxypropyl Cellulose, and Acrylic Acid. *J. Macromol. Sci. Part B* **1991**, *30*, 357–384. [CrossRef]
27. Matsumoto, K.; Iwata, N.; Furumi, S. Cholesteric Liquid Crystals with Thermally Stable Reflection Color from Mixtures of Completely Etherified Ethyl Cellulose Derivative and Methacrylic Acid. *Polymers* **2024**, *16*, 401. [CrossRef]
28. Baba, Y.; Saito, S.; Iwata, N.; Furumi, S. Synthesis and Optical Properties of Completely Etherified Hydroxypropyl Cellulose Derivatives. *J. Photopolym. Sci. Technol.* **2021**, *34*, 549–554. [CrossRef]
29. Justice, R.S.; Schaefer, D.W.; Vaia, R.A.; Tomlin, D.W.; Bunning, T.J. Interface Morphology and Phase Separation in Polymer-Dispersed Liquid Crystal Composites. *Polymer* **2005**, *46*, 4465–4473. [CrossRef]
30. Kroeger, A.; Zhang, B.; Rosenauer, C.; Schlüter, A.D.; Wegner, G. Solvent Induced Phenomena in a Dendronized Linear Polymer. *Colloid Polym. Sci.* **2013**, *291*, 2879–2892. [CrossRef]
31. Vegso, K.; Siffalovic, P.; Jergel, M.; Nadazdy, P.; Nadazdy, V.; Majkova, E. Kinetics of Polymer-Fullerene Phase Separation during Solvent Annealing Studied by Table-Top X-Ray Scattering. *ACS Appl. Mater. Interfaces* **2017**, *9*, 8241–8247. [CrossRef]
32. Yano, S.; Iwata, K.; Kurita, K. Physical Properties and Structure of Organic-Inorganic Hybrid Materials Produced by Sol-Gel Process. *Mater. Sci. Eng. C* **1998**, *6*, 75–90. [CrossRef]
33. Xu, H.; Tang, S.; Chen, J.; Yin, P.; Pu, W.; Lu, Y. Thermal and Phase-Separation Behavior of Injection-Molded Poly(L-Lactic Acid)/Poly(D-Lactic Acid) Blends with Moderate Optical Purity. *Polym. Bull.* **2012**, *68*, 1135–1151. [CrossRef]
34. Salminen, L.; Karjalainen, E.; Aseyev, V.; Tenhu, H. Phase Separation of Aqueous Poly(Diisopropylaminoethyl Methacrylate) upon Heating. *Langmuir* **2022**, *38*, 5135–5148. [CrossRef] [PubMed]

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Article

Bioplastic Production Using Natural Extracts with Cellulose Assisted by Experimental and Computational Screening

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Abstract: The increasing demand for sustainable and environmentally friendly materials has prompted intensive research into developing bioplastics as viable alternatives to conventional petroleum-derived plastics. Here, we report a novel approach to bioplastic production by employing plant extract-based solvents to partially dissolve cellulose, a fundamental biopolymer precursor. Using plant-derived solvents addresses concerns surrounding the environmental impact of traditional solvent-based processes, as per the principles of green chemistry. Using computational screening, some natural products were identified from the integrated database resource MEGx. Six natural sources were selected based on their molecular weight, high pKa, and chemical classification. Thin-layer chromatography (TLC) and column chromatography confirmed the presence of molecules in the extracts. Bioplastics were prepared with 1, 3, 6, 10, and 15 wt.% plant extract concentrations. Control samples without conventional dissolved and positive controls were also studied to compare their properties with novel bioplastics. Chemical characterization and biodegradability tests were performed. Degradation in water and soil tests for 35 days showed that the biodegradability of the bioplastics with natural extracts at higher concentrations was faster than that of the control samples. By day 35, bioplastics containing 15 wt.% of the D1 W extract showed rapid degradation, with higher weight loss compared with the conventional controls. The positive control (C4), containing NaOH and glycerol, degraded more slowly than the plant extract-based formulations. Also, the test indicated that the natural dissolvent's influence on the water uptake of the material produced a better performance than the control samples. The surfaces of the bioplastic formulations were analyzed using a scanning electron microscope (SEM) at different magnifications. The findings presented here hold promise for advancing the field of bioplastics and contributing to the sustainable utilization of plant resources for eco-friendly material production.

Keywords: bioplastic; cellulose; natural dissolvent; plant extract

1. Introduction

Since the 1950s, around 8.3 billion tons of fossil fuel-based plastic have been generated, and it should be highlighted that 80% of it is not recyclable, affecting the environment [1]. Plastic waste generates byproducts associated with phthalates and bisphenols in ground-water and soil contamination [2]. The global environmental crisis, driven by the ubiquitous presence of petroleum-based plastics in our ecosystems, has spurred a dire need for sustainable alternatives [3]. Bioplastics are commercial polymer products obtained from natural sources [4]. Bioplastics have emerged as a promising solution due to the biodegradability of plastic and its reduced environmental impact. Among the various bioplastic precursors, cellulose (Figure 1), a ubiquitous biopolymer abundant in plants, holds great potential [5]. Cellulose is a polymer of repeating glucose units linked by glycosidic bonds [6]. However, the dissolution of cellulose into a workable form for bioplastic production remains a challenge [7].

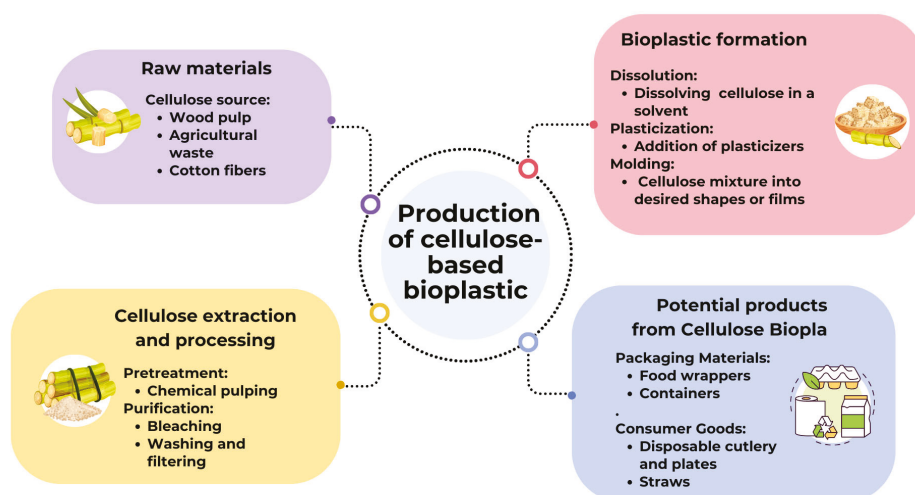


Figure 1. Production of cellulose-based bioplastic and its potential products.

Conventionally, sodium hydroxide action on glycosidic linkages typically results in depolymerization because glycosidic bonds are the primary covalent linkages connecting glucose units in cellulose chains [8]. When this solvent is introduced, it weakens the interactions between hydroxyl groups within and between cellulose molecules, reducing the crystalline order and promoting swelling and partial dissolution [9]. In the viscose process, the alkali cellulose is derivatized into cellulose xanthate through a reaction with carbon disulfide (CS_2) [10]. This step is crucial for creating a cellulose solution that can be used to produce bioplastics. An alkaline solution of sodium could partially dissolve 13% of the biopolymer from cellulose [11].

Glycerol is used primarily as a plasticizer in the formulation of cellulose-based bioplastics to improve flexibility and prevent brittleness [12]. Glycerol is a naturally occurring substance that is biodegradable, contributing to the overall sustainability of bioplastics [13]. It is safe for various applications, including food packaging and medical products, making it suitable for use where human exposure is possible [13]. As a byproduct of biodiesel production, glycerol is often less expensive than traditional petroleum-based plasticizers, reducing production costs for bioplastics [14]. It is particularly effective with hydrophilic materials, such as starch, enhancing the mechanical properties of bioplastic formulations [15]. There-

fore, glycerol's combination of sustainability, safety, and performance makes it a valuable bioplasticizer in the evolving landscape of eco-friendly materials. Cellulose, though abundant and biodegradable, tends to form rigid structures when processed alone, limiting its usability in plastic applications [16]. Glycerol reduces the intermolecular forces between cellulose chains, enhancing their mobility and making the material more pliable [17]. Its hygroscopic nature also helps retain moisture, preventing the bioplastic from becoming brittle over time [18].

Traditional methods of cellulose dissolution involve using harsh chemicals such as NaOH and solvents, often contributing to pollution and environmental risks [19]. Plant extract-based solutions have gained considerable attention in the quest for greener and more sustainable approaches. These solvents, derived from renewable plant sources such as agricultural residues, offer the promise of cellulose dissolution with reduced ecological harm. Recent research has discussed the role of hydrophobic interactions and H-bonding in the dissolution of cellulose [20]. New studies have the main goal of developing a new aqueous-based solvent system for cellulose's partial or total dissolution [21].

This research aimed to study cellulose solvents derived from plant extracts, with a focus on generating bioplastics. Hemp has a higher cellulose content and yields more cellulose than other wood sources [22]. A hemp cellulose-based polymer was mixed with plant extract-based solvents and a plasticizer to form a bioplastic material with the desired properties. By harnessing the use of computational screening to select potential molecules with high pKa, hydroxyl groups for high solubility in water, and no toxicity, which has never been explored for the production of bioplastics, this research aimed to contribute to the bioplastics industry using an environmentally responsible approach (Figure 2) [23].

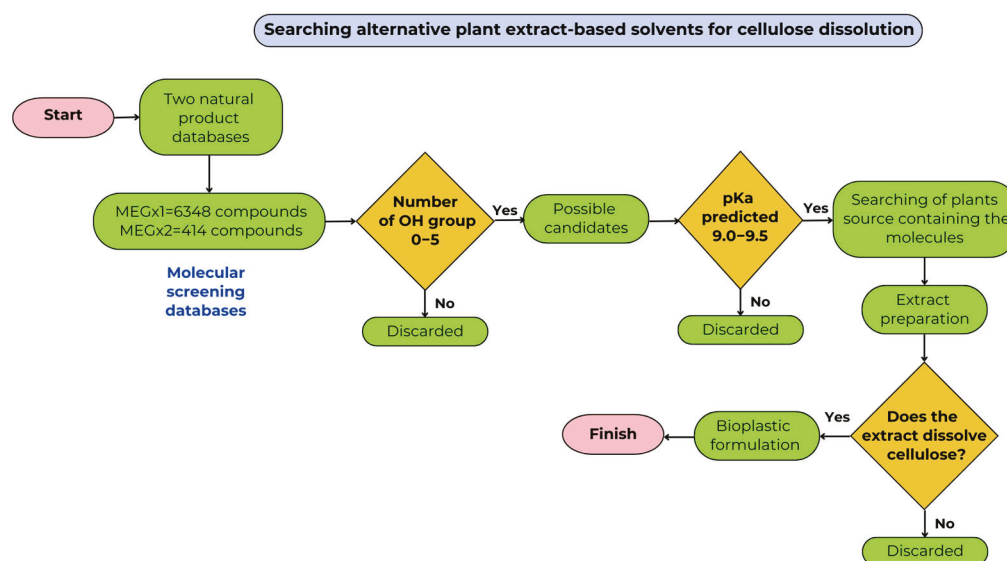


Figure 2. Diagram for searching for alternative plant extract-based solvents for partial dissolution of cellulose used in bioplastic formulation.

Our research tested six plant extract-based dissolvents derived from phenylalanine (quinoa, D1 W), abrine (guayaba, D2 W), alpha-Boswellic acid acetate (palo santo, D3 W), bellericagenin B (Ivory Coast almond, D4 W), obtusol (cacalosuchil, D5 W), and 1-linoleoyl-sn-glycerol-3-phosphorylcholine (soya, D6 W) were used for cellulose bioplastic, focusing on their physical, molecular, and chemical properties. The outcomes of this study not only contribute to the advancement of bioplastic technology but also align with the global drive toward sustainable material production, offering a glimpse into a more eco-conscious future for the plastics industry.

2. Results

2.1. Chemical Characterization of Plant Extracts

2.1.1. FTIR

After extracting the natural sources of our six chosen molecules (see Section 3), we performed IR of these extracts to determine whether the desired products were present. The chemical structures of the target molecules are presented in Figure 3.

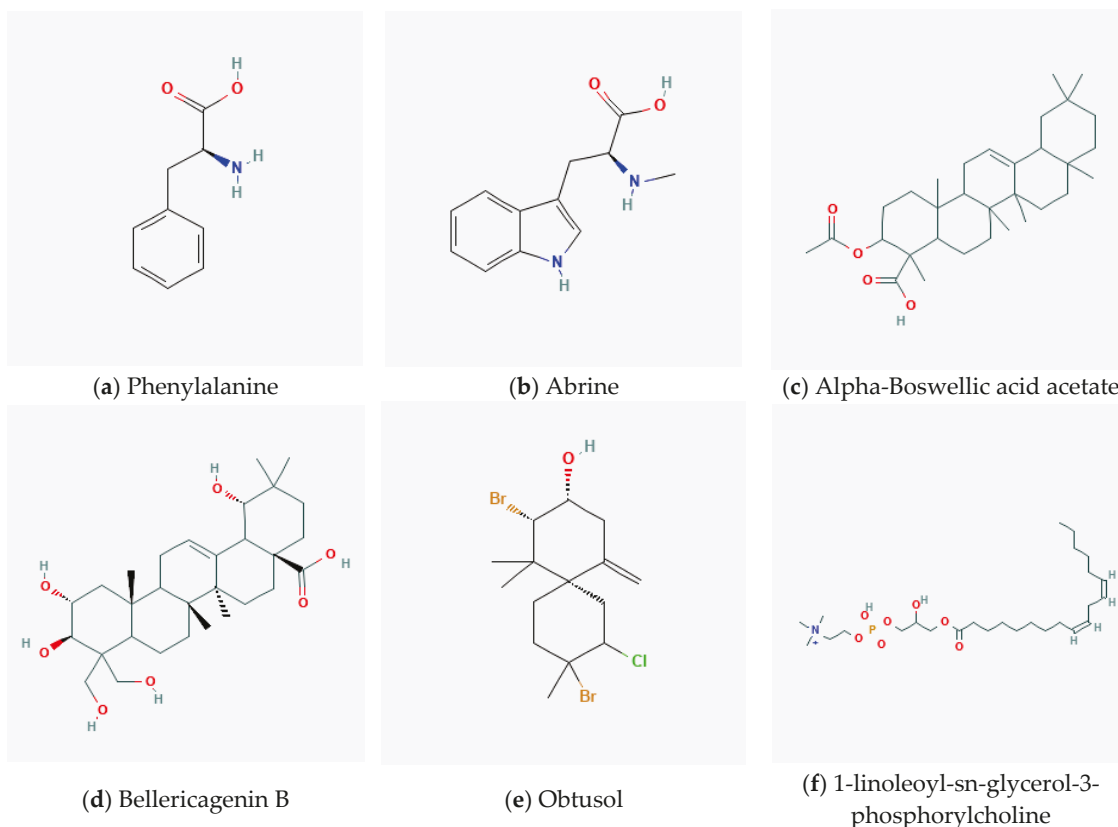


Figure 3. Chemical structures of target molecules obtained from plant extracts: phenylalanine (quinoa, D1 W), abrine (guayaba, D2 W), alpha-Boswellic acid acetate (palo santo, D3 W), bellericagenin B (Ivory Coast almond, D4 W), obtusol (cacalosuchil, D5 W), and 1-linoleoyl-sn-glycerol-3-phosphorylcholine (soya, D6 W). Information obtained from <https://pubchem.ncbi.nlm.nih.gov/> (accessed on 2 June 2025).

The absorption spectra of the dried extracts obtained in the range of 4000 and 650 cm^{-1} are shown in Figure 4. The FTIR of the D1 W molecule shows the characteristic peaks at 3386 cm^{-1} that correspond to NH_2 , 3282 cm^{-1} is the $-\text{OH}$ group, the peak at 2922 cm^{-1} shows C-H stretching of the alkyl group, there is a characteristic peak of the ring aromatic at 1999 cm^{-1} , the peak at 1632 cm^{-1} corresponds to $\text{C}=\text{O}$, and C-C stretching is shown in the peak at 1399 cm^{-1} , C-O stretching in the peak at 1328 cm^{-1} , and the C-H group in the peak at 1040 cm^{-1} , determining the presence of our molecule in the extract. The D2 W molecule has the characteristic peaks at 3441 cm^{-1} corresponding to the $-\text{OH}$ group, the distinctive peak of the aromatic ring in 1993 and 1913 cm^{-1} , and a peak at 1718 cm^{-1} corresponding to $\text{C}=\text{O}$ stretching as well. Like N-H, the peak at 1590 cm^{-1} shows the presence of the $-\text{COO}$ group, C-C stretching is ascribed to the peak at 1398 cm^{-1} , and the C-O group corresponds to the peak at 1237 cm^{-1} , characteristic of the molecules. The peak at 1028 cm^{-1} is shown for N-C stretching. Finally, the peaks at 817 cm^{-1} and 770 cm^{-1} correspond to C-H bending, showing the molecule could be present.

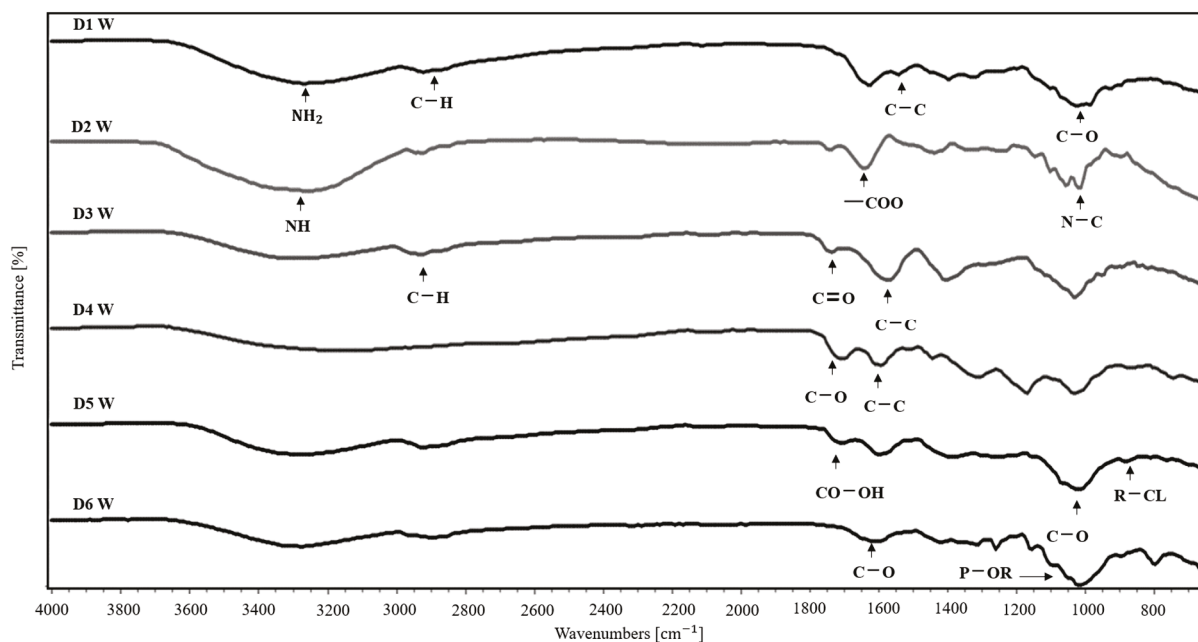


Figure 4. FTIR spectra of extracts: phenylalanine (quinoa, D1 W), abrine (guayaba, D2 W), alpha-Boswellic acid acetate (palo santo, D3 W), bellericagenin B (Ivory Coast almond, D4 W), obtusol (cacalosuchil, D5 W), and 1-linoleoyl-sn-glycerol-3-phosphorylcholine (soya, D6 W).

The D3 W molecule shows the characteristic peaks at 2930 cm^{-1} corresponding to the $-\text{CH}$ group, the peak of the aromatic ring at 1994 cm^{-1} , and $\text{C}=\text{O}$ stretching at 1744 cm^{-1} , and the peak at 1579 cm^{-1} confirms the presence of the $\text{C}-\text{C}$ stretching of the aromatic ring, as well as the $\text{C}-\text{H}$ group in the 1405 cm^{-1} peak, characteristic of the molecule. The 1030 cm^{-1} peak is shown for the cyclohexane structure, determining the presence of alpha-Boswellic acid acetate in the extract. The FTIR of the D4 W molecule shows the peak at 3433 cm^{-1} of the $-\text{OH}$ group, the peak at 2927 cm^{-1} for $-\text{CH}$, and the peak at 1712 cm^{-1} corresponding to $\text{C}=\text{O}$. The 1602 cm^{-1} peak shows the presence of the $\text{C}-\text{C}$ group and the $\text{C}-\text{O}$ group at 1318 cm^{-1} , characteristic of the molecule. The 1174 cm^{-1} peak is shown for the $\text{COO}-$ structure, corresponding to the chemical molecule's structure and the functional groups of bellericagenin B.

Furthermore, the D5 W molecule shows the characteristic peak at 3380 cm^{-1} corresponding to the $-\text{OH}$ group, the distinct peak at 2922 cm^{-1} concerning $-\text{CH}$, the 1713 cm^{-1} peak corresponding to $\text{CO}-\text{OH}$, the 1600 cm^{-1} peak belonging to the $\text{C}-\text{C}$ group of the aromatic group, and the $\text{C}-\text{O}$ stretch group in the 1023 cm^{-1} peak, characteristic of the molecule. The 764 cm^{-1} peak shows the structure of $\text{C}-\text{Cl}$, and the 651 cm^{-1} peak that corresponds to $\text{C}-\text{Br}$ is demonstrated. Therefore, the functional groups of the molecule of interest are shown. The FTIR of the D6 W molecule presented a peak at 3418 cm^{-1} corresponding to the $-\text{OH}$ group, the characteristic peak at 2922 cm^{-1} belonging to $-\text{CH}$, and the peak at 2300 cm^{-1} corresponding to $\text{P}-\text{H}$ stretching are also shown. The 1639 cm^{-1} peak shows the presence of the $\text{N}-\text{H}$ group, as well as the $\text{C}-\text{O}$ group in the 1584 cm^{-1} peak, characteristic of the molecule. The 1038 cm^{-1} peak shows the structure of $\text{P}-\text{OR}$ and CH_2 .

2.1.2. Phytochemical Screening

The samples were prepared at a concentration standard of 100 mg/mL , and the results of the preparation reagents are presented in Table 1. Phytochemical screening confirmed the presence of phytoconstituents like alkaloids, flavonoids, glycosides, phenols, lignins, saponins, sterols, tannins, anthraquinone, and reducing sugar [3]. To identify certain molecules of interest, a phytochemical study of the extracts was carried out to determine

the presence or absence of the main groups of metabolites [24]. The extracts in aqueous solutions were assessed in a phytochemical analysis.

Table 1. Phytochemical profiles of the molecules in the extracts D1 W, D2 W, D3 W, D4 W, D5 W, and D6 W.

Phytochemical Analysis	Test	D1 W	D2 W	D3 W	D4 W	D5 W	D6 W	Ref.
Reducing sugars	Benedict's test	X	X	X	X	X	-	[18]
	Fehling's test	X	X	X	-	X	X	[18]
Proteins and amino acids	Ninhydrin test	X	-	X	-	-	X	[19]
	Xanthoproteic test	X	X	X	X	X	X	[19]
Proteins	Biuret test	-	-	X	-	-	X	[20]
Terpenoids	Salkowski test	X	X	X	X	X	-	[19]
Diterpenes	Copper acetate test	X	X	X	-	X	-	[21]
Saponins	Foam test	-	X	X	X	X	X	[19]
Glycosides	10% NaOH test	X	X	-	-	X	-	[21]
	Keller–Kilani test	-	X	X	-	X	-	[21]
Alkaloids	Dragendroff test	-	-	X	X	X	-	[22]
Coumarins and lactones	NaOH test	X	-	-	-	-	-	[23]
Flavonoids	Alkaline reagent test	X	X	X	X	-	-	[21]
Phenols	Ferric chloride test	X	X	-	X	X	-	[24]
Quinones	Bontrager test	-	X	X	X	X	-	[25]
	Sulfuric acid test	-	X	X	-	-	X	[25]

(-) absence; (X) presence.

2.1.3. Thin-Layer Chromatography Analysis

TLC is a widely used analytical tool because of its simplicity, relatively low cost, high sensitivity, and separation speed [25]. The TLC results are presented in Table 2. The TLC results of molecule D1 W were performed with the extract and the standard of the molecule (phenylalanine). The mobile phase corresponded to a butanol/acetic acid/water mixture, as described in [26], with the proportion adjusted to match the polarity of the molecule. This resulted in retention factors (Rf) of $R_{f\text{standard}} = 0.68$ and $R_{f\text{D1 W}} = 0.65$, demonstrating that phenylalanine was present in the extract. Similarly, the D2W extract was subjected to analysis and was found to be 0.59, while the Rf for the commercial abrine molecule standard was determined to be 0.67. This discrepancy suggests the absence of the abrine molecule in the D2 W extract, as evidenced by the ninhydrin 2% + ethanol revealing technique.

Table 2. Mobile phase, Rf values, and solvent developers set for each crude extract in TLC analysis.

Crude Extract	Mobile Phase	Retention Factor (Rf)	Developer
D1 W	n-butanol/acetic acid/water	0.65732	Ninhydrin 2% + ethanol
D2 W	n-butanol/acetic acid/water	0.59523	Ninhydrin 2% + ethanol
D3 W	Toluene/ethyl acetate	0.40000	Vanillin sulfuric acid ethanol
D4 W	Hexane/acetone	0.53703	Iodine
D5 W	Chloroform/ethanol	0.38596	Iodine
D6 W	Toluene/ethyl acetate	0.78947	Vanillin sulfuric acid ethanol

The TLC of the D3 W molecule was performed, and a retention factor of $R_{f_{D3 W}} = 0.40$ was obtained on the silica plate using toluene/ethyl acetate, with vanillin sulfuric acid as the developer for terpenoid molecules [27]. The D4 W molecule was performed using a mobile phase of hexane/acetone [28]. As a result, the retention factor of $R_{f_{D4 W}} = 0.53$ corresponded to the band of interest [29], whose identification was performed with the iodine developer and with UV light at 254 nm. In the case of D5 W, the $R_{f_{D5 W}} = 0.38$ could correspond to the molecule when the plate was exposed to UV light and the iodine chamber [30]. To confirm the presence of the molecule of interest, D6 W, the developer vanillin sulfuric acid ethanol was heated on the silica plate at 100 °C. The band corresponding to $R_{f_{D6 W}} = 0.78$ could confirm the presence of the molecule in the extract [31].

2.1.4. Column Chromatography

The column chromatography procedure employed for plant extracts represents a technique used for the isolation and identification of bioactive compounds within the extract under investigation [32]. Initially, 50 mL of the mobile phase, previously utilized in thin-layer chromatography (TLC), was prepared. The fully dried plant extract sample was subsequently combined with the mobile phase n-butanol/acetic acid/water to facilitate the distribution of the sample D1 W (see Figure 5). The solvent system was introduced into the column at consistent rates under gravity to fractionate D1 W. Each fraction was collected individually and sequentially numbered for subsequent analysis by thin-layer chromatography. Five fractions, each of 3.5 mL, were then stored for further examination. As a result, F2 showed the related molecule phenylalanine in the plant extract because the $R_{f_{\text{standard}}}$ was 0.662, and the $R_{f_{F2-D1 W}}$ was 0.657. Through this technique, the purity of the molecule in the plant extract was separated and identified.

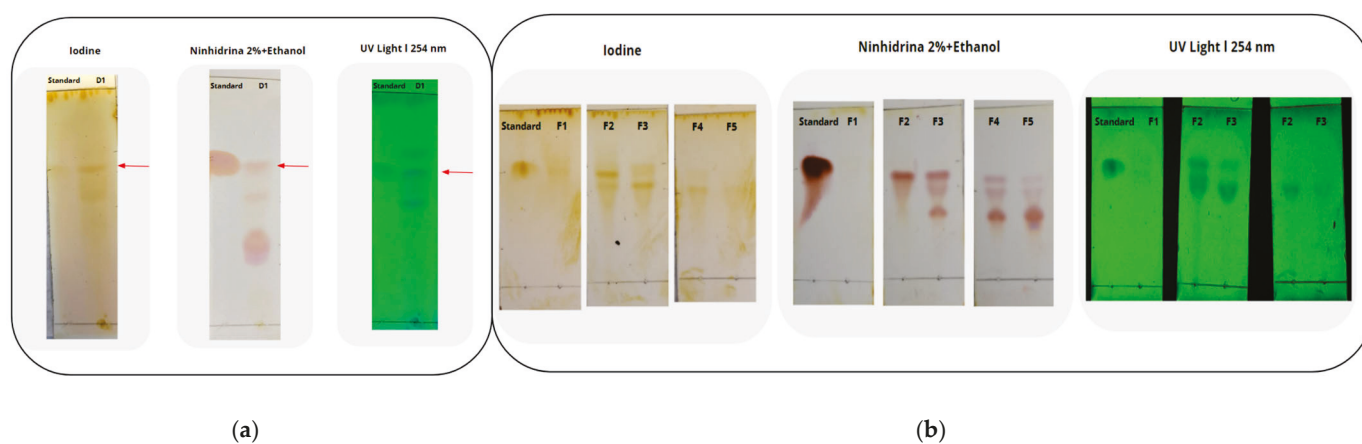


Figure 5. The complete extract D1 W presents the (a) composition and then the TLC of the five fractions (F1–F5) obtained from the column chromatography of the extract D1 W (b). It was identified that the F2 fraction contained the phenylalanine molecule (see red arrow), which is like the standard.

2.1.5. Metabolic Profile

The data were preprocessed in the MZmine software, version 3.9.0 (MZmine 3 Documentation), for non-targeted metabolomics of DDA-type data. Some of the parameters used in the software were a minimum MS1 peak height of 40 amplitude and an MS/MS abundance cutoff of 10 amplitude. Also, blank peaks present in the samples were removed. The results are presented in Table 3.

Table 3. Identification and characterization of metabolites via mass spectrometry: analysis of phenylalanine (D1 W) and 20-hydroxyecdysone.

IL *	RT (min.)	[M + H] ⁺ (<i>m/z</i>)	Molecular Formula	Error (ppm)	MS2 Score *	Identified Metabolite Name
1	1.61	(120.0803 fragment ion)	C ₉ H ₁₁ NO ₂	−3.33	-	Phenylalanine
2	2.92	445.294 [M-2H ₂ O + H] ⁺	C ₂₇ H ₄₄ NO ₇	1.79	0.72	20-hydroxyecdysone

* IL—identification level according to [33].

For the standardless identification of metabolites (level 2) the Global Natural Products Social Molecular Networking (GNPS) library (GNPS Documentation (ccms-ucsd.github.io)) was used, resulting in .mgf or .mzML files that were converted from the Waters raw datasets according to the online instructions (Mass Spectrometry File Conversion—GNPS Documentation (ccms-ucsd.github.io)). The mass tolerance of the precursor ion and that of the fragment ion were set to 0.02 Da. Subsequently, a molecular network was established in which edges were filtered to have more than three matching fragments and a cosine score greater than 0.70.

Level 1 identification: Level 1 metabolite identification of phenylalanine was carried out according to the minimum standards proposed for the metabolomics standards initiative (MSI), utilizing two criteria: (a) the alignment of the retention times with those of the authentic standard and (b) the match in *m/z* with a minimum of 10 ppm.

Level 2 metabolite identification: The raw profile MS/MS Waters files (*.raw) were mass-corrected in the MassLynx software V4.1 (see Figure 6). Each group of files (*.raw) was processed in MZmine 3.7.0 for 20 eV (ID = 9c3cd3dd39e44b8abba692c19ec8e17a) of collision energy to generate a list of feature height (*.csv) and spectra files (*.mgf) for level 2 metabolite identification using feature-based molecular networking (FBMN) algorithms on the Global Natural Products (GNPS) platform. Finally, the sample D1 W chromatogram showed a peak at 1.615 min, indicating that the compound in the sample had the same retention behavior in the chromatographic system as the standard phenylalanine [34]. Mass spectrometry provided the ion fragment *m/z* 120.0806 for the phenylalanine standard, corresponding to its molecular structure. The ion fragment for the sample was *m/z* 120.0803, which was almost identical to the standard. When the sample was analyzed alongside the standard (chromatogram B), the retention time and ion fragments remained consistent, further confirming the presence of phenylalanine in D1 W.

2.2. Preparation of Cellulose-Based Bioplastic Samples

The cellulose was extracted from hemp with a yield of ±47.5% by mass. This extracted cellulose was then employed in the preparation of bioplastic materials. The preparation of the bioplastic commenced following the extraction and characterization of the six extracts. Cellulose, glycerol, and the plant extract were used as solvents in the bioplastic formulation. The concentration of the plant extract was varied to assess its effect. Each mixture was stirred magnetically for 60 min at 80 °C, followed by hot pressing at 240 °C to form bioplastic films. The films were subsequently cut into 1.791 cm diameter circular discs. Cellulose-based bioplastics were successfully produced using the extract-based natural solvent, except for the D2 W samples and the control C2, as illustrated in Figure 7. Notably, control sample C2 (without the plant extract) and sample D2W exhibited poor film-forming properties, resulting in brittle materials that were difficult to manipulate, unlike other formulations. This suggests that while cellulose dissolution occurred to some degree in all

cases, the presence and type of plant extract significantly influenced the behavior of the final bioplastic.

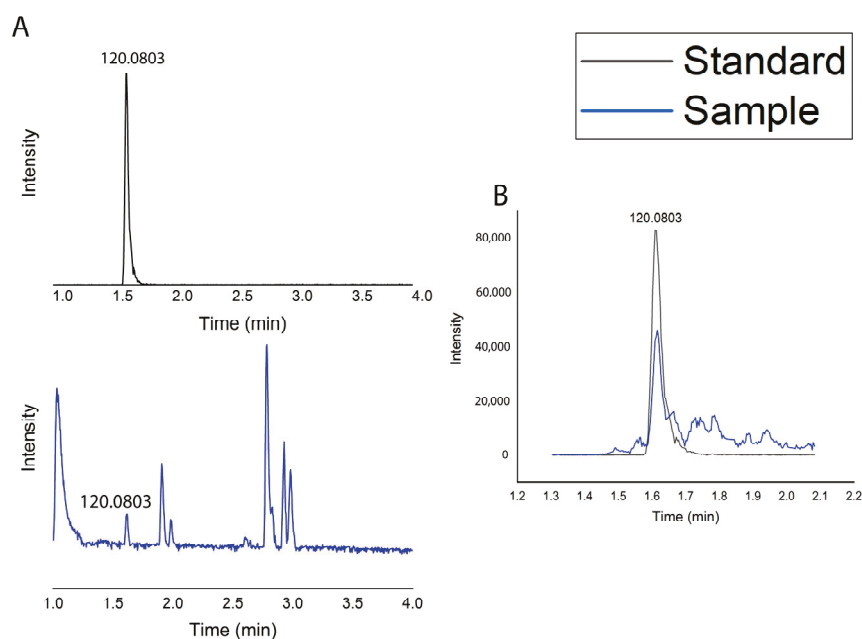


Figure 6. Chromatograms: (A) standard phenylalanine 1.615 min ion fragment (m/z 120.0806) and sample 1.615 min ion fragment (m/z 120.0803); (B) sample along with standard.

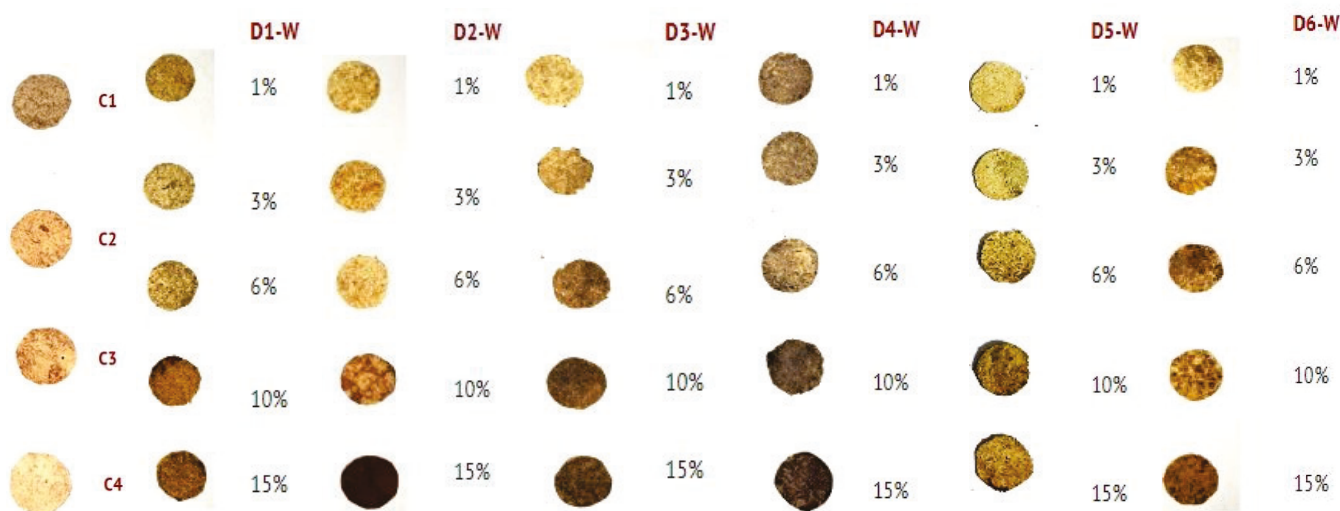


Figure 7. Bioplastic films using glycerol and a plant extract solvent concentration. Bioplastics with the plant extracts phenylalanine (quinoa, D1 W), abrine (guayaba, D2 W), alpha-Boswellic acid acetate (palo santo, D3 W), bellericagenin B (Ivory Coast almond, D4 W), obtusol (cacalosuchil, D5 W), and 1-linoleoyl-sn-glycerol-3-phosphorylcholine (soya, D6 W) compared with controls C1 (cellulose), C2 (cellulose + glycerol), C3 (cellulose + NaOH), and C4 (cellulose + NaOH + glycerol).

2.3. Chemical Properties of the Bioplastics

The FTIR spectra of the cellulose bioplastics at 15 wt.% of natural dissolvent can be seen in Figure 8a. In the spectrum of C1 (cellulose), the O-H peaks ($3300\text{--}3500\text{ cm}^{-1}$) usually show a shift toward lower frequencies compared with pure water due to the formation of hydrogen bridges between water and the hydroxyl groups of cellulose [35]. Also, in the range $1400\text{--}1200\text{ cm}^{-1}$, there is a small peak characteristic of the stretching vibration of C-H in cellulose. The sample C2 (cellulose + glycerol) registered C-O stretching in the $1125\text{--}1000\text{ cm}^{-1}$ region related to the alcohol of glycerol after a plasticizing reaction

with cellulose [36]. The control C3 (cellulose + NaOH) registered a peak in the range of $700\text{--}650\text{ cm}^{-1}$ belonging to the COH functional group after adding NaOH [37]. The positive control C4 (cellulose + NaOH + glycerol) integrated the peaks of --OH , C-H, and CO; these results show the correct incorporation of the compounds in the bioplastic film.

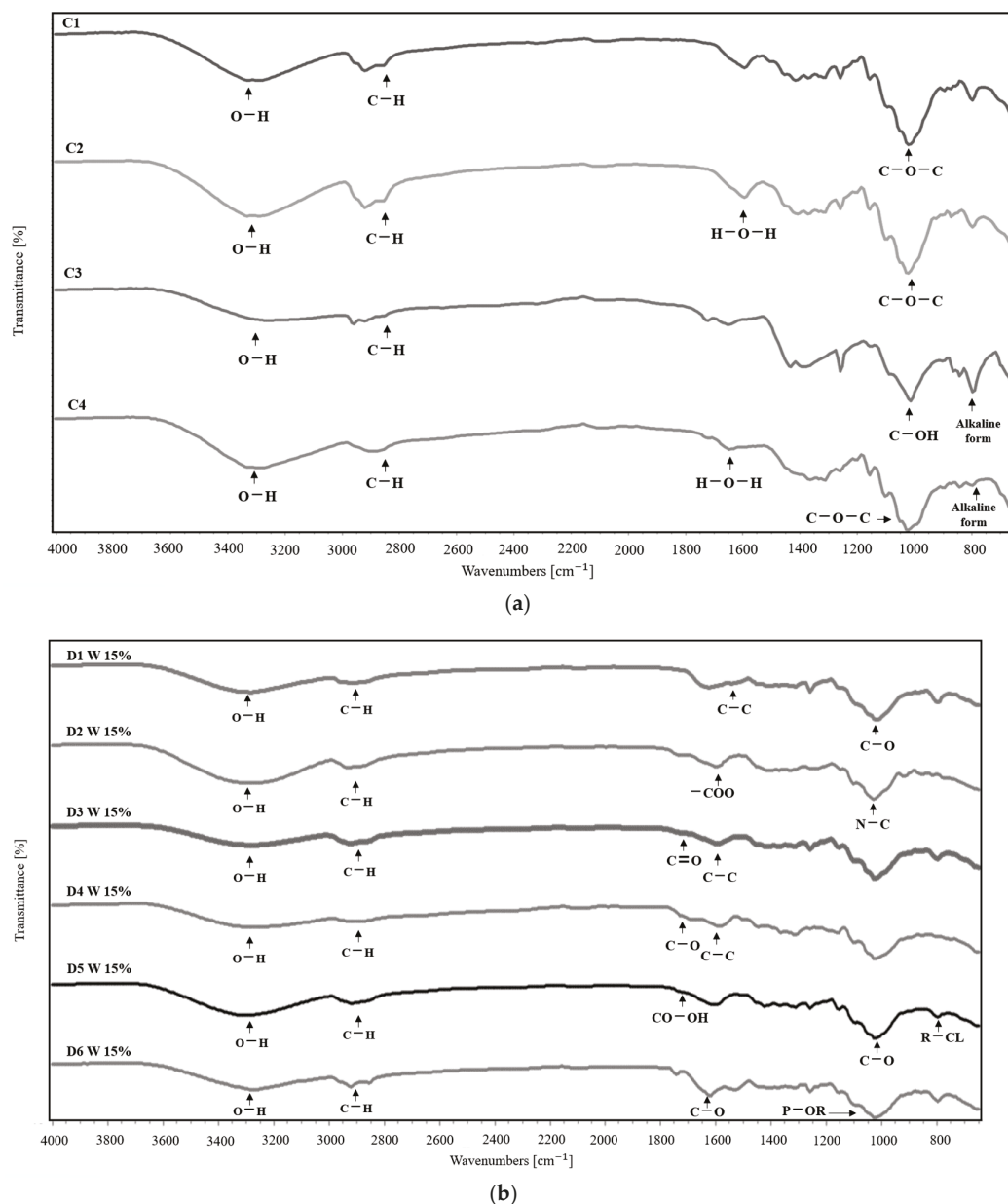


Figure 8. (a) FTIR spectra of bioplastic films used as controls: C1 (cellulose), C2 (cellulose + glycerol), C3 (cellulose + NaOH), and C4 (cellulose + NaOH + glycerol). (b) FTIR spectra of bioplastic films using natural partial dissolvent of cellulose D1 W, D2 W, D3 W, D4 W, D5 W, and D6 W at 15 wt.%.

The presence of the functional groups C=O , C-C , C-O , and COO^- is shown, which correspond to the chemical structure of the solvent molecule D4 W. Therefore, the incorporation of the solvent extract into the structure of the films D4 W 15% is demonstrated. Then, the bioplastic formulated with the D5 W extract shows the peaks C-C , C-O , C-Cl , and C-Br , indicating the presence of the extract solution in the film. Finally, the presence of the peaks corresponding to the functional groups of P-H , N-H , and C-O is observed, as well as the functional groups of P-OR and CH_2 , characteristic of the D6 W extract. These functional groups demonstrate the molecule's presence as a solvent in the bioplastics (Figure 8b).

The FTIR analysis of the bioplastic films with the natural extract D1 W and glycerol shows a significant difference in the functional groups when using bioplastic films with different concentrations from 0 to 15 wt.% compared with the negative control C1 (cellulose) and the positive control C4 (cellulose + NaOH + glycerol).

2.4. Surface Analysis of Bioplastics

A scanning electron microscope (SEM) was employed to acquire detailed information regarding the structure, morphology, size, shape, and surface modifications of the bioplastics [28,29]. The high magnification and resolution capabilities of the SEM facilitated a thorough examination and analysis of the bioplastic film surface. The results reveal distinct structural variations in both the cross-section and upper surface of the bioplastic, as illustrated in Figure S1 (see the Supplementary Materials). The SEM analysis of the film with the highest concentration of D1 W showed semi-organized fiber arrangements on the surface. The cross-sectional view revealed a semi-organized arrangement of fibers within the inner portion of the film. In contrast, the surface of the D2 W film appeared rough, and the cross-sectional analysis showed a disorganized arrangement of internal fibers.

The films prepared with D3 W exhibited a rough surface with noticeable pores in certain areas, as well as accumulations associated with the extract. The cross-sectional analysis revealed a disorganized distribution of internal fibers. In the case of D4 W, the SEM images showed a surface with hierarchical fibers and a limited presence of pores. However, the cross-sectional view indicated a disorganized distribution of fibers within the film, with no clear orientation. For D5 W, the results suggest some fibrillar intertwining and residual solvent on the surface, which may be related to the solubility of the extract. The cross-sectional analysis revealed a distinct internal distribution of components. Additionally, the images of the film with the D6 W extract showed a non-uniform fiber distribution and a porous structure. The surface appeared rough, and the fibers were oriented in a disorganized manner.

2.5. Biodegradability of Bioplastics

Degradation in Soil and Water

The biodegradability of the cellulose bioplastics, formulated with a natural solvent and glycerol, was assessed using the soil burial method [38]. Soil, being rich in microorganisms, facilitates plastic biodegradation more effectively compared with other environments such as water and air [38]. The bioplastics demonstrated a need for elevated temperatures and extended degradation periods to degrade efficiently. The rate of polymer biodegradation is influenced by the chemical nature of the polymer, environmental conditions, and soil composition.

The results presented in Figure 9 demonstrate that cellulose bioplastics formulated with the plant extract D1 W at concentrations of 6, 10, and 15 wt.% exhibited accelerated degradation rates compared with the commercial control, C4. This observation suggests that incorporating the plant extract as a solvent at intermediate-to-higher concentrations enhances the biodegradability of cellulose bioplastics in both soil and water environments. By day 35, the bioplastics containing the plant extract showed significant disintegration. In contrast, the negative control, C1, exhibited the least weight loss in both soil and water. Bioplastics formulated without a conventional solvent, using only a traditional plasticizer (C2), demonstrated a slower degradation rate compared with films that included a traditional solvent (C3). Additionally, the avocado bioplastic (C5) was also evaluated.

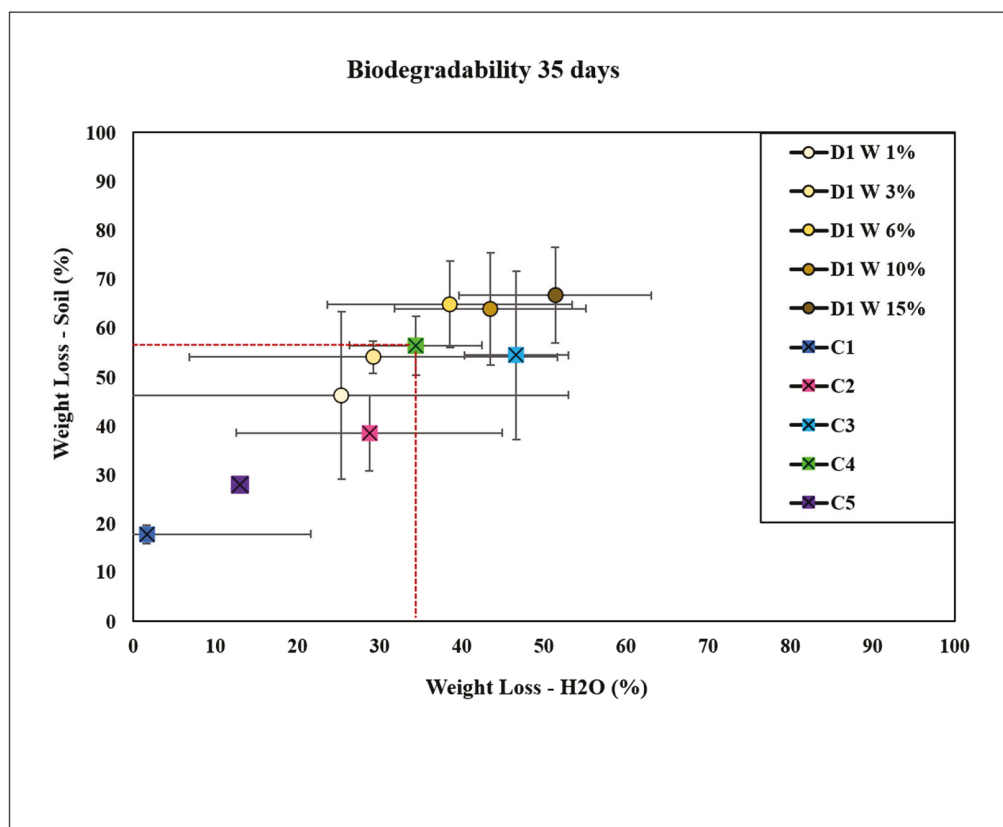


Figure 9. Bioplastics with plant extract D1 W degraded in soil and water at concentrations of 1, 3, 6, 10, and 15 wt.% compared with controls C1 (cellulose), C2 (cellulose + glycerol), C3 (cellulose + NaOH), C4 (cellulose + NaOH + glycerol), and C5 (bioplastic of avocado).

The observed differences were statistically significant in both water and soil, indicating that the presence of the cellulose solvent, rather than the conventional plasticizer, is crucial for accelerating the degradation process. The extremely low *p*-values further confirm statistically significant differences between the groups in both water and soil degradation studies, underscoring that the various treatments or conditions substantially influenced weight loss due to biodegradation. Furthermore, the significant differences observed among the different concentrations of D1, as well as between the positive and negative controls, suggest that the concentration of D1 substantially impacted the biodegradation rates.

The results presented in Figure 10 illustrate the degradation values of bioplastics containing 15 wt.% of extracts D1 W, D2 W, D3 W, D4 W, D5 W, and D6 W after 35 days. Bioplastic films incorporating extracts D3 W and D4 W exhibited relatively lower weight loss in both soil and water environments compared with the commercial control, C4. This suggests that these specific extract formulations may enhance the durability of bioplastics under these conditions. Conversely, bioplastic films containing extract D5 W displayed a distinct degradation pattern, with slower degradation in soil relative to C4 but accelerated degradation in water. This contrasting behavior indicates that the effect of extract D5 on bioplastic degradation was dependent on the environmental medium. Additionally, bioplastic films containing extracts D1 W, D2 W, and D6 W demonstrated more rapid degradation rates in both soil and water environments. Although we did not have evidence of abrine in the extract used for bioplastic D2W, it is possible that the extract had other molecules that could act as a dissolvent for cellulose and accelerate the degradation of D2W.

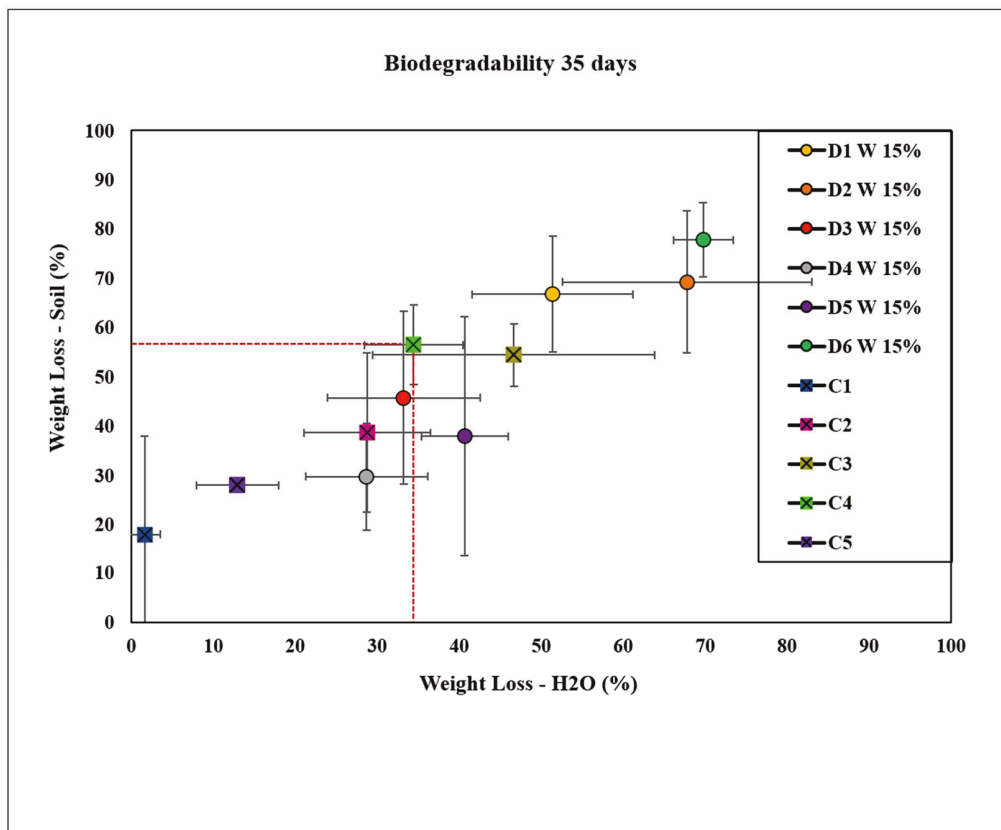


Figure 10. Degradation in soil and water of bioplastics with plant extracts D1 W, D2 W, D3 W, D4 W, D5 W, and D6 W at a concentration of 15 wt.% compared with controls C1 (cellulose), C2 (cellulose + glycerol), C3 (cellulose + NaOH), C4 (cellulose + NaOH + glycerol), and C5 (bioplastic of avocado).

Tukey's HSD test for weight loss in water revealed a significant difference between C1 and D1. Additionally, C1 had a significantly different mean compared with all other groups (D1 to D6, C2, C3, C4, and C5). Extract D2 exhibited significant differences from most other groups, with the exception of D6. Extracts D3, D4, and D5 displayed varied significance when compared with different groups. This variability underscores the complex nature of factors influencing the degradation kinetics of bioplastics.

In terms of weight loss in soil, C1 showed significant differences from all other groups except D4 and C5, indicating that the negative control experienced much lower weight loss compared with most other treatments and controls. The partial dissolution of cellulose occurred during the mixing step with the base (NaOH in the plant extract). It did not occur in C1 and C2, which were our controls without the base, and this can explain the slower degradation of the samples without the base. Extract D1 was significantly different from D3, D4, D5, and C1. Extract D2 also showed substantial differences from D3, D4, D5, and C1, but not from D6. Extract D3 exhibited significant differences from D4, D5, and D6. Extracts D4 and D5 showed substantial differences compared with multiple groups, reflecting their varied effectiveness compared with other treatments and controls. Extract D6 demonstrated significant differences from C1, C2, C3, C4, and C5, suggesting it had the highest weight loss among the treatments.

2.6. Water Uptake of Bioplastics

Water absorption is defined as the amount of water taken up by a bioplastic. It is calculated as the ratio of the weight of water absorbed to the weight of the dry material [11]. The results presented in Figures 11 and 12 indicate that an increased amount of natural fiber leads to a higher degradation rate. This occurs because the natural fiber absorbs water,

which accelerates the hydrolytic degradation process [39]. Assessing water absorption in cellulose bioplastic films is crucial for evaluating the stability of these films in aqueous environments, which impacts their long-term performance.

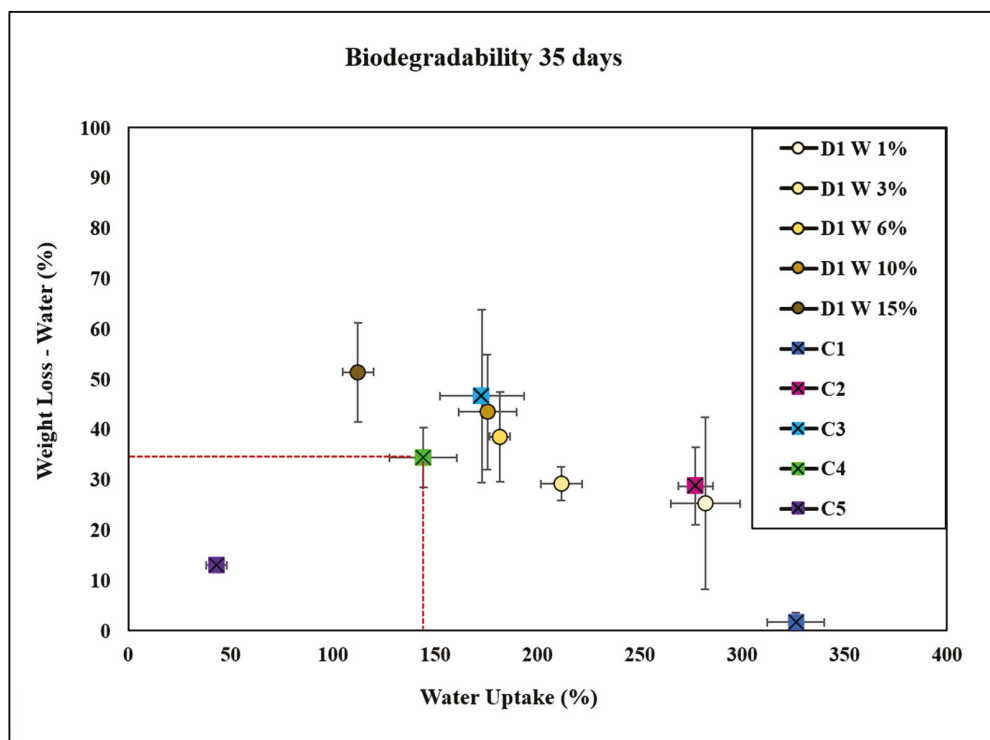


Figure 11. Water uptake and degradation of bioplastics with the plant extract D1 W at concentrations of 1, 3, 6, 10, and 15 wt.% compared with controls C1 (cellulose), C2 (cellulose + glycerol), C3 (cellulose + NaOH), C4 (cellulose + NaOH + glycerol), and C5 (bioplastic of avocado).

The C4 bioplastic film exhibited a water uptake of 150% and a weight loss of 35%. In contrast, the C1 bioplastic film showed a higher water uptake of 325% (see Figure 11). This difference suggests that the inclusion of a conventional plasticizer and cellulose solvent reduced the water uptake. This effect can be attributed to the interaction between glycerol and NaOH, which likely altered the structural properties of the bioplastic film. Specifically, the plasticizer increased the flexibility of the film, while the cellulose solvent facilitated the partial dissolution and dispersion of cellulose molecules, resulting in a more compact and less porous structure [39]. Consequently, the film exhibited reduced water uptake compared with the control cellulose C1 due to the decreased availability of free spaces within its structure for water molecules to penetrate and be absorbed, demonstrating that the alkaline solution and the natural extract were required to form the bioplastic.

Figure 9 illustrates that at lower concentrations of D1 W, the water uptake and degradation rates were lower than those observed for C4. In contrast, at the highest concentration of 15% extract D1 W, the water uptake was lower than that of C4, and the degradation in water was more efficient than that of C4. This result can be attributed to the increased concentration of active components in the extract, which likely enhanced the hydrophobicity of bioplastic film. The higher concentration of extract D1 W may also have introduced more effective degradation-promoting components, leading to improved degradation rates in water compared with C4. Additionally, C1 exhibited significantly higher water uptake than all other groups. D1 W 15% demonstrated a significantly lower water uptake than all other groups except C5. Meanwhile, C1 had significantly lower weight loss compared with all other groups. D1 W 15% exhibited significantly higher weight loss than C1 but lower

than certain other concentrations, such as D1 W 6% and D1 W 10%. D1 W 6% and D1 W 10% showed significantly higher weight loss compared with C1, C2, and C5. Comparisons between D1 W 1%, D1 W 3%, and the other concentrations showed variability in weight loss, but the differences were not consistently significant.

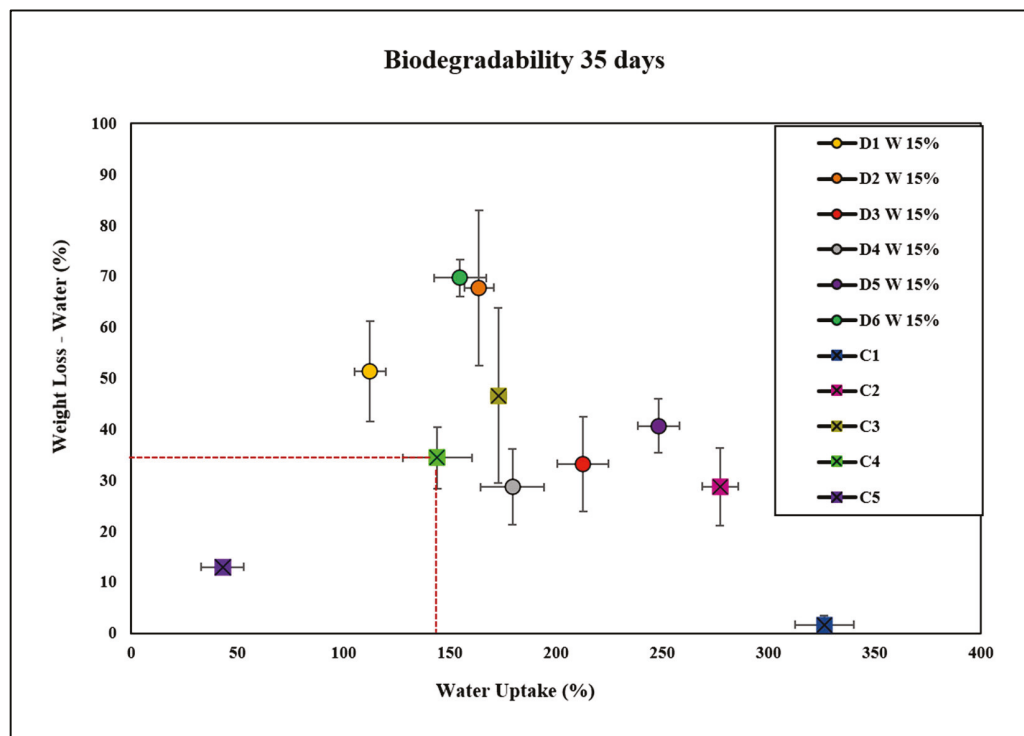


Figure 12. Water uptake and degradation of bioplastics with the plant extracts D1 W, D2 W, D3 W, D4 W, D5 W, and D6 W at a concentration of 15 wt.% compared with controls C1 (cellulose), C2 (cellulose + glycerol), C3 (cellulose + NaOH), C4 (cellulose + NaOH + glycerol), and C5 (bioplastic of avocado).

The negative control C1 group exhibited significantly higher water uptake compared with all other groups. The D1 W 15% group demonstrated significantly lower water uptake compared with C1, C2, and D3, but significantly higher water uptake compared with D4, D5, D6, and C4. D2 W 15% showed a significantly higher water uptake than D1 W 15%, D4, D5, D6, and C4, but lower than C1. The D3 W 15% group had the highest water uptake among the D1 concentrations, significantly higher than all other groups except C1. The D4 W 15% group exhibited significantly higher water uptake compared with D1 W 15%, D5, D6, and C4, but lower than D2 W 15% and D3 W 15%. D5 W 15% showed a significantly higher water uptake than D1 W 15%, D2 W 15%, D4 W 15%, D6 W 15%, and C4, but not compared with C1. D6 W 15% had a significantly higher water uptake compared with D1 W 15%, D2 W 15%, D4 W 15%, and C4, but lower than C1. Overall, C1 exhibited the highest water uptake among all groups, with significant differences from every other group. C2 and C3 also had significantly higher water uptake compared with the D1 concentrations and C4. The principal findings about the biodegradability of the bioplastics studied are presented in Table 4.

Table 4. Biodegradability of bioplastics, including principal conclusions about degradation rate, water uptake, and certain observations.

Bioplastic Type	Composition	Degradation Rate (%)	Water Uptake (%)	Observations
C1	Cellulose (negative control)	Lowest	Highest (325%)	Exhibits the least weight loss in both soil and water, indicating lower biodegradability. Shows the highest water uptake among all groups.
C2	Cellulose + glycerol	Moderate	Moderate	Demonstrates a slower degradation rate compared with C3. Shows higher water uptake than D1 and D6 at 15 wt.%.
C3	Cellulose + NaOH	Moderate	Moderate	Slightly faster degradation than C2. Exhibits higher water uptake compared with C4 but lower than C1.
C4	Cellulose + NaOH + glycerol (commercial control)	Faster	Lower (150%)	Exhibits a faster degradation rate compared with C1, C2, and C3. Lower water uptake compared with C1 but higher than C5.
C5	Bioplastic of avocado	Lower	Lowest	Shows the lowest weight loss among all controls and D1 concentrations, indicating low biodegradability. Water uptake is lower compared with other groups.
D1 W 1%	Plant extract D1 W (1 wt.%)	Lower	Higher	At low concentrations, it exhibits reduced biodegradability and higher water uptake compared with D1 W 15%.
D1 W 3%	Plant extract D1 W (3 wt.%)	Lower	Higher	Similar to D1 W 1%, with moderate water uptake and degradation rates. Differences in weight loss compared with D1 W 6% and 10% were not consistently significant.
D1 W 6%	Plant extract D1 W (6 wt.%)	Higher	Moderate	Higher degradation rate compared with D1 W 1% and 3%. Water uptake is moderate, significantly higher than C1.
D1 W 10%	Plant extract D1 W (10 wt.%)	Higher	Moderate	Has a degradation rate similar to D1 W 6%, with higher weight loss compared with C1, C2, and C5. Moderate water uptake.
D1 W 15%	Plant extract D1 W (15 wt.%)	Highest	Lower	Exhibits the highest degradation rates in both soil and water, especially compared with C1. Lower water uptake than C1 and D1 W 1%.
D2 W 15%	Plant extract D2 W (15 wt.%)	High	Moderate	High degradation rates in both soil and water. Water uptake is higher than D1 W 15%, D4, D5, D6, and C4, but lower than C1.
D3 W 15%	Plant extract D3 W (15 wt.%)	Moderate	High	Exhibits relatively lower weight loss but higher water uptake compared with C4. High water uptake indicates enhanced water absorption capacity without necessarily increasing degradation.
D4 W 15%	Plant extract D4 W (15 wt.%)	Lower	High	Lower degradation rates but higher water uptake than C4, suggesting components in D4 W may inhibit degradation while promoting water absorption.
D5 W 15%	Plant extract D5 W (15 wt.%)	Variable	High	Show slower degradation in soil but accelerated in water compared with C4. It indicates that D5 W effect is environment dependent. High water uptake but does not necessarily increase degradation.
D6 W 15%	Plant extract D6 W (15 wt.%)	High	Moderate	High degradation rates similar to D1 W 15% and D2 W 15%. Water uptake is higher than C4 but lower than D3 W 15%.

The D1 W 15% group showed significant differences from other concentrations (D3 W 15%, D4 W 15%, and D5 W 15%), although these differences were less pronounced. D2 W 15% and D6 W 15% showed the highest weight loss among D1 concentrations and compared with several controls. D1 W 15% showed lower weight loss compared with higher D1 concentrations (D2 W 15% and D6 W 15%) but higher compared with C1. C1 consistently showed the lowest weight loss, significantly different from all other groups, highlighting its role as the negative control. C5 had the lowest weight loss among all controls and D1 concentrations, indicating it might be an outlier or less effective in weight loss. C2 and C3 exhibited higher weight loss compared with C1 and C4, but this was not as high as that of D2 W 15% and D6 W 15%.

Bioplastic films containing extracts D1 W, D6 W, and D2 W exhibited similar levels of water uptake and higher degradation rates compared with the positive control, C4. This can be attributed to the composition of these extracts, which likely contained molecules that increased the hydrophilicity of the films, allowing for water molecules to penetrate more easily and accelerate degradation. Conversely, films with extracts D3 W and D5 W showed similar weight loss but higher water uptake compared with C4, indicating that these extracts enhanced the water absorption capacity of the bioplastic, leading to swelling without necessarily increasing degradation. The film with extract D4 W had lower degradation rates but higher water uptake than C4, suggesting that D4 W may contain components that inhibit degradation while promoting water absorption, potentially acting as barriers to degradation or altering the film's structural integrity.

2.7. pH Change in Cause Medium

The effects of the water pH and immersion time on the water absorption behavior and mechanical properties of the bioplastic films were studied using a benchtop pH meter (Benchtop pH Meter APERA Instrument). The six molecules selected by computational screening had acid dissociation constant (pKa) values that were generally basic, so the changes in the water pH over time were monitored during degradation tests. The results are presented in Figure S2 (Supplementary Materials). Samples C3 and C4 showed significantly different pH variations compared with the other samples, exhibiting a more alkaline pH during the water degradation test. This difference was due to the inclusion of sodium hydroxide in these formulations, which acted as both a conventional cellulose solvent and a standard alkalizing agent in this study [7].

In contrast, bioplastics C1 and C2 did not show significant pH fluctuations. Among the samples containing plant extracts, no significant pH differences were observed compared with the positive control, except for D1 W at a concentration of 15 wt.%. However, the pH change for these extracts was less than that observed for C3 and C4. Plant extracts often contain organic acids and other compounds that act as buffering agents, stabilizing the pH. Thus, the plant extract solvent in D1 W at 15 wt.% likely had a less pronounced alkalizing effect on the solution, resulting in a lower pH change compared with the NaOH-containing formulations.

The statistical test revealed significant differences in pH levels across various time points, indicating that the pH fluctuated depending on the extract concentration and immersion duration. The control samples C3 and C4, containing NaOH, showed higher pH variability, maintaining an elevated pH due to their strong alkalizing effect. The lower extract concentrations (1% to 6%) exhibited moderate pH changes, suggesting a minimal impact due to their lower chemical activity or buffering capacity. In contrast, higher extract concentrations (10% and 15%) displayed marked pH increases over time, likely due to a cumulative interaction with water. Each extract demonstrated a distinct pH profile, reflecting its unique chemical composition. Some extracts, such as D2 W and D5 W, showed

stable pH levels, possibly due to buffering agents. In contrast, control samples C1 and C2, containing commercial bioplastic and glycerol, showed minimal pH alterations, consistent with expectations for non-alkalizing components.

3. Materials and Methods

3.1. Computational Screening

Computational methods have revolutionized problem solving across diverse fields by leveraging advanced high-performance computing systems. The ability to process vast quantities of data and construct models predicting molecular properties or biological activities based on structure–property relationships is notable for several reasons: Firstly, these studies offer a more cost-effective and time-efficient approach than traditional experimental methods, accelerating the discovery and development of new molecules with specific desired properties. The popularity of quantitative structure–activity relationship (QSAR) and quantitative structure–property relationship (QSPR) methods stems from their established reliability and effectiveness in predicting molecular behavior [40].

The decision to use compounds from the Analyticon MEGx database, available at <https://ac-discovery.com> (accessed on 15 February 2024), for screening was likely based on the database containing a diverse and representative set of compounds relevant to this research objective. The choice to use two specific datasets, MEGx_1 and MEGx_2, from the database was based on the characteristics of the compounds included. MEGx_1, with its larger size of 6348 compounds, provides a comprehensive dataset for a broad screening of properties or activities. Meanwhile, MEGx_2, consisting of 414 compounds, is more focused and curated, possibly containing compounds of specific interest or relevance to this research.

The aim of using the software program PaDEL, version 2.21, a descriptor for substructure fragment analysis with a specific fingerprint, SubFPC12, associated with the OH group, was to identify molecular fragments or patterns in the examined compounds similar to conventional solvents [33]. This analysis provided insights into the structural features of the compounds relevant to their properties, such as solubility or toxicity. Furthermore, the evaluation of acute oral toxicity using the software program (developed in Java 1.8) indicated a focus on assessing the potential toxicity of the compounds of this research [41]. The EPA (Environmental Protection Authority) and GHS (Globally Harmonized System) classification systems provided a comprehensive approach to toxicity evaluation, considering both environmental and human health hazards.

3.2. Extract Source Screening

The selection of six natural sources was based on computational screening and the literature reporting the presence of the identified molecules. Table 5 presents the compound name, chemical classification, molecular weight, pKa, and natural source of the molecules. After source identification, quinoa (*Chenopodium quinoa*), guayaba (*Psidium guajava*), palo santo (*Bursera graveolens*), Ivory Coast almond (*Terminalia ivorensis*), cacalosuchil (*Plumeria rubra*), and soya (*Glycine max*) were obtained from the local supermarket.

3.3. Other Reagents

The hemp (*Cannabis sativa* L.) biomass used in this study was acquired from CannAndes S.A. (Tabacundo, Ecuador). Merck-Milipore (Darmstadt, Germany) supplied sodium hydroxide, ethanol, methanol, and glycerol.

Table 5. Molecules identified by computational screening.

Code	Compound Name	Chemical Class	Molecular Weight (g/mol)	OH Groups	Predicted pKa	Source
D1	Phenylalanine	Amino acids and peptides	165.192	0	9.48	Quinoa
D2	Abrine	Amino acids and peptides	218.256	0	9.34	Guayaba
D3	Alpha-Boswellic acid acetate	Terpenoids	498.748	0	9.06	Palo santo
D4	Bellericagenin B	Terpenoids	520.707	5	9.02	Ivory Coast almond
D5	Obtusol	Terpenoids	442.728	2	9.01	Cacalosuchil
D6	1-linoleoyl-sn-glycerol-3-phosphorylcholine	Aliphatic natural product	426.729	1	9.11	Soya

3.4. Preparation of Cellulose Bioplastic

3.4.1. Cellulose Extraction

Cellulose was extracted from hemp through a series of treatments, including alkalization, acid hydrolysis, and bleaching [42]. Initially, the hemp biomass was subjected to crushing and cleaning to eliminate any contaminants. The resultant residue was then treated with hydrochloric acid. Following this, the biomass underwent alkalization using sodium hydroxide. Subsequently, the biomass was bleached with chlorine for 24 h at an ambient temperature. The final residue was then thoroughly washed, dried at room temperature, and then lyophilized. This method effectively yielded a high purity and quantity of cellulose by removing non-cellulosic components. The cellulose was then stored at room temperature. The yield of hemp cellulose was calculated as follows [43]:

$$\text{Cellulose yield (\%)} = \frac{\text{Mass of cellulose obtained}}{\text{Initial mass of hemp}} \times 100 \quad (1)$$

3.4.2. Obtention of Plant Extract

The sample collection was conducted based on the results of computational screening. Biomass was obtained from six distinct sources for the extraction of each molecule. These samples were initially stored at room temperature (25 °C), then crushed into a flour-like powder, packaged, and stored. Two of the biomass samples were washed, dried in an oven at 40 °C for 48 h and then crushed and subsequently stored at room temperature. Following collection, the maceration method (see Table 6) was employed for extraction due to the coarse powder consistency of the biomass material [44].

Table 6. Maceration method quantities.

Source	Amount Biomass (g)	Water (mL)	Time
Quinoa	200	1000	1 day
Guayaba	280	350	1 day
Palo santo	125	500	7 days
Ivory Coast almond	50	500	7 days
Cacalosuchil	50	500	7 days
Soya	200	1000	1 day

Distilled water (W) was employed as the solvent for molecule extraction. It was added to the biomass samples, with the maceration period varying from 24 h to 5 days, depending on the source, and continuous agitation was maintained [45]. Following the maceration period, the samples were subjected to centrifugation at 6000 rpm for 30 min. The supernatant was collected, filtered, frozen at −80 °C, and subsequently lyophilized. The resulting powdered extracts were stored and labeled as D1 W, D2 W, D3 W, D4 W, D5 W, and D6 W.

3.4.3. Cellulose Bioplastics

The general formulation for the cellulose bioplastics comprised glycerol as a standard plasticizer, plant extract, hemp cellulose, and water (see Table 7). For comparison purposes, the control sample C1 served as the negative control, while C4 acted as the positive control. Each sample was magnetically stirred for 60 min at 80 °C. Following this, the mixture was subjected to hot pressing (Dabpress, Shenzhen, China) at 240 °C for 10 min to form the bioplastic films, which were then cut into circular discs with a diameter of 1.791 cm [46]. The films were subsequently dried at room temperature to complete the bioplastic formation. The glycerol content was maintained consistently across all samples.

Table 7. Cellulose bioplastic formulations with plant extract. C1: negative control; C2: conventional plasticizer control; C3: conventional dissolvent control; C4: positive control.

Code	Natural Extract	Hemp Cellulose (g)	NaOH (g)	Glycerol (g)	Water (mL)
D1 W, D2 W, D3 W, D4 W, D5 W, D6 W	1% (0.1 g), 3% (0.3 g), 6% (0.6 g), 10% (1.0 g), 15% (1.5 g)	1	-	1	10
C1	-	1	-	-	10
C2	-	1	-	1	10
C3	-	1	0.6	-	10
C4	-	1	0.6	1	10

3.5. Characterizations of Plant Extract

3.5.1. Fourier-Transform Infrared Spectroscopy of Plant Extract

The Fourier-transform infrared spectroscopy (FTIR) spectrum of each extract was recorded in the infrared region, covering wavelengths from 4000 and 650 cm^{-1} [47]. This method was employed to identify functional groups. A small amount of plant extract was analyzed using the Cary 630 Fourier-transform infrared spectrometer (Agilent Technologies, Stockport, UK) to elucidate the structural characteristics and the intensity of the absorption spectra related to molecular composition or chemical functional groups [48]. The resulting spectral data were then documented.

3.5.2. Phytochemical Analysis

A preliminary qualitative phytochemical analysis was performed to identify secondary metabolites in aqueous extracts from six distinct sources. This study specifically focused on detecting amino acids, proteins, and terpenoids. For this purpose, 100 mg of the powdered plant samples was dissolved in 5 mL of type 1 water (analytical grade). Each extract was then evaluated for the presence of reducing sugars, proteins, amino acids, terpenoids, diterpenes, saponins, glycosides, alkaloids, coumarins, lactones, flavonoids, phenols, and quinones [49].

3.5.3. Thin-Layer Chromatography

The powdered extract was employed for thin-layer chromatography (TLC) profiling. Both the samples and standards were diluted in type 1 water. The prepared aqueous extract was then shaken and filtered to obtain a particle-free solution, which was used for TLC analysis on 20 × 20 cm aluminum sheets coated with silica gel 60 F₂₅₄ [50]. The sample was applied to the plate by a capillary. The plates, loaded with samples, were developed in a chamber containing a solvent mixture with a specific solvent system (see Table 8). TLC was performed on extracts and pure samples of the target compound. After development, the plates were dried and visualized in a UV chamber at 254 nm. Iodine and other revelators

were used, and photographs of the plates were taken. The R_f values of each spot were calculated using Equation (2):

$$R_f \text{ value} = \frac{\text{distance molecule traveled}}{\text{distance solvent traveled}} \quad (2)$$

Table 8. Thin-layer chromatography procedure for the crude extracts with the detailed mobile phase and their proportions.

Crude Extracts	Mobile Phase	Secondary Metabolite	Solvent System
D1 W	n-butanol/acetic acid/water	Amino acid	3:3:1
D2 W	n-butanol/acetic acid/water	Amino acid	3:3:1
D3 W	Toluene/ethyl acetate	Terpenoids	9.3:0.7
D4 W	Hexane/acetone	Terpenoids	2:1
D5 W	Chloroform/ethanol	Terpenoids	10:1
D6 W	Toluene/ethyl acetate	Aliphatic natural product	9.3:0.7

3.6. Characterizations of Bioplastics

3.6.1. Fourier-Transform Infrared Spectroscopy of Bioplastics

The FTIR spectra were used to identify the functional groups of the cellulose bioplastics in the FTIR Cary 630 Fourier-transform infrared spectrometer (Agilent Technologies, Stockport, UK) [51]. The films were placed in a sample holder and scanned in the 4000–600 cm^{-1} range.

3.6.2. Scanning Electron Microscope (SEM)

The surface morphology of the bioplastic film was checked by using a scanning electron microscope (JEOL USA Inc, Peabody, MA, USA). The structure of the cross-section and upper surface was analyzed [52]. The sample was examined under magnifications such as 50 $\times$, 100 $\times$, and 400 $\times$.

3.6.3. Degradation in Water Test

The solubility of the cellulose bioplastics was analyzed by immersing the samples in water [42]. The samples, which were prepared as circular discs with a diameter of 1.791 cm, were initially dried in an oven at 40 °C for two hours. Each sample was weighed to determine its initial mass (M_1) and then placed in a plastic cup containing 100 mL of distilled water. The samples were left at room temperature for 6 h, 1 day, 3 days, 8 days, 21 days, and 35 days. After these time intervals, the samples were dried in an oven (RebelK, RebelK PCD-E9000, Washington, DC, USA) at 40 °C for 24 h. The final samples were weighed for their final weight (M_2). The percentage of solubility in water was calculated using Equation (3):

$$\text{H}_2\text{O Degradation} = \frac{(M_1 - M_2)}{M_1} * 100 \quad (3)$$

3.6.4. Absorption of Water Test

The water absorption by the bioplastics was determined using a slightly modified ASTM D570-98 method [53]. The bioplastic samples, prepared as circular discs with a diameter of 1.791 cm, were initially dried in an oven at 40 °C for 2 h to obtain their dry weight (M_1). The samples were then immersed in 100 mL of distilled water at room temperature for 6 h, 1 day, 3 days, 8 days, 11 days, and 35 days. The bioplastics were retrieved by filtering the water and their final weight (M_2). The absorption of water was found using the following Equation (4):

$$\text{H}_2\text{O Absorption} = \frac{(M_2 - M_1)}{M_1} * 100 \quad (4)$$

3.6.5. Biodegradability Test

The biodegradation of the cellulose bioplastics was assessed using the soil burial method [53]. The samples, prepared as circular discs with a diameter of 1.8 cm, were initially weighed (M_1) using a digital balance. The samples were buried in garden soil and placed in plastic cups. They were maintained in these cups for 3, 8, 21, and 35 days, with the soil moisture and temperature controlled to mimic natural conditions: the temperatures ranged from ambient conditions at night to 37 °C during the day. After the designated periods, the samples were retrieved from the soil, cleaned of residual soil, and dried in an oven at 37 °C for 24 h. The final weight (M_2) was then recorded, and the percentage of biodegradation was calculated using Equation (5):

$$\text{Degradation in Soil} = \frac{(M_1 - M_2)}{M_1} * 100 \quad (5)$$

3.6.6. Column Chromatography Fractionation

The D1 W plant extract was subjected to silica gel column chromatography to isolate phytoconstituents of interest [47]. The elution systems employed included butanol, acetic acid, and water. A concentrated aqueous extract of 30 mg was fractionated using column chromatography on silica gel (60–120 mesh) [54]. The fractions were collected and subjected to further analysis.

3.6.7. Metabolomic Profile

Data acquisition was performed by Waters model Acquity I-Class Ultra Performance Liquid Chromatography (Waters, Milford, MA, USA) coupled to Tandem Mass Spectrometry (UPLC-MS/MS) equipment for LC modules coupled to a XEVO G2-XS QTOF quadrupole time-of-flight mass spectrometer. Chromatographic separation was achieved using a Waters ACQUITY UPLC CSH C18 column (50 × 3 mm), with a 1.7 µm particle size. The mobile phase was 0.1% formic acid in water (Solvent A) and acetonitrile (Solvent B). The flow rate and sample injection volume were set at 0.5 mL/min and 5 µL, respectively. The solvent gradient program was set as follows: 0.0–0.5 min, 1–99% B; 0.5–8.0 min, 99–100% B; 8.0–9.0 min, 100% B; 9.00–9.01 min, 100–1% B; and 9.01–10 min, 1% B. Electrospray ionization (ESI) was performed in positive mode for MS data acquisitions. Unified tune parameters were set for the MS and fast data-dependent acquisition (Fast DDA) experiments: the capillary voltage was kept at 0.5 kV; the source temperature was 120 °C; the desolvation temperature was 450 °C; the nitrogen desolvation gas flow rate was 900 L h^{−1}; the cone gas flow rate was kept at 0 L h^{−1}, and argon was used as the collision gas. Both MS and Fast DDA data were acquired in resolution mode. The data were calibrated using an external reference (Waters Corporation, Milford, MA, USA, LockSpray™) by the constant infusion of a leucine-enkephalin solution (1 ng/L) at a 10 µL/min flow rate. Data acquisition was performed using the MassLynx V4.1 software (Waters, Milford, MA, USA). In Fast-DDA mode, the mass range of the full MS and MS2 scans were m/z 100–1200 and 50–1200 Da, respectively.

3.6.8. Statistical Analysis

After the samples were characterized, the results of the physiochemical properties were analyzed, tabulated, and represented graphically. These results were analyzed statistically using Microsoft Excel 2019.

4. Conclusions

In this study, we investigated the feasibility of utilizing plant extract-based solvents for the partial dissolution of cellulose, a crucial step in the production of bioplastics. Our

findings indicate that these plant extract-based solvents, derived from renewable plant sources, demonstrated notable effectiveness in partially dissolving hemp cellulose for the preparation of bioplastic, suggesting their potential utility in sustainable bioplastic production. This research successfully demonstrated the synthesis of bioplastics using a dissolvent obtained from plant extract-based solvents. The resulting bioplastics exhibited promising chemical and physical properties, making them viable candidates for a range of applications. This suggests a significant step forward in the development of environmentally friendly alternatives to traditional petroleum-based plastics for single-use plastic products. Future studies should aim to optimize these aspects to make plant extract-based solvents even more practical and competitive in large-scale bioplastic production.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/molecules30132752/s1>. Figure S1. SEM images of the bioplastic films of quinoa (D1 W), guayaba (D2 W), palo santo (D3 W), Ivory Coast almond (D4 W), cacalosuchil (D5 W), and soya (D6 W) at the highest concentration of 15 wt.% at different magnifications; Figure S2. pH change in degradation in water test at 0 h, 6 h, 1 day, 3 days, 8 days, 21 days, and 35 days of the bioplastics films with plant extract and conventional plasticizers.

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References

1. Kong, U.; Rawi, N.F.M.; Tay, G.S. The Potential Applications of Reinforced Bioplastics in Various Industries: A Review. *Polymers* **2023**, *15*, 2399. [CrossRef] [PubMed]
2. Ramadan, M.; Cooper, B.; Posnack, N.G. Bisphenols and phthalates: Plastic chemical exposures can contribute to adverse cardiovascular health outcomes. *Birth Defects Res.* **2020**, *112*, 1362–1385. [CrossRef] [PubMed]
3. Kumar, R.; Verma, A.; Shome, A.; Sinha, R.; Sinha, S.; Jha, P.K.; Kumar, R.; Kumar, P.; Shubham; Das, S.; et al. Impacts of Plastic Pollution on Ecosystem Services, Sustainable Development Goals, and Need to Focus on Circular Economy and Policy Interventions. *Sustainability* **2021**, *13*, 9963. [CrossRef]
4. Rosenboom, J.-G.; Langer, R.; Traverso, G. Bioplastics for a circular economy. *Nat. Rev. Mater.* **2022**, *7*, 117–137. [CrossRef]
5. Narancic, T.; Cerrone, F.; Beagan, N.; O’connor, K.E. Recent Advances in Bioplastics: Application and Biodegradation. *Polymers* **2020**, *12*, 920. [CrossRef]
6. Wang, B.-T.; Hu, S.; Yu, X.-Y.; Jin, L.; Zhu, Y.-J.; Jin, F.-J. Studies of Cellulose and Starch Utilization and the Regulatory Mechanisms of Related Enzymes in Fungi. *Polymers* **2020**, *12*, 530. [CrossRef]

7. Acharya, S.; Liyanage, S.; Parajuli, P.; Rumi, S.S.; Shamsheina, J.L.; Abidi, N. Utilization of Cellulose to Its Full Potential: A Review on Cellulose Dissolution, Regeneration, and Applications. *Polymers* **2021**, *13*, 4344. [CrossRef]
8. Zhou, J.; Zhang, L. Solubility of Cellulose in NaOH/Urea Aqueous Solution. *Polym. J.* **2000**, *32*, 866–870. [CrossRef]
9. Wu, C.; Li, J.; Zhang, Y.; Li, X.; Wang, S.; Li, D. Cellulose Dissolution, Modification, and the Derived Hydrogel: A Review. *ChemSusChem* **2023**, *16*, e202300518. [CrossRef]
10. Gondhalekar, S.C.; Pawar, P.J.; Dhumal, S.S.; Thakre, S.S. Mechanism of xanthation reaction in viscose process. *Cellulose* **2019**, *26*, 1595–1604. [CrossRef]
11. Przypis, M.; Wawoczny, A.; Gillner, D. Biomass and Cellulose Dissolution—The Important Issue in Renewable Materials Treatment. *Appl. Sci.* **2023**, *13*, 1055. [CrossRef]
12. Hidayati, S.; Zulferiyenni; Maulidia, U.; Satyajaya, W.; Hadi, S. Effect of glycerol concentration and carboxy methyl cellulose on biodegradable film characteristics of seaweed waste. *Heliyon* **2021**, *7*, e07799. [CrossRef] [PubMed]
13. Ben, Z.Y.; Samsudin, H.; Yhaya, M.F. Glycerol: Its properties, polymer synthesis, and applications in starch based films. *Eur. Polym. J.* **2022**, *175*, 111377. [CrossRef]
14. Wang, H.; Li, H.; Lee, C.K.; Nanyan, N.S.M.; Tay, G.S. A systematic review on utilization of biodiesel-derived crude glycerol in sustainable polymers preparation. *Int. J. Biol. Macromol.* **2024**, *261*, 129536. [CrossRef]
15. Benitez, J.J.; Florido-Moreno, P.; Porras-Vázquez, J.M.; Tedeschi, G.; Athanassiou, A.; Heredia-Guerrero, J.A.; Guzman-Puyol, S. Transparent, plasticized cellulose-glycerol bioplastics for food packaging applications. *Int. J. Biol. Macromol.* **2024**, *273*, 132956. [CrossRef]
16. Santana, R.F.; Bonomo, R.C.F.; Gandolfi, O.R.R.; Rodrigues, L.B.; Santos, L.S.; Pires, A.C.d.S.; de Oliveira, C.P.; Fontan, R.d.C.I.; Veloso, C.M. Characterization of starch-based bioplastics from jackfruit seed plasticized with glycerol. *J. Food Sci. Technol.* **2018**, *55*, 278–286. [CrossRef]
17. Fauziyah, S.N.; Mubarak, A.S.; Pujiastuti, D.Y. Application of glycerol on bioplastic based carrageenan waste cellulose on biodegradability and mechanical properties bioplastic. *IOP Conf. Ser. Earth Environ. Sci.* **2021**, *679*, 012005. [CrossRef]
18. Vieira, M.G.A.; da Silva, M.A.; Dos Santos, L.O.; Beppu, M.M. Natural-based plasticizers and biopolymer films: A review. *Eur. Polym. J.* **2011**, *47*, 254–263. [CrossRef]
19. Amarakoon, M.; Alenezi, H.; Homer-Vanniasinkam, S.; Edirisinghe, M. Environmental Impact of Polymer Fiber Manufacture. *Macromol. Mater. Eng.* **2022**, *307*, 2200356. [CrossRef]
20. Norgren, M.; Costa, C.; Alves, L.; Eivazi, A.; Dahlström, C.; Svanedal, I.; Edlund, H.; Medronho, B. Perspectives on the Lindman Hypothesis and Cellulose Interactions. *Molecules* **2023**, *28*, 4216. [CrossRef]
21. Kihlman, M.; Medronho, B.F.; Romano, A.L.; Germgård, U.; Lindman, B. Cellulose Dissolution in an Alkali Based Solvent: Influence of Additives and Pretreatments. *J. Braz. Chem. Soc.* **2013**, *24*, 295–303. [CrossRef]
22. Lawson, L.; Degenstein, L.M.; Bates, B.; Chute, W.; King, D.; Dolez, P.I. Cellulose Textiles from Hemp Biomass: Opportunities and Challenges. *Sustainability* **2022**, *14*, 15337. [CrossRef]
23. Zamora-Mendoza, L.; Guamba, E.; Miño, K.; Romero, M.P.; Levoyer, A.; Alvarez-Barreto, J.F.; Machado, A.; Alexis, F. Antimicrobial Properties of Plant Fibers. *Molecules* **2022**, *27*, 7999. [CrossRef] [PubMed]
24. Gul, R.; Jan, S.U.; Faridullah, S.; Sherani, S.; Jahan, N. Preliminary Phytochemical Screening, Quantitative Analysis of Alkaloids, and Antioxidant Activity of Crude Plant Extracts from Ephedra intermedia Indigenous to Balochistan. *Sci. World J.* **2017**, *2017*, 5873648. [CrossRef]
25. Fukunaga, Y.; Ogawa, R.; Homma, A.; Okada, T. Thin Layer Chromatography-Freeze Surface-Enhanced Raman Spectroscopy: A Powerful Tool for Monitoring Synthetic Reactions. *Chem. A Eur. J.* **2023**, *29*, e202300829. [CrossRef]
26. Dar, A.; Ahmad, M.N.; Samin, G.; Jahangir, M.M.; Rehman, R.; Anwar, J.; Al-thagafi, Z.T.; Meraf, Z.; Jaber, M.M.; Dar, A.A. Separation of Amino Acids, Dyes, and Pigments Using Novel Pressurized Circular TLC Assembly for Secure Medical Imaging Applications. *Int. J. Anal. Chem.* **2023**, *2023*, 9914633. [CrossRef]
27. Harish, D.; Solanki, N. Chromatographic methods used for characterization of boswellic acids. *MOJ Drug Des. Dev. Ther.* **2018**, *2*, 195–201. [CrossRef]
28. Skalicka-Woźniak, K.; Garrard, I. Counter-current chromatography for the separation of terpenoids: A comprehensive review with respect to the solvent systems employed. *Phytochem. Rev.* **2014**, *13*, 547–572. [CrossRef]
29. Nasser, A.L.M.; Mazzolin, L.P.; Hiruma-Lima, C.A.; Santos, L.S.; Eberlin, M.N.; Brito, A.R.M.d.S.; Vilegas, W. Preparative Droplet Counter-Current Chromatography for the Separation of the New Nor-Seco-Triterpene and Pentacyclic Triterpenoids from *Qualea Parviflora*. *Chromatographia* **2006**, *64*, 695–699. [CrossRef]
30. Salim, M.F.H.; Nugraha, I.M.A.D.P.; Adilla, F.; Yanti, L.P.D. Chromatography Profiles of Terpenoid Compounds in The Extract of Sambiloto (*Andrographis paniculata*) Herb from Various Solvents. *Walisono J. Chem.* **2021**, *4*, 74–80. [CrossRef]
31. Heftmann, E. *Chromatography: Fundamentals and Applications of Chromatography and Related Differential Migration Methods*, 6th ed.; Elsevier: Amsterdam, The Netherlands, 2004.
32. Annadurai, P. Extraction and Isolation of bioactive compounds from Lantana camara leaves by column Chromatographic techniques. *Res. J. Pharm. Technol.* **2021**, *14*, 1607–1611. [CrossRef]

33. Yap, C.W. PaDEL-descriptor: An open source software to calculate molecular descriptors and fingerprints. *J. Comput. Chem.* **2011**, *32*, 1466–1474. [CrossRef] [PubMed]
34. Deng, C.; Deng, Y.; Wang, B.; Yang, X. Gas chromatography–mass spectrometry method for determination of phenylalanine and tyrosine in neonatal blood spots. *J. Chromatogr. B* **2002**, *780*, 407–413. [CrossRef]
35. Portella, E.H.; Romanzini, D.; Angrizani, C.C.; Amico, S.C.; Zattera, A.J. Influence of Stacking Sequence on the Mechanical and Dynamic Mechanical Properties of Cotton/Glass Fiber Reinforced Polyester Composites. *Mater. Res.* **2016**, *19*, 542–547. [CrossRef]
36. Hanry, E.L.; Redzwan, N.F.M.; Badeges, N.F.A.K.; Surugau, N. Characterization of biofilms developed from alginate extracted from *Padina* sp. incorporated with calcium chloride (CaCl₂). *J. Phys. Conf. Ser.* **2022**, *2314*, 012022. [CrossRef]
37. Oh, S.Y.; Yoo, D.I.; Shin, Y.; Kim, H.C.; Kim, H.Y.; Chung, Y.S.; Park, W.H.; Youk, J.H. Crystalline structure analysis of cellulose treated with sodium hydroxide and carbon dioxide by means of X-ray diffraction and FTIR spectroscopy. *Carbohydr. Res.* **2005**, *340*, 2376–2391. [CrossRef]
38. Krishnamurthy, A.; Amritkumar, P. Synthesis and characterization of eco-friendly bioplastic from low-cost plant resources. *SN Appl. Sci.* **2019**, *1*, 1432. [CrossRef]
39. Phosri, S.; Kunjiek, T.; Mukkhakang, C.; Suebthep, S.; Sinsup, W.; Phornsirigarn, S.; Charoeythornkhajhornchai, P. Biodegradability of bioplastic blown film in a marine environment. *Front. Mar. Sci.* **2022**, *9*, 917397. [CrossRef]
40. Oliveros R, D.A.; Machado, R.A.; Mora, J.R. Quantitative structure–property relationship analysis of the spectrochemical series by employing electronic descriptors from DFT calculations. *Mol. Phys.* **2022**, *120*, e2040629. [CrossRef]
41. García-Jacas, C.R.; Marrero-Ponce, Y.; Cortés-Guzmán, F.; Suárez-Lezcano, J.; Martínez-Rios, F.O.; García-González, L.A.; Pupo-Meriño, M.; Martínez-Mayorga, K. Enhancing Acute Oral Toxicity Predictions by using Consensus Modeling and Algebraic Form-Based 0D-to-2D Molecular Encodes. *Chem. Res. Toxicol.* **2019**, *32*, 1178–1192. [CrossRef]
42. Steven, S.; Fauza, A.N.; Mardiyati, Y.; Santosa, S.P.; Shoimah, S.M. Facile Preparation of Cellulose Bioplastic from *Cladophora* sp. Algae via Hydrogel Method. *Polymers* **2022**, *14*, 4699. [CrossRef] [PubMed]
43. Sayakulu, N.F.; Soloi, S. The Effect of Sodium Hydroxide (NaOH) Concentration on Oil Palm Empty Fruit Bunch (OPEFB) Cellulose Yield. *J. Phys. Conf. Ser.* **2022**, *2314*, 012017. [CrossRef]
44. Abubakar, A.R.; Haque, M. Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes. *J. Pharm. Bioallied Sci.* **2020**, *12*, 1–10. [CrossRef] [PubMed]
45. Nortjie, E.; Basitere, M.; Moyo, D.; Nyamukamba, P. Extraction Methods, Quantitative and Qualitative Phytochemical Screening of Medicinal Plants for Antimicrobial Textiles: A Review. *Plants* **2022**, *11*, 2011. [CrossRef]
46. Wang, Q.; Cai, J.; Zhang, L.; Xu, M.; Cheng, H.; Han, C.C.; Kuga, S.; Xiao, J.; Xiao, R. A bioplastic with high strength constructed from a cellulose hydrogel by changing the aggregated structure. *J. Mater. Chem. A* **2013**, *1*, 6678–6686. [CrossRef]
47. Kumar, S.S.; Manoj, P.; Giridhar, P. Fourier transform infrared spectroscopy (FTIR) analysis, chlorophyll content and antioxidant properties of native and defatted foliage of green leafy vegetables. *J. Food Sci. Technol.* **2015**, *52*, 8131–8139. [CrossRef]
48. Zamora-Mendoza, L.; Vispo, S.N.; De Lima, L.; Mora, J.R.; Machado, A.; Alexis, F. Hydrogel for the Controlled Delivery of Bioactive Components from Extracts of *Eupatorium glutinosum* Lam. Leaves. *Molecules* **2023**, *28*, 1591. [CrossRef]
49. Barthwal, R.; Mahar, R. Exploring the Significance, Extraction, and Characterization of Plant-Derived Secondary Metabolites in Complex Mixtures. *Metabolites* **2024**, *14*, 119. [CrossRef]
50. Kowalska, T.; Sajewicz, M. Thin-Layer Chromatography (TLC) in the Screening of Botanicals—Its Versatile Potential and Selected Applications. *Molecules* **2022**, *27*, 6607. [CrossRef]
51. Fathanah, U.; Lubis, M.R.; Nasution, F.; Masyawi, M.S. Characterization of bioplastic based from cassava crisp home industrial waste incorporated with chitosan and liquid smoke. *IOP Conf. Ser. Mater. Sci. Eng.* **2018**, *334*, 012073. [CrossRef]
52. Wang, Q.; Guo, J.; Xu, D.; Cai, J.; Qiu, Y.; Ren, J.; Zhang, L. Facile construction of cellulose/montmorillonite nanocomposite biobased plastics with flame retardant and gas barrier properties. *Cellulose* **2015**, *22*, 3799–3810. [CrossRef]
53. Shafqat, A.; Al-Zaqri, N.; Tahir, A.; Alsalmeh, A. Synthesis and characterization of starch based bioplastics using varying plant-based ingredients, plasticizers and natural fillers. *Saudi J. Biol. Sci.* **2021**, *28*, 1739–1749. [CrossRef] [PubMed]
54. Gini, T.; Jothi, G.J. Column chromatography and HPLC analysis of phenolic compounds in the fractions of *Salvinia molesta* mitchell. *Egypt. J. Basic Appl. Sci.* **2018**, *5*, 197–203. [CrossRef]

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Article

Functionalisation of Lignin-Derived Diols for the Synthesis of Thermoplastic Polyurethanes and Polyester Resins

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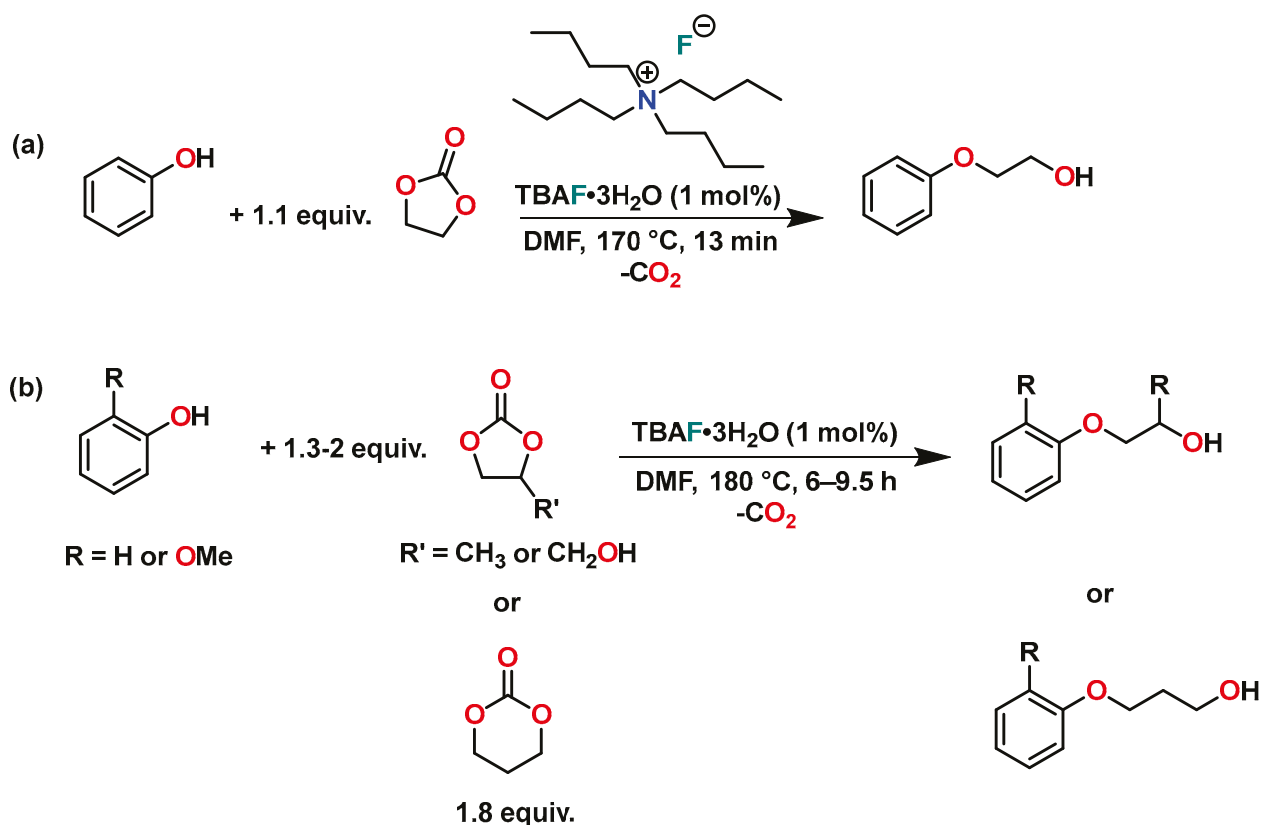
Abstract: The functionalisation of lignin-derived phenolics (guaiacol, 4-propylguaiacol, eugenol, isoeugenol, phenol, *m*-cresol, catechol, syringol, syringaldehyde, and vanillin) for the synthesis of thermoplastic polyurethanes (PUs) and polyester (PE) resins is herein described. Diols were synthesised from phenolics in a one-step reaction using either glycerol carbonate or ethylene carbonate as a greener, solvent-free synthetic route. Nine of the diols were selected for the synthesis of PUs, and two of the diols were used for the synthesis of PE resins, with their physical and thermal properties characterised. Analysis of the PUs by differential scanning calorimetry (DSC) confirmed their amorphous nature, while thermogravimetric analysis (TGA) suggested improved thermal stability for all PUs with the addition of an alkyl or aldehyde substituent on the benzene ring regardless of the diisocyanate used. However, lower PU thermal stabilities were observed with the use of an aliphatic diisocyanate over an aromatic diisocyanate in the absence of an additional substituent. Analysis of the PEs by DSC also confirmed that the clear resins were all amorphous, and gel permeation chromatography (GPC) revealed significantly higher molecular weights and dispersities when an aliphatic diacid was utilised over an aromatic diacid.

Keywords: lignin; biobased; phenols; glycerol carbonate; ethylene carbonate; polyurethanes; polyesters

1. Introduction

Approximately 70 million tonnes of lignin is discarded from the pulp and paper industry each year [1]. As a result, extensive efforts towards lignin valorisation to synthesise new materials has been undertaken [2]. Lignin, one of the main constituents of lignocellulose, is a complex 3-D amorphous macromolecule that is made of various phenylpropanoid units [3]. These units consist of monomeric phenolics, particularly *p*-hydroxyphenyl (H), guaiacyl (G), or syringyl (S) subunits, where their composition varies depending on the lignin source [4]. One common method of lignin valorisation is the depolymerisation of lignin into monomers, usually through methods such as pyrolysis. Due to the 3D network structure of lignin, the majority of the products from lignin pyrolysis are phenolics, such as guaiacol, 4-propylguaiacol, eugenol, catechol, phenol, and syringol, to name a few [5]. These phenolics can be further tailored to specialty polymers derived from biomass sources [6].

Organic carbonates are of interest as greener candidates for alkylating agents, particularly replacing carcinogenic compounds like alkyl halides. They offer the advantage of being non-toxic, biodegradable, and good solvents due to their high boiling point [7]. Organic carbonates such as ethylene carbonate, propylene carbonate, and glycerol carbonate convert aromatic hydroxyl (OH) groups to hydroxy alkylated chains to increase compound reactivity for further functionalisation [8]. A study by Kao et al. utilised ethylene carbonate to synthesise a range of diols derived from phenolic compounds with the release of CO₂ as a by-product [9]. They reacted their specific phenolics with 1.1 equivalents of ethylene carbonate, 1 mol% of tetrabutylammonium fluoride (TBAF•3H₂O) at 170 °C under nitrogen (N₂) and dimethyl formamide (DMF) as a solvent from anywhere between 13 min and 21 h to produce the hydroxy alkylated compounds in yields ranging from 66 to 98% (Scheme 1a). They also tested the scope of other cyclic carbonates, including propylene carbonate, glycerol carbonate, and trimethylene carbonate, on phenol or guaiacol to give the target compounds in moderate to high yields (Scheme 1b) [9].



Scheme 1. (a) Reaction of phenol with ethylene carbonate and TBAF•3H₂O and (b) reaction of phenol or guaiacol with propylene carbonate, glycerol carbonate or trimethylene carbonate from Kao et al. [9].

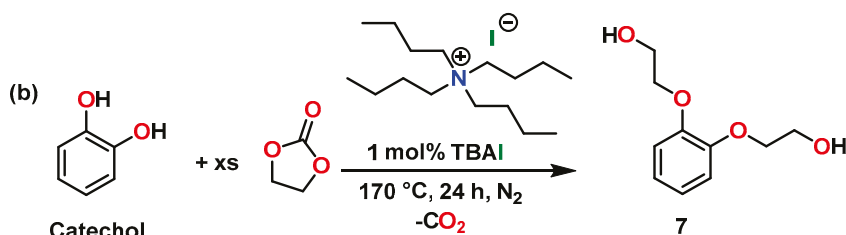
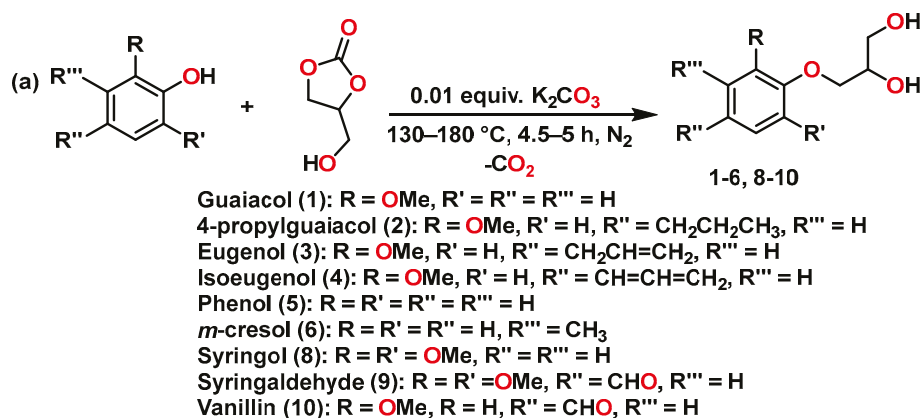
Among these organic carbonates, glycerol carbonate is of interest since it can be derived from the reaction between glycerol and CO₂ [10]. Glycerol is formed as a by-product in the formation of biodiesel and there are demands to repurpose captured CO₂ from the atmosphere, making both of them available in high abundance [11,12]. Previous work in our group utilised a one-step reaction to form organic diols with glycerol carbonate acting as both the reagent and the solvent with either eugenol or vanillin [13]. Potassium carbonate (K₂CO₃) was used as a base, and the reaction was heated between 130 and 180 °C for anywhere between 5 and 24 h. While the eugenol-based diol was successfully synthesised,

the vanillin-based diol was found to undergo a self-condensation polymerisation and was not investigated further for polymer production.

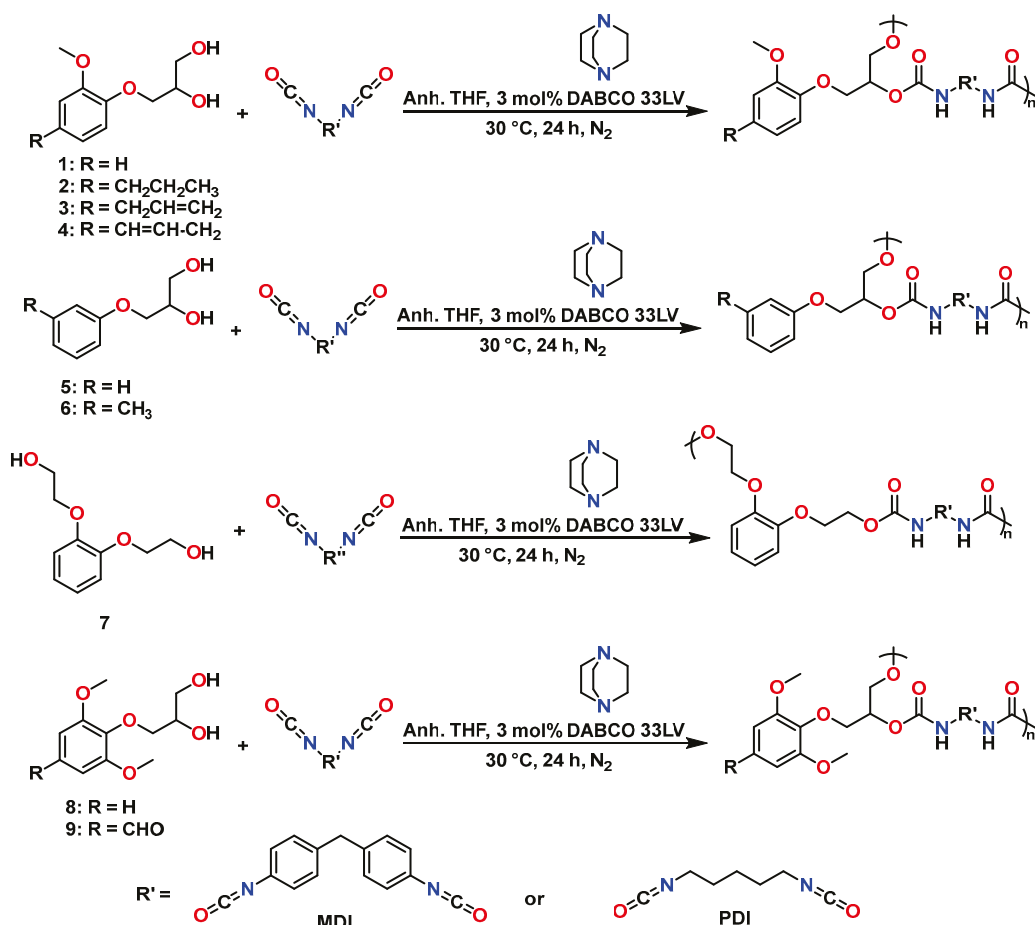
Polyester (PE) resins are known to have great low-temperature fixability, meaning they can easily be repaired at lower temperatures. They have been utilised as binder resins in toner production as they can allow for more controlled particle sizes and molecular weights [14]. Due the successful synthesis of the eugenol-based diol, Kosalka et al. selected it as the monomer of choice for PE co-block synthesis [13]. A PE block co-polymer was synthesised from eugenol using either succinic (SA), terephthalic (TA) or isophthalic acid (IA) as the diacid source under argon (Ar) for 48 to 72 h at high temperatures to be used for industrial scale toner production. It was found that only SA was successful at polymerising the eugenol diol, and the resulting polymer was found to have a high dispersity ($\bar{D}$) that readily crosslinked upon further heating, making it unsuitable for applications in toners [13].

In addition to PEs, polyurethanes (PUs) are used in diverse markets such as packaging, automotive, construction, and electronics, to name a few [15]. All PUs can be classified as either thermoplastics or thermosets. Thermoplastic PUs can be synthesised from diols or polyols (such as hydroxyl terminated PEs) and diisocyanates with a functionality of two, forming compounds that not only have improved solubility but are also thermo-reprocessable, meaning they soften when heated while retaining their shape when cooled, allowing for them to be remelted and reused. Thermoset PUs are synthesised from the reaction between polyols and polyisocyanates with higher functionalities, which leads to crosslinked materials that are unable to be thermo-reprocessed or insoluble [16]. Thermoplastic PUs are also known to have high ductility and excellent biocompatibility, allowing them to be used in structures that require flexibility such as wearable devices [17], making them more desirable than thermoset PUs. Previous work in our group explored the synthesis of thermoplastic PUs from diols derived from 4-hydroxybenzaldehyde, vanillin or syringaldehyde [18]. The diols were synthesised in a two-step reaction from either a hydroxy alkylation with ethylene carbonate followed by a reduction with sodium borohydride (NaBH_4), or a reduction with NaBH_4 followed by Williamson–Ether synthesis with 1,3-dibromopropane. The PUs were then synthesised by reacting these diols with either methylene diphenyl diisocyanate (MDI) or pentamethylene diisocyanate (PDI) as the diisocyanate under N_2 for 24 h at ambient temperatures, resulting in polymers with moderate to high molecular weights and good thermal stabilities [18]. However, abiding by the 12 principles of green chemistry [19], the two-step synthesis previously outlined results in diols with more derivatives produced, resulting in lower atom economy, while also requiring more energy for synthesis due to the multiple steps employed.

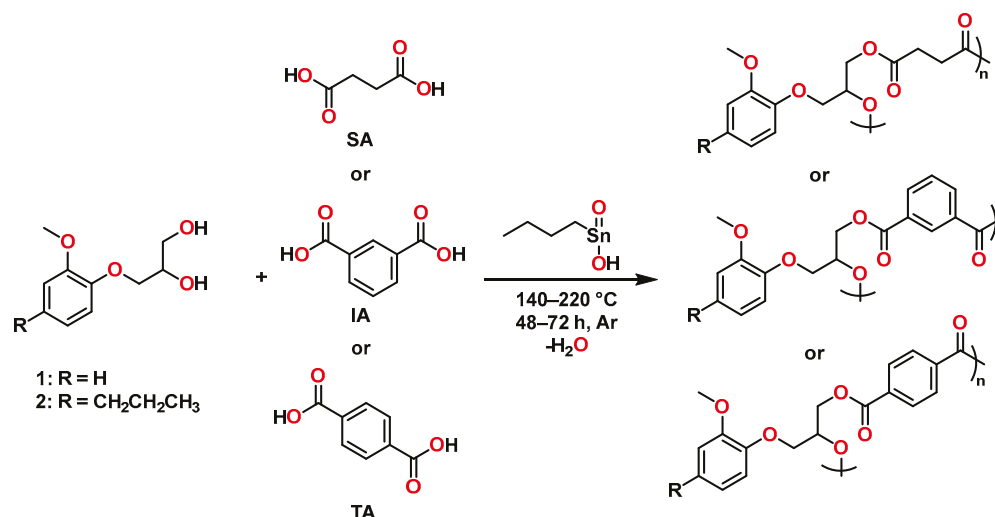
The overall objective of this study is to synthesise various diols in a one-step solvent-free process from lignin-derived monomers using either glycerol carbonate or ethylene carbonate (Scheme 2) for the synthesis of PUs and PE resins for potential biobased applications. Lignin-based phenolics were reacted with glycerol carbonate, while catechol, which contains two phenolic OH groups, was reacted with ethylene carbonate to obtain the desired diols. These diols then underwent a one-step addition polymerisation with one of two diisocyanates (MDI or PDI) to synthesise PUs (Scheme 3). Select diols also underwent a one-step condensation polymerisation with one of three carboxylic acids (SA, IA or TA) to prepare high-molecular-weight PE resins (Scheme 4).



Scheme 2. (a) Reaction of phenolics with glycerol carbonate. (b) Reaction of catechol with ethylene carbonate.



Scheme 3. Reaction of lignin-based diols with diisocyanates to prepare PUs.



Scheme 4. Reaction of lignin-based diols with diacids to yield PE resins.

2. Results and Discussion

2.1. Lignin-Based Diol Synthesis

The synthesis of diols 1–6 and 8–10 followed a literature procedure from Kosalka et al. [13] (Scheme 2a). The respective phenol and glycerol carbonate were added in a 1:1 ratio, and K₂CO₃ was added in catalytic amounts as the base for the initial deprotonation step. The mixture was then refluxed over a temperature range of 130 to 180 °C for either 4.5 or 5 h under N₂. Once cooled to room temperature, the diol initially formed as an oil and sometimes solidified to a waxy solid after leaving it to further dry. Purification was performed using either recrystallisation techniques or flash column chromatography to yield the diol as either a powder, an oil, or a glassy solid. The synthesis of diol 7 followed a modified procedure from Kao et al. [9] (Scheme 2b). Ethylene carbonate was added in a slight excess of catechol along with catalytic amounts of tetrabutylammonium iodide (TBAI) as a phase transfer catalyst in the absence of DMF. The reaction proceeded under N₂ for 24 h at 170 °C, after which diol 7 was obtained after recrystallising it with diethyl ether (Et₂O). The resulting diols and their respective yields are shown in Figure 1.

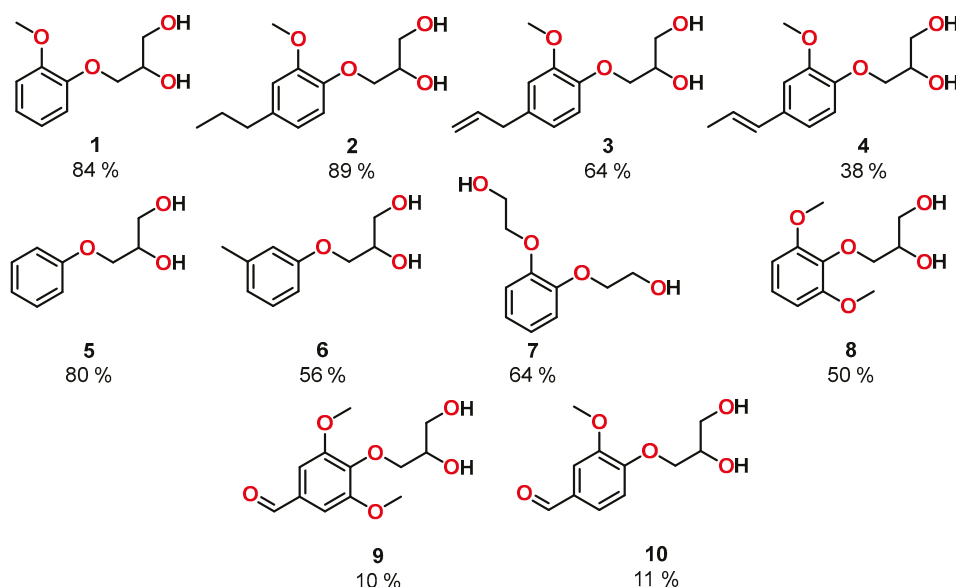


Figure 1. Structure and yields of diols 1–10.

Analysis by ^1H and ^{13}C NMR for diols 1–3, 5–8 and 10 matched previously reported literature data [9,11,13,20–22]. For diol 4, analysis by ^1H NMR revealed a multiplet at ~ 6.9 ppm for the aromatic protons, a doublet at ~ 6.3 ppm for the trans alkene proton, a doublet at ~ 6.1 ppm for the cis alkene proton, two multiplets at ~ 4.1 and ~ 3.7 ppm for the newly formed alkylated protons, a singlet at ~ 3.8 ppm for the methoxy protons, two broad singlets at ~ 2.3 and ~ 3.0 ppm for the aliphatic OH protons, and a doublet of doublets at ~ 1.9 ppm for the methyl protons (Figure S1). Confirmation of the protons interacting with each other was performed using 2D Correlation Spectroscopy (COSY) NMR, justifying the previously made ^1H NMR assignments (Figure S2). Analysis by ^{13}C NMR showed the ipso aromatic carbons at ~ 132 , ~ 147 and ~ 150 ppm containing the alkene, ether, and methoxy substituents, respectively, with the vinyl carbons appeared at ~ 124 and ~ 130 ppm. The protonated aromatic carbon signals were revealed at ~ 109 , ~ 115 and ~ 119 ppm. The newly synthesised alkylated carbons were observed at ~ 64 , ~ 70 and ~ 72 ppm, similar to what was seen with diols 6–8. The signals at ~ 56 and ~ 18 ppm represents the methoxy and methyl carbons, respectively (Figure S3). Further confirmation of the successful synthesis of diol 9 was observed by high resolution mass spectrometry (HRMS) using the direct analysis in real time (DART) method, where the calculated and found masses closely matched (Figure S4).

For diol 9, the ^1H NMR spectra revealed a singlet resonance at ~ 9.9 ppm for the aldehyde signal, a doublet at ~ 7.1 ppm for the aromatic protons, multiplets at ~ 3.8 , ~ 4.0 and ~ 4.1 ppm for the newly formed alkylated protons, a doublet at ~ 3.9 ppm for the methoxy protons, and a broad singlet at ~ 2.7 ppm for the aliphatic OH protons (Figure S5). These assignments were confirmed by 2D COSY NMR (Figure S6). The ^{13}C NMR spectra showed signals at ~ 191 ppm for the aldehyde carbon, the ipso aromatic signals at ~ 132 , ~ 142 , and ~ 153 ppm containing the aldehyde, ether, and methoxy substituents, respectively, the protonated aromatic carbons at ~ 107 ppm, the new alkylated carbons at ~ 63 , ~ 71 and ~ 76 ppm, and the methoxy carbons at ~ 56 ppm (Figure S7). Analysis by DART-HRMS showed that the calculated and found masses closely matched, further confirming the successful synthesis of diol 9 (Figure S8).

The presence of unreacted starting material and, in some cases, a potential second product contributed to the lower yields of select diols, particularly those that required purification by column chromatography to isolate the target diol. This second product, which was observed by TLC, was suspected to be a disubstituted phenolic, which was previously proposed by Tabanelli et al. due to the intramolecular rearrangement of the glycerol carbonate moiety, particularly the carbonate group over the free OH group [7]. In addition, diol 10, which was previously synthesised by Kosalka et al. [13], was found to undergo self-polymerisation when reacted for 12 h, whereas we found that the same diol remained stable after a 4.5 h reaction. The shorter time frame might allow for the aldehyde group to remain stable and not trigger polymerisation compared to longer times. Although the synthesis of diol 10 was successful, the yield was extremely low compared to the other diols apart from diol 9, which was derived from syringaldehyde. In both cases, TLC analysis suggested that most of the product remained as unreacted starting material, which may be attributed to the aldehyde group. Since the reaction time cannot be increased at the risk of self-polymerisation, diol 10 was not investigated further for polymer synthesis.

2.2. PU Synthesis and Characterisation

Diols 1–9 underwent a polyaddition reaction to synthesise PUs with a diisocyanate, 1,4-diazabicyclo[2.2.2]octane (DABCO) 33LV as the catalyst, and anhydrous tetrahydrofuran (THF) as the solvent (Scheme 3) [18]. The diisocyanates selected were MDI and PDI, which

were added in an equimolar amount of the diol, along with catalytic amounts of DABCO 33LV in dry THF. The reactions proceeded for 24 h under N₂ after were then purified by precipitation into cold methanol (MeOH) for the MDI-containing PUs or hexanes for the PDI-containing PUs. After precipitation, the resulting PUs were either powders, clear gel-like materials, or gummy resins in low to high yields. The gel-like materials appear as a sticky substance that resembles a gel while the gummy resins collectively joined together in the shape of chewing gum during precipitation. The characterisation data is summarised in Table 1.

Table 1. Characterisation data of PUs.

Sample	Diisocyanate	Appearance	Yield (%)	T _g (°C)	T _d (5%) (°C)	T _d (50%) (°C)
PU-1a	MDI	Gummy	94	53.2	185	348
PU-1b	PDI	Gel	75	6.6	209	279
PU-2a	MDI	Powder	33	-	215	331
PU-2b	PDI	Gel	27	100.9	140	319
PU-3a	MDI	Gummy	52	81.5	224	335
PU-3b	PDI	Powder	44	-	212	350
PU-4a	MDI	Powder	94	242.6	155	338
PU-4b	PDI	Gummy	61	112.2	145	316
PU-5a	MDI	Gummy	58	111.7	222	339
PU-5b	PDI	Gummy	66	11.8	206	285
PU-6a	MDI	Gummy	12	-	142	336
PU-6b	PDI	Gel	17	116.7	124	317
PU-7a	MDI	Powder	98	109.9	284	335
PU-7b	PDI	Gel	33	9.7	193	320
PU-8a	MDI	Gummy	83	115.4	239	337
PU-8b	PDI	Gel	30	−8.3	175	253
PU-9a	MDI	Gummy	92	127.9	143	362
PU-9b	PDI	Powder	57	68.4	176	335

Analysis by ¹H NMR for the MDI-containing PUs showed additional signals at ~7.1 and ~7.3 ppm for the aromatic MDI backbone as well as an additional signal at ~3.8 ppm for the methylene bridge in MDI (Figures S9, S13, S17, S20, S24, S28, S32, S36 and S40). For the PDI-containing PUs, ¹H NMR spectroscopy showed additional signals between ~2.9 and ~3.1 ppm and ~1.2 to 1.4 ppm for the aliphatic PDI backbone (Figures S11, S15, S19, S22, S26, S30, S34, S38 and S42). However, due to the poor solubility of PU-3b, the ¹H NMR spectrum could not be integrated, although the location of the signals confirmed its structure. Analysis by ¹³C NMR for the PUs did not always reveal all carbon signals due to poor solubility in dimethyl sulfoxide (DMSO). However, new signals were typically observed at ~120 and ~129 ppm for the aromatic MDI carbons (Figures S10, S14, S18, S21, S25, S29, S33, S37 and S41) and ~24 and ~30 ppm for the aliphatic PDI carbons (Figures S12, S16, S23, S27, S31, S35 and S39). Additionally, the poor solubility of PU-3b and PU-9b resulted in no ¹³C NMR spectra being obtained for either PU as both compounds swelled in DMSO-d₆ when heated, although ¹H NMR spectroscopy sufficiently confirmed the target structures. However, due to their

overall poor solubility in organic solvents, GPC analysis could not be performed on these polymers.

Fourier transform infrared spectroscopy (FTIR) analysis confirmed the N-H stretch at $\sim 3300\text{ cm}^{-1}$ and the C=O urethane stretches at $\sim 1700\text{ cm}^{-1}$ for the MDI-containing PUs (Figure S43) and at $\sim 1690\text{ cm}^{-1}$ for the PDI-containing PUs (Figure S44). C-H stretching was also observed for both sets of PUs at ~ 2800 and $\sim 2900\text{ cm}^{-1}$, although the stretches were sharper in the PDI-containing PUs due to the aliphatic carbons present.

Analysis by DSC revealed higher glass transition temperatures (T_g) for the MDI-containing PUs than the PDI-containing PUs (Figure 2a,b) due to the rigidity of the aromatic rings in MDI compared to the flexibility of the aliphatic carbons in PDI. T_g s were not observed for PU-2a, PU-6a and PU-3b, which may be due to them having very weak T_g s that are not visible by DSC. While no noticeable trend was observed for the MDI containing PUs in terms of their T_g , it was found that the PDI containing PUs derived from diols **1**, **5**, **7** and **8**, which all lacked a substituent, exhibited the lowest T_g s compared to the remaining PDI PUs. This suggests that the addition of a substituent on the benzene ring improves the thermal properties of the PUs, particularly when the polymer exhibits a high degree of flexibility.

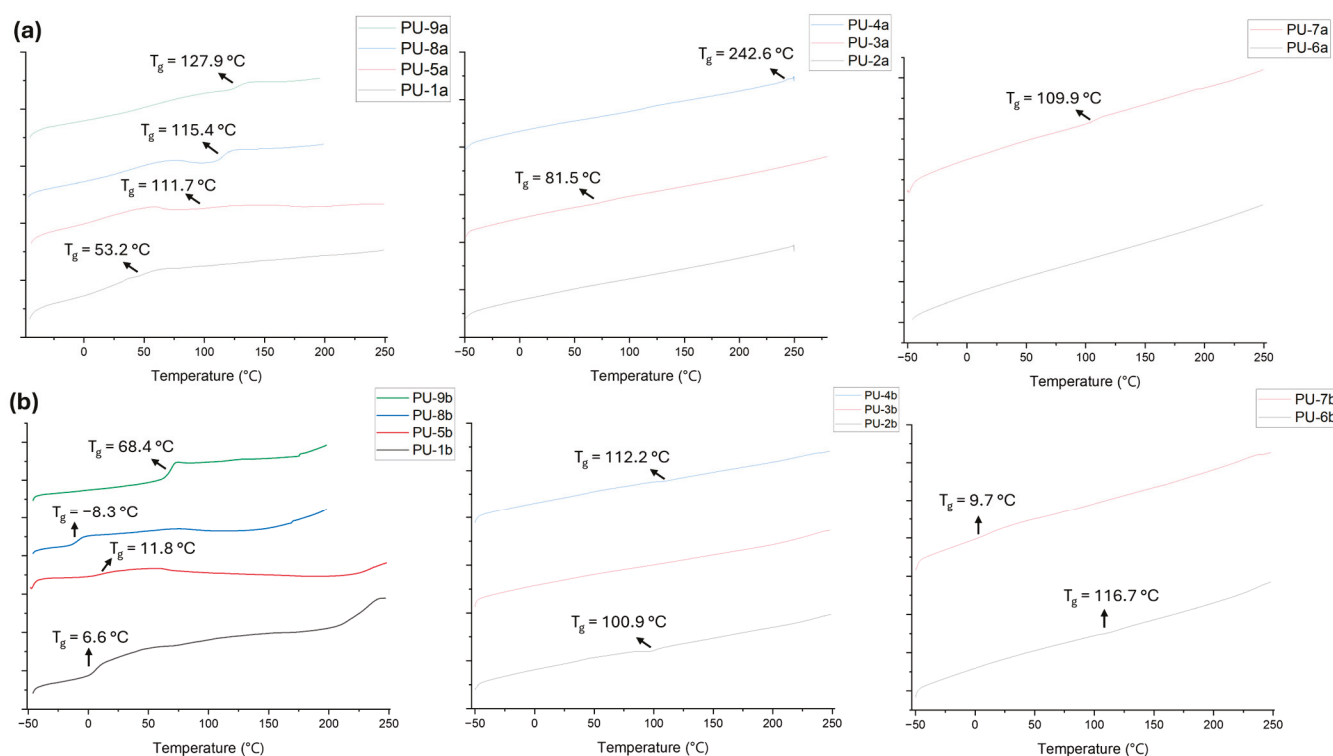


Figure 2. DSC traces of (a) PU-1a to PU-9a and (b) PU-1b to PU-9b (exotherm down).

Analysis by TGA on the thermal decomposition (T_d) of these polymers revealed a similar trend. For the PUs synthesised from diols **1**, **5** and **8**, the MDI-containing polymers demonstrated significantly higher thermal stability at 50% weight loss compared to the PDI-containing polymers by ~ 50 to $80\text{ }^{\circ}\text{C}$ (Figure 3). However, the addition of an alkyl, aldehyde or even an additional hydroxyalkyl substituent on the benzene ring showed similar thermal stabilities regardless of the diisocyanate used, suggesting that substituents improve the overall thermal stability of the PUs. This could lead to these particular monomers also being used in polyol formulations for PU foams to potentially improve their mechanical properties, which was also previously explored in our group [18]. The derivative weight changes for all PUs exhibited two to three decomposition temperatures

(Figures S45 and S46), representing the breakdown of the aromatic rings and urethane linkages [18,23].

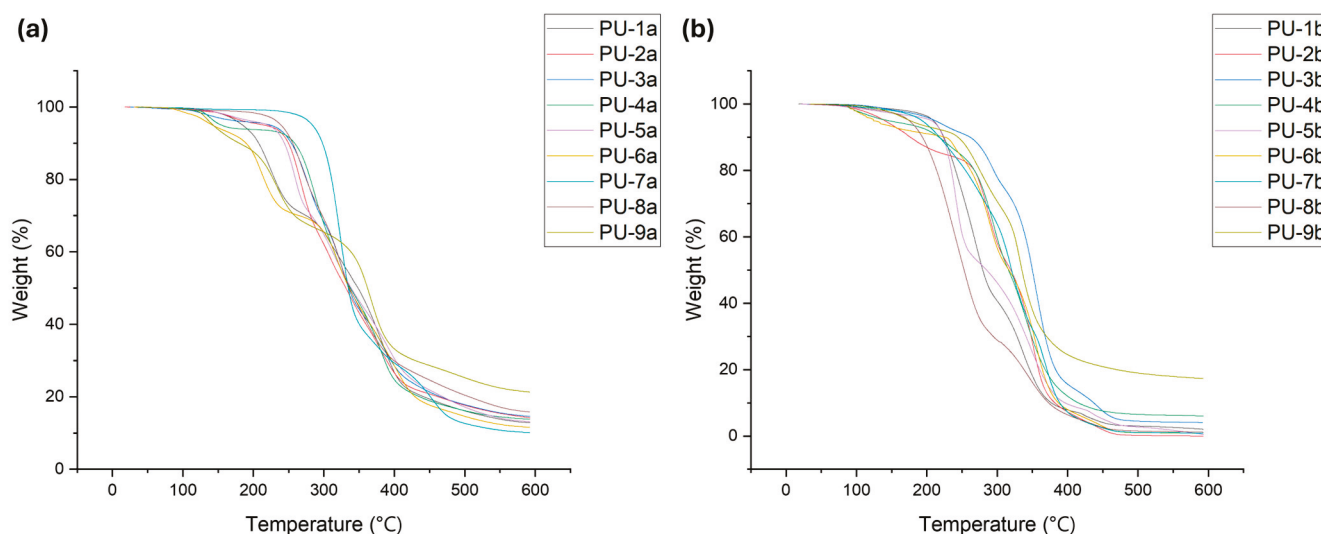


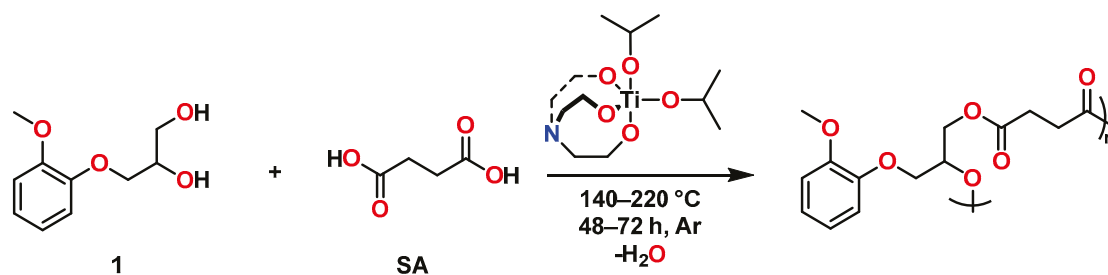
Figure 3. TGA curves of (a) PU-1a to PU-9a and (b) PU-1b to PU-9b.

2.3. PE Synthesis and Characterisation

Diols **1** and **2** were selected for polycondensation reactions since they could be obtained in yields greater than 80% to compare against the PE resin previously synthesised from diol **3** in our group (Scheme 4) [13]. Carboxylic acids utilised in these reactions were SA, TA and IA. Diol **1** was successfully polymerised with SA (PE-1a), TA (PE-1b) and IA (PE-1c) to yield a yellow glassy solid. Purification was attempted using toluene and methanol mixtures; however, these procedures proved ineffective.

Analysis by ^1H and ^{13}C NMR confirmed the successful synthesis of all three PE resins. In addition to the signals corresponding to the polymeric aromatic and methoxy regions, signals unique to each carboxylic acid utilised were identified. For PE-1a, ^1H NMR spectra showed a new singlet at ~ 2.7 ppm, representing the protons coming from SA (Figure S47), while ^{13}C NMR spectra showed two new signals at ~ 171.8 ppm, representing the C=O groups from SA (Figure S48). For PE-1b, the TA aromatic protons were observed as a singlet at ~ 8.0 ppm (Figure S49) while new carbon signals were observed at ~ 165 ppm for the C=O resonance and ~ 129 – 133 ppm for the aromatic carbons of TA (Figure S50). For PE-1c, the IA protons appeared as multiplets at ~ 7.4 , 8.2 , and 8.7 ppm (Figure S51) and the IA carbons appeared at ~ 165 ppm for the C=O resonance and between 125 – 133 ppm for the aromatic carbons (Figure S52). There is also evidence of diol **1** being present in all three PE resins due to using an excess amount to achieve the desired acid value.

The reaction with SA was also repeated using a tetraalkyl titanate catalyst instead of a tin catalyst (Scheme 5). This was carried out to evaluate other options instead of using a tin-based catalyst as tin species are known to have detrimental environmental effects. ^1H NMR analysis showed identical signals to that of PE-1a (Figure S53), suggesting a successful synthesis. Although the reaction was successful, titanium catalysts are susceptible to water, which is the by-product of this reaction. Therefore, if lignin-derived diols requiring higher and longer reaction times are employed, the tin catalyst will continue to be used as the titanium catalyst may degrade.



Scheme 5. Reaction of **1** with SA and a titanium catalyst.

Diol **2** was also reacted with all three diacid species (Scheme 4), where SA was found to be the only diacid successfully yielding the desired PE resin. This is potentially due to the heating requirements being too intense when employing either TA or IA. This result was also previously observed when diol **3** was reacted with these same carboxylic acids, suggesting the alkyl substituent plays a role in the overall reaction mechanism [13]. The resulting polymer (PE-2) crosslinks before any useful material is generated, thereby making NMR analysis inconclusive. As this work was focused on producing bio-based materials suitable for toner production, PE resins tailored with specific thermal properties such as softening point (T_s) and T_g 's were desired. Table 2 summarises the properties of PE-1a, PE-1b, PE-1c and PE-2 in comparison to PE-3.

Table 2. Characterisation data of PE resins.

Sample	Diacid	Yield (%)	T_g (°C)	T_s (°C)	M_w (g/mol)	M_n (g/mol)	$\bar{D}$ (M_w/M_n)
PE-1a	SA	91	17.7	100.1	26,440	3730	7.1
PE-1b	TA	94	41.8	102.9	3900	2790	1.4
PE-1c	IA	89	49.5	104.1	5070	2530	2.0
PE-2	SA	88	20.1	<100	94,500	2840	33.3
PE-3 [13]	SA	90	29.9	<100	132,900	1850	71.9

A patent by Shinji et al. specified that the ideal T_s for PE toners should be between 100 °C and 160 °C and the ideal T_g should be between 50 °C and 70 °C [24]. PE-1a, PE-1b and PE-1c, all polymerised from diol **1**, displayed similar T_s 's slightly higher than 100 °C, whereas PE-2 was found to have a T_s less than 100 °C, which was also observed for PE-3. The DSC data showed significantly different results depending on the diacid source. Compounds PE-1b and PE-1c are similar in nature relative to PE-1a. The difference in T_g between either PE-1b or PE-1c and PE-1a is almost 30 °C, which can be attributed to the flexibility of the organic acids. PE-1a has flexibility due mainly to the ethyl chain, where it can bend and rotate, which is also why PE-2 and PE-3 exhibit similar T_g 's, although PE-3 has a higher T_g by ~10 °C. PE-1b and PE-1c are much more rigid due to the aromatic species and as such, require higher energy inputs relative to PE-1a to initiate intramolecular rotation. The GPC data showed that PE-1a had a higher molecular weight (M_w) and dispersity ($\bar{D}$) compared to PE-1b and PE-1c, which may be due to the use of SA compared to IA or TA. The M_w and $\bar{D}$ of PE-2 were significantly higher than the PEs synthesised from diol **1**, although it was still not as high as PE-3. Unfortunately, PE-2 does not solidify and is gummy, limiting its applications. This high M_w may indicate that, as the polymer reaction conditions are intensified, the polymer chains grow to the point where they can no longer remain in the melt and precipitates. This may explain why the material solidifies after several days of growth. Due to its low T_g and liquid state at room temperature, PE-2 is not suitable for toner production. However, PE-1b and PE-1c show some promise

in applications for toner production due to their relatively lower molecular weights and dispersities and their higher T_g 's and T_s 's that are within or close to the ideal range of toner properties specified by Shinji et al. [24].

3. Materials and Methods

3.1. Materials

Glycerol carbonate was supplied by Huntsman Corporation in Houston, TX, USA. Ethylene carbonate was purchased from Alfa Aesar in Ward Hill, MA, USA. MDI, PDI, and Fascat 4100 was supplied by ChemPoint in Bellevue, MA, USA. All other materials, reagents and catalysts were purchased from MilliporeSigma Canada in Oakville, ON, Canada. Anhydrous tetrahydrofuran (THF) was dried using an SPS solvent purification solvent. All other reagents and solvents were reagent grade and used as received. All reactions carried out in a N_2 or Ar environment were performed using standard Schlenk line techniques.

3.1.1. General Synthesis of Diols 1–6 and 8–10

For exact reaction conditions, please refer to the Supplementary Materials. In a 50 mL round bottom flask, the phenol was added along with glycerol carbonate (1 equiv.) and K_2CO_3 (0.01 equiv.). The mixture was refluxed under N_2 at 130 °C for 30 min, and the temperature was increased by 10 °C every 30 min until a temperature of 180 °C was reached. At 180 °C, the reaction proceeded for an additional 2–2.5 h. The material was extracted using H_2O or brine and ethyl acetate (EtOAc) (~50 mL $\times$ 3), and the organic layer was dried using sodium sulfate (Na_2SO_4). The solvent was decanted and removed via rotary evaporation and the product was purified by either recrystallisation or flash column chromatography.

3.1.2. Synthesis of Diol 7

In a 100 mL 3 neck round bottom flask, catechol (1.101 g, 0.010 mol), ethylene carbonate (2.113 g, 0.024 mol), and TBAI (1 mol%, 0.037 g) were added together, and the reaction was heated at 170 °C for 24 h under N_2 . After cooling to RT, the material hardened into an orange amber solid. The product was then recrystallised using Et_2O to yield a brown powder. Yield: 64% (1.260 g).

3.2. General Preparation of PUs

The diol (0.50–1.00 g) was added to a 100 mL 3-neck round bottom flask along with anhydrous THF (10–20 mL) and DABCO 33LV (3 mol% based on diol amount). MDI or PDI (1 equiv.) was added under N_2 , and the reaction stirred at 30 °C for 24 h, after which the mixture was precipitated or washed in cold MeOH (MDI) or hexanes (PDI) to remove any unreacted diol or diisocyanate. Yield: PU-1a: 94% (2.22 g); PU-1b: 75% (1.38 g); PU-2a: 33% (0.67 g); PU-2b: 27% (0.40 g); PU-3a: 52% (1.06 g); PU-3b: 44% (0.73 g); PU-4a: 94% (1.92 g); PU-4b: 61% (1.01 g); PU-5a: 58% (1.45 g); PU-5b: 66% (1.26 g); PU-6a: 12% (0.28 g); PU-6b: 17% (0.31 g); PU-7a: 98% (2.22 g); PU-7b: 33% (0.58 g); PU-8a: 83% (1.73 g); PU-8b: 30% (0.50 g); PU-9a: 92% (0.91 g); PU-9b: 57% (0.46 g).

3.3. General Preparation of PEs

3.3.1. Preparation of PE-1a, PE-1b and PE-1c

In a 500 mL 3-neck flask, 1 (50.00 g, 0.25 mol) and SA (25.32 g, 0.21 mol) were added, resulting in a 1.15:1 ratio of diol to acid, along with Fascat 4100 (0.50 g, 0.002 mol). Independent experiments were also performed using TA (35.62 g, 0.21 mol) and IA (35.62 g, 0.21 mol) along with 1 (50.00 g, 0.25 mol). Polymerisations involving TA and IA followed

an almost identical protocol. All polymerisations were carried out under an Ar blanket. Using a mechanical stirring rod, the reagents in the ratios mentioned above were heated to 140 °C resulting in a melt. The temperature was kept at 140 °C for 1 h. After the 1 h mark, the temperature was raised by 5 °C/h until a temperature of 180 °C was reached. The reaction then was left overnight to react (11–13 h). The reaction temperature was then increased from 180 °C to 185 °C and allowed to run for 1 h. The temperature was then increased 5 °C/h until 200 °C was reached and then allowed to react again overnight (14–16 h). The temperature was then increased by 5 °C/h until a temperature of 240 °C was reached. The reaction was then allowed to run for 14–30 h. Experiments involving TA and IA were left to run at 240 °C until the acid value was ~15 mg KOH/g. The resulting material was clear with a yellowish tint. Yield: PE-1a: 91% (70.75 g); PE-1b: 94% (81.07 g); PE-1c: 89% (76.76 g).

3.3.2. Evaluation of the Catalyst for PE-1a

The polymerisation of 1 with SA was repeated in the presence of a tetraalkyl titanate catalyst, (titanium (IV) triethanolaminate isopropoxide (8.2 Ti wt%)) (0.50 g, 0.002 mol). The resulting material appeared identical to the material depicted above.

3.3.3. Preparation of PE-2

In a 500 mL 3-neck flask, 2 (50.00 g, 0.21 mol) along with SA (20.89 g, 0.18 mol), resulting in a 1.15:1 ratio of diol to acid and Fascat 4100 (0.50 g, 0.002 mol). Polymerisations were carried out under an Ar blanket. Using a magnetic stirring rod, the reagents were heated to 140 °C resulting in a melt. The temperature was kept at 140 °C for 1 h, after which the temperature was raised by 5 °C/h until a temperature of 180 °C was reached. The reaction was left overnight to react (11–13 h). The colour of the material did not noticeably change. The reaction temperature was increased from 180 °C to 185 °C and allowed to run for 1 h. The temperature was then increased by 5 °C/h until 200 °C was reached. It was then allowed to react again overnight (14–16 h). The colour of the material had not changed after the second day of reacting. The temperature was then increased by 5 °C/h until a temperature of 220 °C was reached. The reaction was then allowed to run for 14–30 h. In all attempts, at some point after 48 h, the material in the melt precipitated out and was found to be insoluble in any solvent and incapable of melting. Prior to solidification, the resulting material was clear with a yellowish tint and was quite soft. Yield: 88% (57.47 g).

3.4. Characterisation Techniques

Flash column chromatography was carried out on silica gel (Silica Gel 60, 40–63 µm, EMD, MilliporeSigma, Oakville, ON, Canada). Reactions and chromatographic purifications were monitored by thin layer chromatography (TLC). Silica-coated aluminium plates (Alugram Sil G/UV₂₅₄, Macherey-Nagel, Düren, Germany) were used for TLC tests. Plates were visualised with UV light. ¹H, ¹³C and 2D NMR were carried out on a 400 MHz Bruker Avance II spectrometer (Billerica, MA, USA) using CDCl₃ or DMSO-d₆ as the solvent with the TOPSPIN software package. ¹H spectra (400 MHz) and ¹³C spectra (101 MHz) were referenced against the internal standard tetramethylsilane (TMS). NMR data were reported as: chemical shift δ (ppm) relative to the solvent, multiplicity, number of protons, and coupling constant *J* (Hz). Mass spectrometry data was obtained on a JEOL AccuTOF 4G (DART) mass spectrometer (Tokyo, Japan) at the Advanced Instrumentation for Molecular Structure Mass Spectrometry Laboratory at the University of Toronto with the JEOL msAxel software package. FTIR analysis was performed on an Agilent Cary 630 FTIR Spectrometer (Santa Clara, CA, USA) using the MicroLab PC software package. For the PU's, DSC was completed on a DSC Q20 TA Instrument (New Castle, DE, USA) with a flow rate of 40 mL/min at a heating rate of 10 °C/min in an aluminium Tzero pan under a N₂

atmosphere with ~10 mg of sample with the Universal Analysis software package. The third cycle was used for analysis of the sample. TGA was conducted on a PerkinElmer TGA 4000 (Waltham, MA, USA) under a N₂ atmosphere with a flow rate of 40 mL/min and a heating rate of 10 °C/min in a Platinum HT pan with ~10 mg of sample with the TRIOS software package. For the PEs, DSC, GPC and TGA analysis were performed by the analytical staff at Xerox Research Centre of Canada (XRCC). Acid value measurements were performed by titration with a 0.1 N solution of potassium hydroxide in ethanol and 3–5 drops of phenolphthalein as an indicator. Samples were first dissolved in THF, and the volume of titrant used to cause a colour change was recorded to determine the acid value. Polymerisations were considered complete once the acid value of the material was between 15 and 20.

4. Conclusions

Ten diols were synthesised in a one-step reaction using either glycerol carbonate or ethylene carbonate and further reacted to synthesise PUs and PEs. While most of the diols were obtained in moderate to high yields, only the diols containing an aldehyde group exhibited yields lower than 20%. The synthesis of eighteen PUs was confirmed by NMR and FTIR while the synthesis of four PEs was confirmed by NMR. DSC analysis of the PUs and PEs was consistent with the degree of rigidity or flexibility of the diisocyanate or diacid used. TGA analysis of the PUs demonstrated lower thermal stabilities of the PDI-containing PUs compared to the MDI-containing PUs synthesised from diols **1**, **5** and **8**, which only contained methoxy or hydroxy alkylated substituents. Consistent thermal stabilities were observed for the remaining PUs with alkyl or aldehyde substituents regardless of the diisocyanate used, offering their respective starting monomers more promising applications in PU foams. GPC analysis of the PEs showed the highest molecular weights and dispersities when SA was used as a diacid, and the *T_g* values were relatively similar to each other. These results demonstrated the most potential for PE-1b and PE-1c, both derived from diol **1** and aromatic diacids TA or IA, as better candidates for PE-based toners.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/molecules30122604/s1>, Complete synthesis and characterisation details of diols **1–10**; Figures S1–S4: ¹H NMR, 2D COSY NMR, ¹³C NMR spectra and HRMS (DART) analysis of diol **4**; Figures S5–S8: ¹H NMR, 2D COSY NMR, ¹³C NMR spectra and HRMS (DART) analysis of diol **9**; Figures S9–S18: ¹H and ¹³C NMR spectra of PU-1a, PU-1b, PU-2a, PU-2b and PU-3a; Figure S19: ¹H NMR spectra of PU-3b; Figures S20–S41: ¹H and ¹³C NMR spectra of PU-4a, PU-4b, PU-5a, PU-5b, PU-6a, PU-6b, PU-7a, PU-7b, PU-8a, PU-8b and PU-9a; Figure S42: ¹H NMR spectra of PU-9b; Figure S43: FTIR analysis of PU-1a to PU-9a; Figure S44: FTIR analysis of PU-1b to PU-9b; Figure S45: Derivative weight change of PU-1a to PU-9a; Figure S46: Derivative weight change of PU-1b to PU-9b; Figures S47–S52: ¹H and ¹³C NMR of PE-1a, PE-1b and PE-1c; Figure S53: ¹H NMR spectra of PE-1a using titanium catalyst.

Author Contributions: Conceptualisation, R.N.C., J.J.S.K., D.A.F. and G.G.S.; methodology, R.N.C., J.J.S.K. and G.G.S.; validation, R.N.C., B.V.F. and U.D.; formal analysis, R.N.C., J.J.S.K., B.V.F. and U.D.; investigation, R.N.C., J.J.S.K., B.V.F. and U.D.; resources, D.A.F. and G.G.S.; data curation, R.N.C., J.J.S.K., B.V.F. and U.D.; writing—original draft preparation, R.N.C.; writing—review and editing, R.N.C., D.A.F. and G.G.S.; visualisation, R.N.C. and B.V.F.; supervision, R.N.C., D.A.F. and G.G.S. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

Ar	Argon
CDCl ₃	Deuterated chloroform
COSY	Correlation spectroscopy
Đ	Dispersity
DABCO	1,4-diazabicyclo[2.2.2]octane
DART	Direct analysis in real time
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
DMSO-d ₆	Deuterated dimethyl sulfoxide
DSC	Differential scanning calorimetry
Et ₂ O	Diethyl ether
EtOAc	Ethyl acetate
FTIR	Fourier transform infrared spectroscopy
GPC	Gel permeation chromatography
HRMS	High resolution mass spectrometry
IA	Isophthalic acid
K ₂ CO ₃	Potassium carbonate
MeOH	Methanol
MDI	Methylene diphenyl diisocyanate
Mn	Number average molecular weight
MW	Weight average molecular weight
N ₂	Nitrogen
NaBH ₄	Sodium borohydride
Na ₂ SO ₄	Sodium sulfate
NMR	Nuclear magnetic resonance
PDI	Pentamethylene diisocyanate
PE	Polyester
PU	Polyurethane
SA	Succinic acid
TA	Terephthalic acid
TBAI	Tetrabutylammonium iodide
TBAF•3H ₂ O	Tetrabutylammonium fluoride
TGA	Thermogravimetric analysis
Tg	Glass transition temperature
THF	Tetrahydrofuran
TLC	Thin layer chromatography
Ts	Softening point

References

1. Haile, A.; Gelebo, G.G.; Tesfaye, T.; Mengie, W.; Mebrate, M.A.; Abuhay, A.; Limeneh, D.Y. Pulp and Paper Mill Wastes: Utilizations and Prospects for High Value-Added Biomaterials. *Bioresour. Bioprocess.* **2021**, *8*, 35. [CrossRef] [PubMed]

2. Bertella, S.; Luterbacher, J.S. Lignin Functionalization for the Production of Novel Materials. *Trends Chem.* **2020**, *2*, 440–453. [CrossRef]
3. Zhao, S.; Abu-Omar, M.M. Materials Based on Technical Bulk Lignin. *ACS Sustain. Chem. Eng.* **2021**, *9*, 1477–1493. [CrossRef]
4. Carafa, R.N.; Foucher, D.A.; Sacripante, G.G. Biobased Polymers from Lignocellulosic Sources. *Green Chem. Lett. Rev.* **2023**, *16*, 2153087. [CrossRef]
5. Du, L.; Wang, Z.; Li, S.; Song, W.; Lin, W. A Comparison of Monomeric Phenols Produced from Lignin by Fast Pyrolysis and Hydrothermal Conversions. *Int. J. Chem. React. Eng.* **2013**, *11*, 135–145. [CrossRef]
6. Gandini, A.; Lacerda, T.M. From Monomers to Polymers from Renewable Resources: Recent Advances. *Prog. Polym. Sci.* **2015**, *48*, 1–39. [CrossRef]
7. Tabanelli, T.; Giliberti, C.; Mazzoni, R.; Cucciniello, R.; Cavani, F. An Innovative Synthesis Pathway to Benzodioxanes: The Peculiar Reactivity of Glycerol Carbonate and Catechol. *Green Chem.* **2019**, *21*, 329–338. [CrossRef]
8. Sternberg, J.; Pilla, S. Materials for the Biorefinery: High Bio-Content, Shape Memory Kraft Lignin-Derived Non-Isocyanate Polyurethane Foams Using a Non-Toxic Protocol. *Green Chem.* **2020**, *22*, 6922–6935. [CrossRef]
9. Kao, S.-C.; Lin, Y.-C.; Ryu, I.; Wu, Y.-K. Revisiting Hydroxyalkylation of Phenols with Cyclic Carbonates. *Adv. Synth. Catal.* **2019**, *361*, 3639–3644. [CrossRef]
10. de Caro, P.; Bandres, M.; Urrutigoity, M.; Cecutti, C.; Thiebaud-Roux, S. Recent Progress in Synthesis of Glycerol Carbonate and Evaluation of Its Plasticizing Properties. *Front. Chem.* **2019**, *7*, 308. [CrossRef]
11. Truscetto, A.M.; Gambarotti, C.; Lauria, M.; Auricchio, S.; Leonardi, G.; Shisodia, S.U.; Citterio, A. One-Pot Synthesis of Aryloxypropanediols from Glycerol: Towards Valuable Chemicals from Renewable Sources. *Green Chem.* **2013**, *15*, 625–628. [CrossRef]
12. Galletti, G.; Prete, P.; Vanzini, S.; Cucciniello, R.; Fasolini, A.; De Maron, J.; Cavani, F.; Tabanelli, T. Glycerol Carbonate as a Versatile Alkylating Agent for the Synthesis of β -Aryloxy Alcohols. *ACS Sustain. Chem. Eng.* **2022**, *10*, 10922–10933. [CrossRef]
13. Kosalka, J.J.S.; Sacripante, G.G.; Lough, A.J.; Foucher, D.A.; Gossage, R.A. Biomass Utilisation Strategies for Applications in Novel Polymer and Polymer Resin Production. *Polym. From Renew. Resour.* **2017**, *8*, 1–10. [CrossRef]
14. Kinsyo, T.; Nakanishi, H.; Hirai, K.; Noda, H.; Takikawa, T.; Yahiro, S. Development of Polyester Resin Particles for Toner with a Controlled Particle Size Distribution and Shape. *Polym. J.* **2017**, *49*, 593–600. [CrossRef]
15. Alinejad, M.; Henry, C.; Nikafshar, S.; Gondaliya, A.; Bagheri, S.; Chen, N.; Singh, S.K.; Hodge, D.B.; Nejad, M. Lignin-Based Polyurethanes: Opportunities for Bio-Based Foams, Elastomers, Coatings and Adhesives. *Polymers* **2019**, *11*, 1202. [CrossRef]
16. Wendels, S.; Avérous, L. Biobased Polyurethanes for Biomedical Applications. *Bioact. Mater.* **2021**, *6*, 1083–1106. [CrossRef]
17. Xu, T.; Shen, W.; Lin, X.; Xie, Y.M. Mechanical Properties of Additively Manufactured Thermoplastic Polyurethane (TPU) Material Affected by Various Processing Parameters. *Polymers* **2020**, *12*, 3010. [CrossRef]
18. Carafa, R.N.; Fernandes, B.V.; Repiquet, C.; Rana, S.; Foucher, D.A.; Sacripante, G.G. Functionalization of Phenolic Aldehydes for the Preparation of Sustainable Polyesters and Polyurethanes. *Polymers* **2025**, *17*, 643. [CrossRef]
19. Sheldon, R.A. Metrics of Green Chemistry and Sustainability: Past, Present, and Future. *ACS Sustain. Chem. Eng.* **2018**, *6*, 32–48. [CrossRef]
20. Kosalka, J.J.S. Green Resins from Renewable Resources. Master's Thesis, Ryerson University, Toronto, ON, Canada, 2014.
21. Sayyed, I.A.; Thakur, V.V.; Nikalje, M.D.; Dewkar, G.K.; Kotkar, S.P.; Sudalai, A. Asymmetric Synthesis of Aryloxypropanolamines via OsO₄-Catalyzed Asymmetric Dihydroxylation. *Tetrahedron* **2005**, *61*, 2831–2838. [CrossRef]
22. Bredikhin, A.A.; Bredikhina, Z.A.; Antonovich, O.A.; Zakharychev, D.V.; Krivolapov, D.B. Crystallization Features and Spontaneous Resolution of 3-(2,6-Dimethoxyphenoxy)Propane-1,2-Diol: The Case of Stable Conglomerate and Metastable Solid Solution. *J. Mol. Struct.* **2017**, *1144*, 443–450. [CrossRef]
23. Zhao, C.; Huang, C.; Chen, Q.; Ingram, I.D.V.; Zeng, X.; Ren, T.; Xie, H. Sustainable Aromatic Aliphatic Polyesters and Polyurethanes Prepared from Vanillin-Derived Diols via Green Catalysis. *Polymers* **2020**, *12*, 586. [CrossRef] [PubMed]
24. Shinji, K.; Funato, R.; Tajiri, N.; Ito, H.; Iwasaki, H. Polyester Resin for Toner and Process for Its Production. US4866158, 12 September 1989.

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Article

NaOH/Urea-Compatible Chitosan/Carboxymethylcellulose Films: Orthogonal Optimization of Packaging Properties

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Abstract: Chitosan (CS)-based films have demonstrated significant potential as biodegradable packaging materials, but their suboptimal barrier and mechanical properties limit practical applications. In this study, CS/carboxymethyl cellulose (CMC) composite films were prepared using a NaOH/urea-based alkaline system. Optimal ratios (1.5% CS, 2% CMC, 2.5% NaOH, and 4% urea) were determined through an $L_{16}(4^4)$ orthogonal array design. Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD) analyses confirmed the formation of stable physical crosslinks between CS and CMC via hydrogen bonding. These interactions significantly enhanced mechanical properties (tensile strength: 46.08 MPa; elongation at break: 68%), improved thermal stability (maximum decomposition temperature: 304 °C), and superior barrier properties (water vapor transmission rate: 0.26×10^{-5} g/(m²·h·Pa); oxygen transmission rate: 1.12×10^{-4} g/(m²·s)). NaOH concentration exhibited the most pronounced influence on film performance. The composite film combines inherent biodegradability with excellent functional properties, offering a sustainable alternative to conventional petroleum-based packaging materials.

Keywords: NaOH/urea system; carboxymethyl cellulose; chitosan; orthogonal test; biodegradable film

1. Introduction

Global plastic consumption currently stands at 430 million tons annually and is projected to reach 600 million tons by 2025, with non-biodegradable packaging materials dominating the market and causing severe environmental pollution [1]. This has driven research efforts toward biodegradable alternatives. Among these, chitosan (CS), the second most abundant natural polysaccharide, consists of β -(1→4)-linked 2-amino-2-deoxy-d-glucose units [2,3]. CS exhibits exceptional properties, including non-toxicity, biodegradability, biocompatibility, antimicrobial activity, and film-forming capability [4–6].

However, pure CS films suffer from limitations such as poor barrier performance, low mechanical strength, and inadequate thermal stability. They are soluble only in weakly acidic aqueous solutions [7]. In contrast, carboxymethyl cellulose (CMC), an anionic polysaccharide obtained through cellulose etherification [8], is non-toxic, cost-effective, and biodegradable, with high water solubility and excellent film-forming properties [9,10]. CMC films demonstrate high transparency, enhanced mechanical properties, and remarkable biocompatibility [11,12]. The complementary characteristics of CS and CMC suggest that their combination could overcome individual material limitations. Consequently,

CS/CMC composite films fabricated by incorporating CMC into CS represent a promising eco-friendly packaging solution with wide-ranging applications.

Researchers have made significant progress in CS/CMC composite film research. Chen et al. [13] developed hydrochloric acid-dissolved CS/CMC films incorporated with blackberry anthocyanins and tea polyphenols, which showed enhanced tensile strength, improved elongation at break, and reduced UV–visible light transmittance compared to conventional films. Lan et al. [14] fabricated acetic-acid-based CMC/sodium alginate/CS films demonstrating 95.7% and 93.4% antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*, respectively.

Despite these advances, existing studies have primarily focused on acidic systems [14–17], while alkaline systems (e.g., NaOH/urea) remain relatively unexplored. Alkaline systems offer distinct advantages: they eliminate acidic residues' environmental hazards and enhance intermolecular hydrogen bonding [15]. However, the current understanding is limited to single-material dissolution mechanisms, and the mechanism of intermolecular solubility of two-phase molecules and its regulation on the overall performance have not yet been clarified.

For instance, Hu et al. [16] found that NaOH/urea aqueous solutions effectively dissolve chitosan at $-20\text{ }^{\circ}\text{C}$. In this system, urea disrupts intermolecular hydrogen bonds, preventing chain aggregation. Similarly, Cai et al. [17] demonstrated that alkaline solvents dissolve cellulose at low temperatures. Here, hydroxide ions weaken cellulose's intermolecular hydrogen bonds, while urea disrupts the crystalline structure through water–cellulose interactions.

This study aims to develop CS/CMC composite films via a NaOH/urea system, systematically optimize their packaging-related properties through an orthogonal design, and elucidate the role of hydrogen bonding in enhancing performance.

2. Results and Discussion

2.1. Thickness and Apparent Analysis of (CS/CMC) Composite Film

The thickness of CS/CMC composite films ranged from 0.10 to 0.56 mm (Table 1). These variations are influenced by the concentrations of chitosan, carboxymethyl cellulose (CMC), urea, and sodium hydroxide, as well as the water content during film formation.

Table 1. Thickness (mean $\pm$ SD, mm) and visual appearance of CS, CMC, and CS/CMC composite films.

Run No.	Thickness (mm)	Apparent
CS	0.11 ± 0.01	
CMC	0.11 ± 0.01	
A ₁	0.10 ± 0.01	
A ₂	0.15 ± 0.01	
A ₃	0.19 ± 0.02	
A ₄	0.22 ± 0.01	
A ₅	0.16 ± 0.02	
A ₆	0.24 ± 0.02	
A ₇	0.30 ± 0.02	

Table 1. Cont.

Run No.	Thickness (mm)	Apparent
A ₈	0.36 ± 0.03	
A ₉	0.38 ± 0.03	
A ₁₀	0.32 ± 0.02	
A ₁₁	0.27 ± 0.02	
A ₁₂	0.40 ± 0.03	
A ₁₃	0.33 ± 0.03	
A ₁₄	0.38 ± 0.01	
A ₁₅	0.43 ± 0.02	
A ₁₆	0.46 ± 0.02	

As summarized in Table 1, pure CS films exhibit a yellow color, whereas pure CMC films are white. The composite films retain transparency and homogeneity, with their color transitioning from colorless to light yellow as the CS content increases. This color shift may originate from the amino groups in chitosan, which absorb light in the visible spectrum.

2.2. FTIR Analysis of CS/CMC Composite Films

As shown in Figure 1, the pure CS film exhibited characteristic peaks at 3470 cm^{-1} , corresponding to NH and OH symmetric stretching vibrations as well as inter- and intramolecular hydrogen bonding [18]. The observed hydrogen bonding interactions were consistent with previous reports on chitosan-based materials [19]. Two distinct peaks at 1640 cm^{-1} and 1570 cm^{-1} were identified as amide I (C = O stretching vibration in the -NHCO- group) and amide II (NH bending vibration), respectively [18,19]. The presence of these amide signatures confirmed the existence of acetylated amine structures in the chitosan matrix.

In contrast, the pure CMC film exhibited a broader absorption band at 3600 cm^{-1} , which originated from the stretching of O–H groups and inter- and intramolecular hydrogen bonding [19]. The absorption peak at 1610 cm^{-1} was attributed to the symmetric and asymmetric stretching vibrations of carboxyl groups ($-\text{COO}^-$) [18,19].

No new characteristic bands were generated in the FTIR spectra of the composite membranes, suggesting a physical interaction between CS and CMC. Under alkaline conditions, NaOH decomposed into Na^+ and OH^- , which neutralized $-\text{NH}_3^+$ in CS as well as $-\text{COO}^-$ in CMC, changing the charged state of the molecules and affecting the intermolecular interactions [20]. Meanwhile, urea forms hydrogen bonds with CS and CMC and water, which reduces phase separation and promotes uniform dispersion and interaction of CS and CMC in the system [21]. In the CS/CMC films, the intermolecular hydrogen bonding between CMC and CS resulted in peaks of the O–H and N–H stretching bands between 3470 and 3640 cm^{-1} . The amide I band at 1640 cm^{-1} was red-shifted, and the FTIR peaks of CS ($-\text{NH}$ at 3470 cm^{-1}) and CMC ($-\text{COO}^-$ at 1610 cm^{-1}) were shifted by $5\text{--}50\text{ cm}^{-1}$, indicating that intermolecular hydrogen bonding was formed between the amino group in CS and carboxylic acid group in CMC, which produced a strong physical hydrogen bonding interactions. This indicated that the CS/CMC composite film was successfully synthesized. FTIR analysis was critical to confirm the absence of chemical crosslinking and identify hydrogen bonding interactions between CS and CMC, which

directly correlate with mechanical and barrier properties. Furthermore, CS and CMC formed a stabilizing network structure through hydrogen bonding, which has been verified in studies [20,22,23], and provided a structural basis for the excellent mechanical properties, thermal stability, and barrier properties of the composite film. Similar results have been reported in other studies. For example, Wu et al. [24] found that the hydrogen bonding interactions between CS and CMC significantly improved the mechanical properties of the composite membrane. Physical crosslinking via hydrogen bonding was preferred over chemical methods to retain the biocompatibility and biodegradability of CS/CMC, avoiding potential toxicity from crosslinking agents.

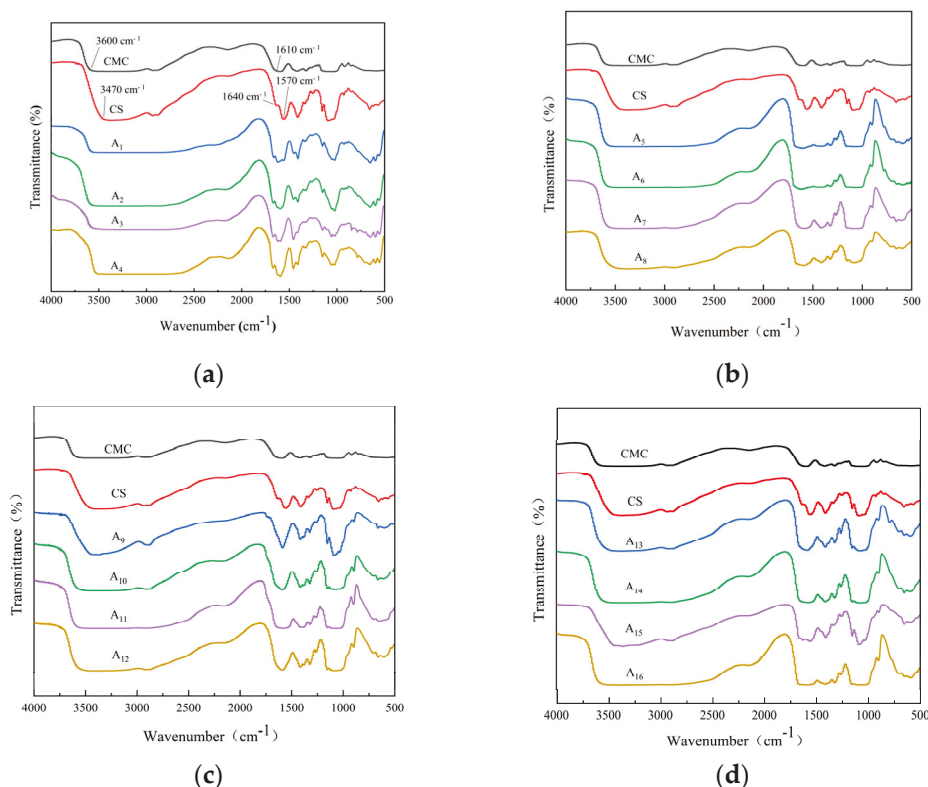


Figure 1. FTIR spectra of chitosan (CS), carboxymethyl cellulose (CMC), and CS/CMC composite films with varying CS additions (a) 1.0%, (b) 1.5%, (c) 2.0%, and (d) 2.5%.

2.3. XRD Analysis of CS/CMC Composite Films

As shown in Figure 2a, the pure CS films exhibited distinct crystallization peaks at $2\theta = 11.38^\circ$, 16.04° , and 20.74° , which were associated with the ordered arrangement of CS molecular chains and intramolecular hydrogen bonding. The pure CMC film showed broad diffraction peaks near $2\theta = 20.66^\circ$, indicating its amorphous nature.

In the CS/CMC composite films (Figure 2b), the characteristic peaks were shifted in position, and the crystallinity was reduced by 22–81% compared to the pure films. The addition of CMC disrupted the orderly arrangement of CS molecular chains, resulting in broader and less intense crystalline peaks. This was attributed to carboxymethyl groups on CMC chains hindering hydrogen bond formation between CS molecules.

The NaOH/urea solvent system was found to significantly influence the crystalline structure. Variations in alkali concentration altered the hydrogen bond network, with excessive NaOH leading to more disordered structures. These observations confirmed that the composite films' crystallinity was strongly dependent on both composition and processing conditions [25].

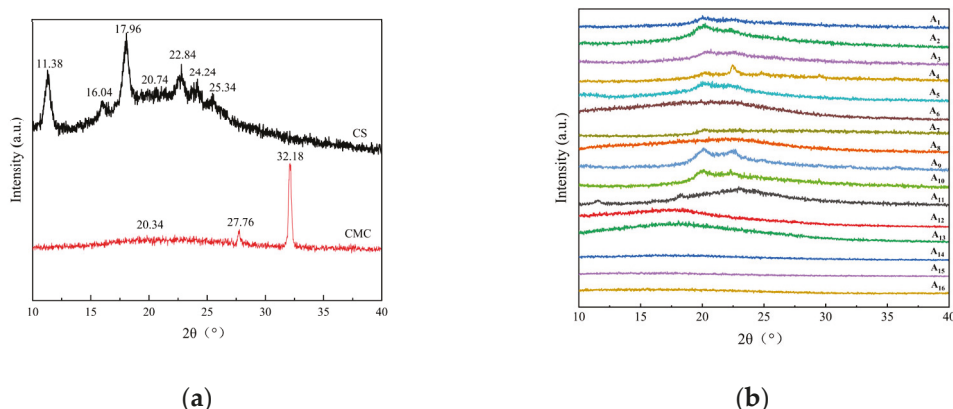


Figure 2. X-ray diffraction (XRD) patterns of (a) pure CS and CMC films, and (b) CS/CMC composite films with varying NaOH concentrations.

2.4. Thermogravimetric Analysis of CS/CMC Composite Films

The thermal stability of the films was evaluated using a thermogravimetric analyzer (STA7200, Hitachi) under a nitrogen atmosphere (flow rate of 20 mL/min). The temperature was increased from 40 to 800 °C at a heating rate of 10 °C/min.

As shown in Figure 3a,b, the thermal decomposition of composite films occurred in four stages. The first stage (50–150 °C) corresponded primarily to volatile loss and water evaporation. The second stage (150–220 °C) involved hydrogen bond breakage between CMC and CS, accompanied by urea decomposition (NH_3 and CO_2 release). The third stage (220–400 °C) is associated with rapid weight loss due to the decomposition of CMC glucose units and CS glucosamine units. The decomposition onset temperature of composite films (A_4 , A_5 , A_7 , etc.) ranged from 245 to 262 °C, 12–18% higher than pure CS (220 °C) and CMC (230 °C). The fourth stage (>400 °C) represents the slow weight loss from residual carbides and ash.

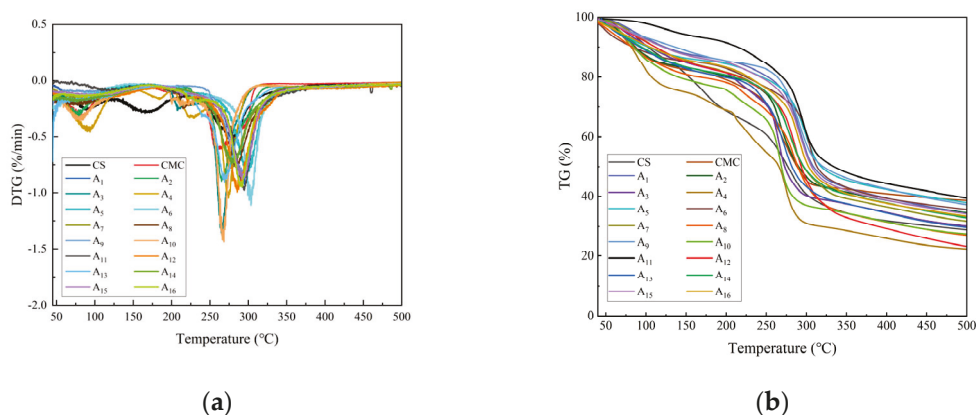


Figure 3. Thermogravimetric analysis of CS, CMC, and CS/CMC composite films: (a) DTG curves, (b) TG curves. Tests were conducted under nitrogen atmosphere at a heating rate of 10 °C/min.

Composite films demonstrated significantly higher decomposition temperatures and enhanced thermal stability compared to pure CS and CMC films. This improvement was attributed to dense hydrogen-bonded networks formed by optimal CS/CMC ratios in the NaOH/urea system, which restricted molecular chain mobility.

2.5. DSC Analysis of CS/CMC Composite Film

As shown in Figure 4a, the pure CS membrane showed two heat absorption peaks at 177 °C and 252 °C, which corresponded to the conformational transition of the molecular

chain and the deacetylation process, respectively [26], and the exothermic peak at 295 °C was attributed to the thermal decomposition reaction of the CS backbone, and this result was in agreement with the results of thermogravimetric analysis. The pure CMC membrane has two heat absorption peaks at 149 °C and 264 °C, as well as an exothermic peak at 324 °C. The cellulose membrane has been characterized by a series of thermal decomposition reactions. The thermal decomposition of cellulose consists of a series of reactions including dehydration, decarboxylation, decarbonylation, and a complex pyrolysis process of glycosidic, C-H, and C-O-C bond breaking [26,27].

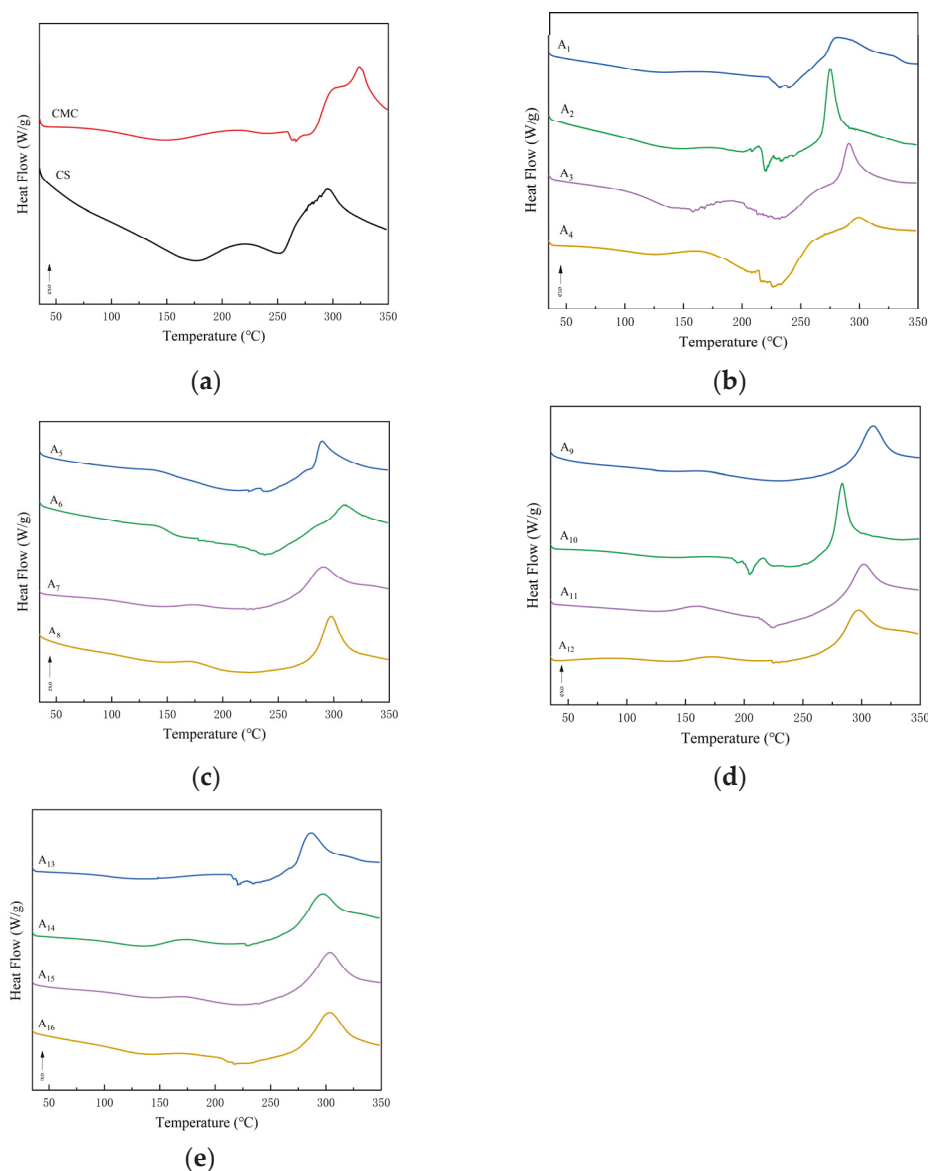


Figure 4. DSC of CS film, CMC film, and CS/CMC composite film with different addition amounts: (a) pure CS and CMC films; (b) composite film with 1.0% CS addition; (c) 1.5% CS addition; (d) 2.0% CS addition; (e) 2.5% CS addition.

As can be seen from Figure 4b–e, the first heat absorption peak of the composite films appeared at 134–160 °C in comparison to the pure CS films, which shifted toward lower temperatures. This phenomenon suggests that the NaOH/urea system breaks some of the intermolecular hydrogen bonds and reduces the initial thermal stability of the molecular chains [28]. The second heat absorption peak temperature was in the range of 194–238 °C, which was lower than that of the pure CS film, and this change reflected the interaction

between CS and CMC, and also confirmed that the incorporation of CMC disrupted the crystalline structure of CS. The exothermic peak temperatures of the composite membranes were in the range of 275–311 °C, fluctuating around the exothermic peak temperature of 295 °C for the pure CS membrane. This indicates that the thermal decomposition behavior of the composite membrane is affected by a combination of factors, including the interaction between CS and CMC, the change in crystallinity, the role of the solvent system, etc., and these factors work together to make the thermal decomposition behavior of the composite membrane change significantly.

In conclusion, the DSC analysis results confirmed the formation of a new composite structure between CS and CMC molecules through the NaOH/urea system. The thermal decomposition behavior of this composite structure retained the characteristics of each component and produced significant synergistic effects. In addition, the results are corroborated by the formation of hydrogen bonds detected by FTIR and the decrease in crystallinity shown by XRD, which provide an important theoretical basis for the synergistic optimization of the mechanical properties, barrier properties, and thermal stability of the composite films.

2.6. Mechanical Properties of CS/CMC Composite Films

The mechanical performance was evaluated through tensile strength and elongation at break measurements (Figure 5). Pure CS film exhibited 31.42 MPa tensile strength and 9.61% elongation, while pure CMC film showed 7.06 MPa and 5.32%. NaOH/urea-prepared composites demonstrated superior properties, with A₇ reaching 64.86% elongation and A₁ achieving 46.08 MPa tensile strength. This enhancement was attributed to optimized CS/CMC mixing and hydrogen-bonded network formation.

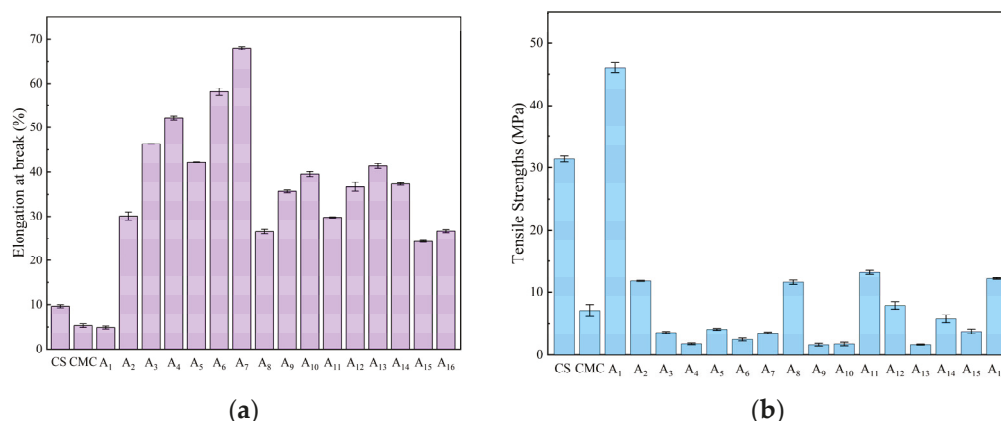


Figure 5. Mechanical properties of CS, CMC, and CS/CMC composite films: (a) elongation at break (%), (b) tensile strength (MPa).

However, maximum tensile strength (46.08 MPa) was achieved only at 1% concentrations of both CS and CMC. Higher CMC concentrations reduced tensile strength significantly (e.g., 11.77 MPa at 1.5% CMC) compared to pure CS films (31.42 MPa). This behavior reflects their distinct roles: CS provides mechanical support through hydrogen bonding sites, while CMC primarily bears external forces. Notably, low concentrations of CMC, NaOH, and urea increased tensile strength, whereas higher concentrations enhanced flexibility (up to 68% elongation) and reduced brittleness. XRD analysis confirmed that reduced crystallinity in composite films led to looser molecular chain arrangement, decreasing rigidity while improving stretchability [29]. This is consistent with the findings of He et al. [30] on cellulose/chitosan composite films prepared in LiOH/urea aqueous solution.

The mechanical properties of CS/CMC composite films prepared under alkaline conditions (A_1 : tensile strength 46.08 MPa, elongation at break 4.88%; A_7 : tensile strength 7.84 MPa, elongation at break 68.00%) were superior to those of Valizadeh et al. [31] prepared in a conventional acetic acid system and close to those of low-density polyethylene (LDPE, tensile strength 8–20 MPa, elongation at break 100–600%). Its high tensile strength is suitable for primary food packaging (e.g., dry product bags), while its high ductility can be applied to flexible packaging (e.g., cling film).

2.7. Moisture Content, Water Absorption, and Solubility of CS/CMC Composite Films

Water content critically influences film flexibility and water resistance in humid conditions. Pure CS films exhibited high water content (Figure 6a) due to strong hydrogen bonding between water molecules and CS functional groups (hydroxyl and amino groups). Composite films showed reduced water content (12.44–31.02%), with A_2 and A_9 representing the highest and lowest values, respectively. This reduction resulted from optimized CS/CMC hydrogen bonding that limited water absorption.

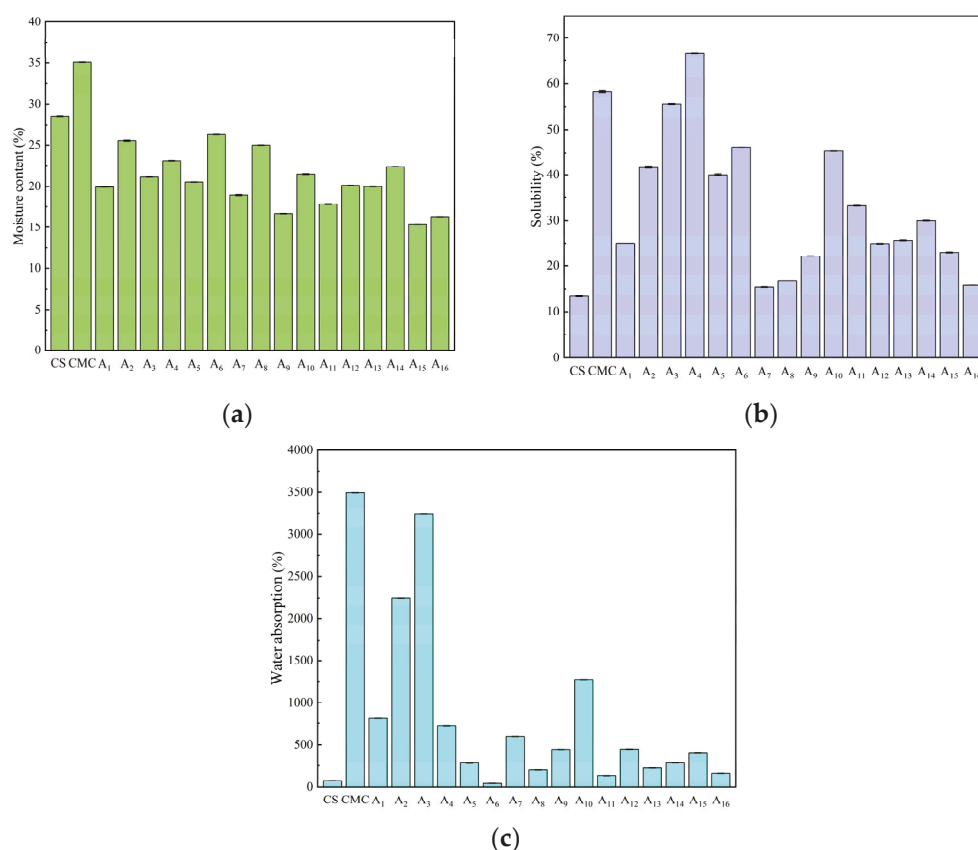


Figure 6. (a) Moisture content, (b) solubility, and (c) water absorption of CS, CMC, and CS/CMC composite films.

Film solubility, closely related to water resistance, showed complex concentration dependence (Figure 6b). The A_4 composite film reached maximum solubility (15.38%), reflecting CMC's inherent hydrophilicity (pure CMC: 58.33%), while A_7 showed minimum solubility (62% reduction vs. pure CS) due to its compact structure. Composite films exhibited significantly greater water absorption than pure CS ($82\text{--}4322\times$ increase, Figure 6c), attributable to the hydrophilic nature of both polymers [32].

Both composition and processing conditions governed water sensitivity. NaOH concentration affected intermolecular cross-linking via pH modification, while urea modulated hydrogen bond stability. Similar results were reported in a study by Liu et al. [20]. Opti-

mal concentrations promoted dense networks that reduced water uptake, whereas excess amounts disrupted these networks, increasing hydrophilicity. These interdependent factors collectively determine the films' water-related performance for packaging applications.

2.8. Water Vapor Permeability (WVP) and Oxygen Transmission Rate (OTR) of CS/CMC Composite Films

Oxygen permeability (OTR) is an important indicator for evaluating the freshness retention ability of composite films. As shown in Figure 7a, the pure CS film exhibited an OP of $5.21 \times 10^{-4} \text{ g}/(\text{m}^2 \cdot \text{s})$, and the incorporation of CMC into CS significantly reduced the OP of CS/CMC composite films.

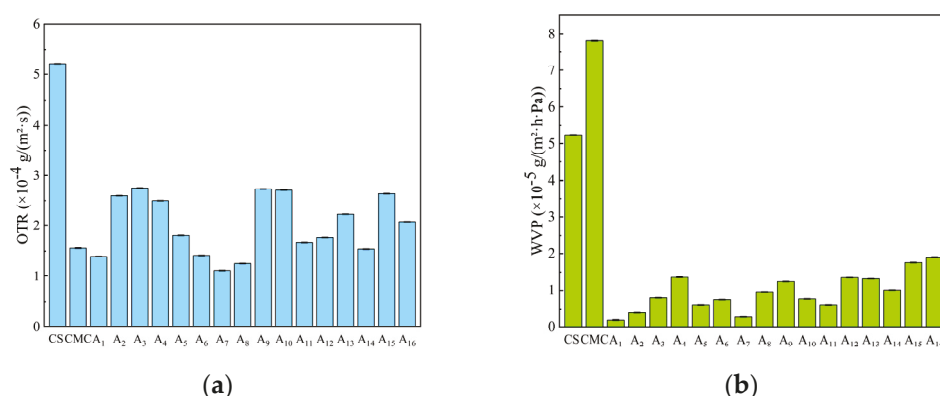


Figure 7. (a) Water vapor transmission rate, and (b) oxygen transmission rate of CS, CMC, and CS/CMC composite films.

Among these, the CS composite film with 1.5% CMC addition demonstrated excellent oxygen barrier performance. The OTR of composite films initially decreased but then increased with further CMC addition, with the A₇ composite film showing the lowest OP and best barrier performance.

This is attributed to the formation of a denser network structure in optimally proportioned NaOH/urea solutions, which reduces oxygen transmission. However, excessive CMC addition disrupts the CS crystalline structure and increases film hydrophilicity, thereby raising oxygen transmission rates.

Effective oxygen barrier performance delays package content oxidation, maintains product quality and freshness, and enhances the value of composite films for food packaging applications.

Water vapor permeability (WVP) is a key parameter for assessing composite film barrier performance, directly influencing packaging material moisture resistance. Figure 7b shows that while pure CS and CMC films exhibit higher WVP values, all CS/CMC composite films demonstrate lower WVP values than single-component films, indicating superior water vapor barrier performance.

This occurs because the inherent hydrophilic groups in CS and CMC create relatively weak water vapor barriers. However, when composited in the NaOH/urea system, the physical cross-linking between the polymer chains produces a denser dry film surface that hinders water vapor transport, thereby reducing the WVP value.

Effective water vapor barrier performance prevents moisture-induced deterioration of packaged contents, prolongs product shelf life, and represents a key advantage for composite film packaging application.

2.9. UV-Blocking Properties

UV-blocking performance is an important index for measuring how well packaging materials protect contents from UV radiation damage. Analysis of transmittance at 280 nm and 320 nm (T_{280} and T_{320}) showed CS/CMC composite films had significantly better UV-shielding performance than pure CS or CMC films.

As shown in Table 2, the composite films A₄, A₉, A₁₂, A₁₃, A₁₄, and A₁₅ had lower 280 nm transmittance than single-component films.

Table 2. Orthogonal test results for mechanical, barrier, and UV-blocking properties of CS, CMC, and CS/CMC composite films.

Run No.	Experimental Design				Response Variables					
	A: CS % (w/v)	B: CMC % (w/v)	C: NaOH % (w/v)	D: Urea % (w/v)	Tensile Strength (MPa)	Elongation at Break (%)	T_{280} (%)	T_{320} (%)	WVP ($\times 10^{-5}$ g/(m ² ·h·Pa))	OTR ($\times 10^{-4}$ g/(m ² ·s))
A ₁	1 (1.0%)	1 (1.0%)	1 (1.0%)	1 (4.0%)	46.08	4.88	2.44	3.80	0.18	1.40
A ₂	1	2 (1.5%)	2 (1.5%)	2 (8.0%)	11.77	30.06	14.48	19.19	0.37	2.60
A ₃	1	3 (2.0%)	3 (2.0%)	3 (12.0%)	3.54	46.27	4.07	7.21	0.79	2.75
A ₄	1	4 (2.5%)	4 (2.5%)	4 (16.0%)	1.76	52.11	0.73	1.29	1.36	2.50
A ₅	2 (1.5%)	1	2	3	4.06	42.22	4.30	5.73	0.61	1.81
A ₆	2	2	1	4	2.45	58.04	6.67	8.40	0.74	1.42
A ₇	2	3	4	1	3.47	68.00	1.34	2.14	0.26	1.12
A ₈	2	4	3	2	11.57	26.64	4.72	6.72	0.96	1.25
A ₉	3 (2.0%)	1	3	4	1.62	35.67	0.77	1.43	1.24	2.73
A ₁₀	3	2	4	3	1.74	39.51	2.54	4.87	0.76	2.71
A ₁₁	3	3	1	2	13.3	29.77	4.36	6.50	0.61	1.66
A ₁₂	3	4	2	1	7.84	36.68	0.36	0.61	1.35	1.77
A ₁₃	4 (2.5%)	1	4	2	1.64	41.34	0.83	1.33	1.32	2.23
A ₁₄	4	2	3	1	5.73	37.30	0.62	1.08	1.01	1.54
A ₁₅	4	3	2	4	3.72	24.25	0.18	0.40	1.75	2.64
A ₁₆	4	4	1	3	12.14	26.73	1.27	2.35	1.90	2.08

The 280 nm and 320 nm values of A₁₂ and A₁₅ films approached 0%, indicating nearly complete UV blockage. This performance is attributed to hydrogen and amide bonding networks between CS and CMC that effectively absorb and scatter UV radiation [33,34], thereby enhancing UV-blocking performance.

Furthermore, NaOH and urea addition optimized film structure by promoting CS-CMC cross-linking, forming denser networks that further enhanced UV barrier performance. These findings agree with the literature reports. For example, Wu et al. [24] demonstrated that CS-CMC hydrogen bonding significantly enhances composite film UV barrier properties. Yang et al. [11] also reported that optimal CS/CMC ratios form dense networks that improve UV-blocking properties.

Compared to the T_{280} (>10%) of CS/CMC films prepared in hydrochloric acid solution [13], alkaline-prepared composite films showed significantly lower T_{280} values.

2.10. Statistical Analysis of Orthogonal Experiment

In this study, mechanical properties, UV-blocking performance, WVP, and OP were evaluated, as shown in Table 2. The orthogonal test results (Tables 3 and 4) were analyzed using range analysis and variance analysis.

Table 3. Range analysis of orthogonal test results.

Response Variable		Experimental Factors			
		A: CS	B: CMC	C: NaOH	D: Urea
Tensile strength (MPa)	k_1	15.79	13.35	18.49	15.78
	k_2	5.39	5.42	6.85	9.57
	k_3	6.13	6.01	5.62	5.37
	k_4	5.81	8.33	2.15	2.39
	R	10.40	7.93	16.34	13.39
	Order of priority Optimization	A1	C > D > A > B		D1
Elongation at break (%)	k_1	33.33	31.03	29.86	36.71
	k_2	48.72	41.23	33.30	31.95
	k_3	35.41	42.07	36.47	38.68
	k_4	32.41	35.54	50.24	42.52
	R	16.32	11.04	20.38	10.57
	Order of priority Optimization	A2	C > A > B > D		D4
T_{280} (%)	k_1	5.43	2.09	3.69	1.09
	k_2	4.16	6.08	4.83	6.10
	k_3	2.01	2.39	2.55	3.05
	k_4	0.73	1.77	1.26	2.09
	R	4.71	4.31	3.57	5.01
	Order of priority Optimization	A4	D > A > B > C		D1
T_{320} (%)	k_1	7.87	3.07	5.26	5.26
	k_2	5.60	8.39	6.48	8.44
	k_3	3.35	3.91	4.11	5.04
	k_4	1.29	2.74	2.26	2.88
	R	6.58	5.64	4.23	5.56
	Order of priority Optimization	A4	A > B > D > C		D4
WVP ($\times 10^{-5}$ g/(m ² ·h·Pa))	k_1	0.68	0.84	0.86	0.70
	k_2	0.64	0.72	1.02	0.82
	k_3	0.99	0.85	1.00	1.02
	k_4	1.50	1.39	0.93	1.27
	R	0.85	0.67	0.16	0.57
	Order of priority Optimization	A2	A > B > D > C		D1
OTR ($\times 10^{-4}$ g/(m ² ·s))	k_1	2.31	2.04	1.64	1.46
	k_2	1.40	2.07	2.20	1.93
	k_3	2.22	2.04	2.07	2.34
	k_4	2.12	1.90	2.14	2.32
	R	0.91	0.17	0.56	0.88
	Order of priority Optimization	A2	A > D > C > B		D1

Data from previous tests were synthesized to determine the optimal composite film formulation. The range value (R) reflects factor importance in the experiment. Larger R values indicate greater factor influence on the measured parameters, typically identifying main factors. Conversely, smaller R values suggest a lesser influence on the response variables.

Table 4. Analysis of variance (ANOVA) for mechanical and barrier properties in the orthogonal experiment.

Source of Variance	Implicit Variable	Sum of Squared Deviations	Degree of Freedom	Mean Square	<i>F</i>	<i>p</i> *
A: CS	Tensile strength	301.945	3	100.648	0.932	0.522
	Elongation at break	694.891	3	231.63	0.650	0.634
	<i>T</i> ₂₈₀	54.411	3	18.137	5.171	0.105
	<i>T</i> ₃₂₀	98.135	3	32.712	5.866	0.090
	WVP	1.875	3	0.625	32.975	0.008
	OTR	2.077	3	0.692	15.418	0.025
B: CMC	Tensile strength	156.147	3	52.049	0.482	0.718
	Elongation at break	322.13	3	107.377	0.301	0.825
	<i>T</i> ₂₈₀	48.159	3	16.053	4.576	0.122
	<i>T</i> ₃₂₀	81.576	3	27.192	4.876	0.113
	WVP	1.083	3	0.361	19.052	0.019
	OTR	0.070	3	0.023	0.519	0.698
C: NaOH	Tensile strength	603.983	3	201.328	1.864	0.311
	Elongation at break	957.717	3	319.239	0.895	0.535
	<i>T</i> ₂₈₀	26.683	3	8.894	2.536	0.232
	<i>T</i> ₃₂₀	36.101	3	12.034	2.158	0.272
	WVP	0.066	3	0.022	1.166	0.451
	OP	0.780	3	0.260	5.793	0.092
D: Urea	Tensile strength	404.415	3	134.805	1.248	0.430
	Elongation at break	231.841	3	77.28	0.217	0.879
	<i>T</i> ₂₈₀	54.645	3	18.215	5.193	0.105
	<i>T</i> ₃₂₀	100.416	3	33.472	6.002	0.088
	WVP1	0.756	3	0.252	13.291	0.031
	OTR	2.063	3	0.688	15.314	0.025

* Note: $p > 0.05$, not significant; $p < 0.05$, significant; $p < 0.01$, highly significant.

Analysis of variance (ANOVA) was employed to assess the statistical significance of each factor's effect on the response variables. Typically, p -values > 0.05 indicate non-significant differences, p -values < 0.05 denote significant factor effects, and p -values < 0.01 represent highly significant effects on the response variables.

ANOVA results demonstrated that NaOH concentration (Factor C, $R = 16.34$) most significantly affected tensile strength by directly modulating intermolecular hydrogen bond density, while urea (Factor D) improved flexibility through crystalline structure disruption (Table 3). Furthermore, ANOVA revealed significant NaOH effects on both WVP ($p = 0.008$) and OP ($p = 0.025$) (Table 4), confirming the alkaline system's crucial role in barrier properties.

By combining the test results in Table 2 with the range analysis in Table 3 and the variance analysis in Table 4 for each factor, we obtained the following results.

For Factor A, which significantly affects both water vapor transmission rate and oxygen transmission rate, A2 was selected. Although Factor A showed no significant effect on tensile strength, elongation at break, T_{280} , and T_{320} , A2 was still chosen based on the range analysis results. In summary, to ensure good overall performance of the composite film, the chitosan concentration should be set at the A2 level.

Factor B significantly affects the water vapor transmission rate, with B2 being optimal. For tensile strength, elongation at break, T_{280} , T_{320} , and oxygen transmission rate, where Factor B shows no significant effect, B3 was selected based on range analysis. The evaluation shows that B2 provides an optimal water vapor transmission rate and better UV-barrier properties and oxygen transmission rate, but poorer mechanical properties. B3 offers

better balanced mechanical properties including tensile strength and elongation at break, moderately good UV barrier properties, and superior water vapor and oxygen transmission rates. Therefore, B3 was selected as the optimal carboxymethyl cellulose concentration to ensure good overall film performance.

Factor C showed no significant effects on the composite film. Based on range analysis of mechanical properties, UV barrier properties, water vapor, and oxygen transmission rates, both C1 and C4 were considered, with C4 being ultimately selected. Thus, the sodium hydroxide concentration was set at the C4 level to ensure good overall film performance.

Factor D most significantly affected the oxygen transmission rate as the primary factor, leading to the selection of D1. It also significantly influenced the water vapor transmission rate as a secondary factor. For tensile strength, elongation at break, T_{280} , and T_{320} , where no significant effects were observed, D1 was still selected based on range analysis. Considering mechanical properties, UV barrier properties, water vapor, and oxygen transmission rates as evaluation criteria, and to ensure good overall performance, the urea concentration was set at the D1 level.

Based on the above analysis, the optimal composite film formulation is A2B3C4D1, consisting of 1.5% chitosan, 2% carboxymethyl cellulose, 2.5% sodium hydroxide, and 4% urea. This formulation demonstrates better mechanical and barrier properties, with moderate additive concentrations that balance all performance indicators.

3. Materials and Methods

3.1. Materials

Chitosan (degree of deacetylation ≥ 95 , determined by elemental analysis according to ASTM F210318 [35]; viscosity: 100–200 mPa·s), glacial acetic acid, and sodium hydroxide were obtained from Shandong Keyuan Biochemical Co., Ltd. (Heze, China). Sodium carboxymethyl cellulose (viscosity: 800–1200 mPa·s) was obtained from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China). Urea and glycerol were supplied by Fuchen Chemical Reagent Co., (Tianjin, China).

3.2. Film Preparation

Pure CS films were prepared by dissolving CS (1.0–2.5 wt%) in 1 vol% acetic acid under stirring at 25 °C for 2 h. Similarly, pure CMC films were obtained by dissolving carboxymethyl cellulose (1.0–2.5 wt%) in deionized water. Glycerol (30 wt% of dry polymer mass) was added to both solutions, followed by 30 min stirring and defoaming. The solutions were cast onto Petri dishes (13 × 13 cm) and dried at 40.0 ± 0.5 °C.

For composite films, CS and CMC solutions were combined at a 1:1 volume ratio. After adding glycerol (30 wt%), the mixture was stirred for 30 min and defoamed (vacuum, 30 min). The resulting solution was cast onto Petri dishes and dried at 40.0 ± 0.5 °C. The dried films were neutralized with 1 M acetic acid or NaOH, rinsed with deionized water, and air-dried. The percentages of each component in the dry film are shown in Table 5.

Table 5. Percentage of each component in dry film.

Compose	A: CS	B: CMC	C: NaOH	D: Urea
Dry film mass percentage ($w/w\%$)	4.3–22.3	4.8–22.6	4.8–22.6	4.3–20.9

Control films were prepared in 1 vol% acetic acid ($\text{pH } 2.5 \pm 0.1$) following the same procedure for comparison.

3.3. Orthogonal Experimental Design

The comprehensive performance of CS/CMC composite films was evaluated through an $L_{16}(4^4)$ orthogonal array design. Key evaluation metrics were selected to represent critical packaging material characteristics, including mechanical properties (tensile strength and elongation at break), water vapor transmission rate (WVP), oxygen transmission rate (OTR), and UV-blocking performance (transmittance at 280 nm and 320 nm). These parameters were chosen to systematically assess the film's mechanical strength, flexibility, moisture resistance, freshness preservation capability, and UV protection efficiency.

3.3.1. Factors and Levels

Four key factors were identified for optimization: CS concentration (Factor A: 1.0–2.5% w/v), CMC concentration (Factor B: 1.0–2.5% w/v), NaOH concentration (Factor C: 2.0–8.0% w/v), and urea concentration (Factor D: 4.0–10.0% w/v). Each factor was assigned four equally spaced levels, with the complete level design presented in Table 6. The $L_{16}(4^4)$ orthogonal array was adopted to efficiently explore this four-factor, four-level design space while maintaining statistical rigor.

Table 6. $L_{16}(4^4)$ orthogonal array design for CS/CMC composite films, including four factors (CS, CMC, NaOH, urea) at four levels.

Level	Factor			
	A: CS % (w/v)	B: CMC % (w/v)	C: NaOH % (w/v)	D: Urea % (w/v)
1	1.0	1.0	1.0	4.0
2	1.5	1.5	1.5	8.0
3	2.0	2.0	2.0	12.0
4	2.5	2.5	2.5	16.0

3.3.2. Experimental Matrix

A 16-run experimental design was constructed using the $L_{16}(4^4)$ orthogonal array. (Table 7), with each experimental group replicated three times to ensure statistical reliability.

Table 7. $L_{16}(4^4)$ orthogonal array design.

Run No.	A: CS % (w/v)	B: CMC % (w/v)	C: NaOH % (w/v)	D: Urea % (w/v)
A ₁	1.0	1.0	1.0	4.0
A ₂	1.0	1.5	1.5	8.0
A ₃	1.0	2.0	1.0	12.0
A ₄	1.0	2.5	2.5	16.0
A ₅	1.5	1.0	1.5	12.0
A ₆	1.5	1.5	1.0	16.0
A ₇	1.5	2.0	2.5	4.0
A ₈	1.5	2.5	2.0	8.0
A ₉	2.0	1.0	2.0	16.0
A ₁₀	2.0	1.5	2.5	12.0
A ₁₁	2.0	2.0	1.0	8.0
A ₁₂	2.0	2.5	1.5	4.0
A ₁₃	2.5	1.0	2.5	8.0
A ₁₄	2.5	1.5	2.0	4.0
A ₁₅	2.5	2.0	1.5	16.0
A ₁₆	2.5	2.5	1.0	12.0

3.4. Characterization and Testing

3.4.1. Thickness and Appearance of CS/CMC Composite Film

Five points were randomly selected on the composite film with a thickness gauge, and the average value was taken as the thickness of the film, in mm. The appearance of the composite film was photographed by a digital camera.

3.4.2. FTIR Analysis

FTIR spectra were recorded using a Nicolet iS10 spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). The films were analyzed in the spectral range of 500–4000 cm^{-1} with a resolution of 4 cm^{-1} . Each spectrum was acquired by averaging 32 consecutive scans to ensure signal-to-noise ratio.

3.4.3. X-Ray Diffraction (XRD)

Crystalline structures were characterized by XRD (Rigaku SmartLab, Tokyo, Japan) with Cu K α radiation ($\lambda = 1.5406 \text{ \AA}$) at 40 kV and 30 mA. Scans were performed in the 2θ range of 10–40° with a scanning speed of 5°/min. The samples were mounted on a zero-background silicon holder to minimize substrate interference. The formula for calculating the degree of crystallinity is as follows:

$$\text{Crystallinity (\%)} = \left(\frac{A_{\text{crys}}}{A_{\text{total}}} \right) \times 100\% \quad (1)$$

where A_{crys} is the XRD integrated area of the crystalline portion; A_{total} is the total XRD integrated area.

3.4.4. Thermogravimetric Analysis

The thermal stability of the films was evaluated using a thermogravimetric analyzer (STA7200, Hitachi, Tokyo, Japan). The temperature was increased from 40 to 500 °C at a heating rate of 10 °C/min under a nitrogen atmosphere with a flow rate of 20 mL/min.

3.4.5. Differential Scanning Calorimetry (DSC)

The composite films were analyzed by differential scanning calorimeter (Q100, TA). The test conditions were nitrogen atmosphere, temperature range 40–350 °C, heating rate 10 °C/min.

3.4.6. Mechanical Properties

Tensile properties were measured according to GB/T 13022-91 [36] using a universal testing machine (CMT6104, MTS Systems, Shenzhen, China). Film specimens (10 × 130 mm) were clamped with a 50 mm gauge length and tested at a crosshead speed of 5 mm/min. Five replicates were measured for each sample group, and the average values of tensile strength and elongation at break were calculated. Film thickness was determined using a digital micrometer.

3.4.7. Moisture Content, Water Absorption and Solubility

The water content of the films was determined using the mass loss method [37]. Film samples (2 cm × 2 cm) were weighed (W_1) and dried at 105 °C until constant weight (W_2), and the moisture content was calculated using Equation (2). For solubility testing, dried films were immersed in distilled water for 24 ± 1 h and removed, and surface moisture was blotted before weighing (W_3). The films were then redried to constant weight (W_4),

and solubility was calculated using Equations (3) and (4). All measurements were recorded with an accuracy of ± 0.01 g.

$$\text{Moisture content}(\%) = \frac{W_1 - W_2}{W_1} \times 100\% \quad (2)$$

$$\text{Water absorption}(\%) = \frac{W_3 - W_2}{W_2} \times 100\% \quad (3)$$

$$\text{Solubility}(\%) = \frac{W_2 - W_4}{W_2} \times 100\% \quad (4)$$

3.4.8. Water Vapor Transmission Rate (WVP)

The water vapor transmission rate was measured according to GB/T 1037-2021 [38], with modifications based on the methodology of Manjushree et al. [39]. Glass vials (10 mL) were filled with anhydrous calcium chloride (dried) and sealed with the test films, maintaining a 3 mm gap between the desiccant and film surface. The assemblies were placed in desiccators containing saturated aqueous solutions at 25 °C. Weight measurements were taken at 24 h intervals (accuracy: ± 0.01 g). The WVP was calculated from the linear regression of mass gain versus time using Equation (5):

$$\text{WVP} = \frac{K \times D}{S \times \Delta P} \quad (5)$$

where K is the slope of the obtained straight line (g/h); D is the thickness of the composite Film (mm); S is the area of the bottle mouth (m^2); ΔP is the vapor pressure on both sides of the composite film at 25 °C (Pa).

3.4.9. Oxygen Permeability (OTR)

The oxygen permeability (OTR) was determined based on iron oxidation, following the deoxidizer adsorption method described by Zhang et al. [40]. A mixture of sodium chloride (1.5 g), activated carbon (1 g), and reduced iron powder (0.5 g) was loaded into brown glass vials (neck diameter: 2 cm). Film samples were heat-sealed over the vial openings, and the assemblies were stored at 25 °C and 90% relative humidity (RH) for 48 h. Triplicate tests were conducted for each sample, and the OTR was calculated using Equation (6).

$$\text{OTR} = \frac{m - m_0}{t \times s} \quad (6)$$

3.4.10. Ultraviolet–Visible Spectroscopy

UV–Vis transmittance spectra of the films were recorded at 25 °C using a UV–Vis spectrophotometer. Film samples were cut into strips (1 cm $\times$ 5 cm). Background correction was performed using an empty cuvette as a reference. Each film strip was mounted on a cuvette, and transmittance was measured from 200 to 800 nm.

4. Conclusions

In this study, CS/CMC composite films prepared in the NaOH/urea system achieved optimal performance at 1.5% CS, 2% CMC, 2.5% NaOH, and 4% urea. The alkaline system avoided acidic solvent residues while enhancing hydrogen bonding, yielding films with exceptional mechanical properties (46.08 MPa tensile strength, 68% elongation), barrier performance (WVP: 0.26×10^{-5} g/($\text{m}^2 \cdot \text{h} \cdot \text{Pa}$); OTR: 1.12×10^{-4} g/($\text{m}^2 \cdot \text{s}$)), and thermal stability (304 °C decomposition temperature). These results position the film as a sustainable alternative to petroleum-based packaging. Future research will systematically evaluate the biodegradability and practical application potential of CS/CMC composite materials by verifying the hydrophilicity of the films, conducting composting degradation experiments, and testing their food preservation effects under real storage conditions.

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References

- Leite, L.S.F.; Le Gars, M.; Azeredo, H.M.; Moreira, F.K.; Mattoso, L.H.; Bras, J. Insights into the effect of carboxylated cellulose nanocrystals on mechanical and barrier properties of gelatin films for flexible packaging applications. *Int. J. Biol. Macromol.* **2024**, *280*, 135726. [CrossRef] [PubMed]
- Ambaye, T.G.; Vaccari, M.; Prasad, S.; van Hullebusch, E.D.; Rtimi, S. Preparation and applications of chitosan and cellulose composite materials. *J. Environ. Manag.* **2022**, *301*, 113850. [CrossRef]
- Wang, Y.; Xu, T.; Qi, J.; Liu, K.; Zhang, M.; Si, C. Nano/micro flexible fiber and paper-based advanced functional packaging materials. *Food Chem.* **2024**, *458*, 140329. [CrossRef]
- Qin, Y.; Wang, Y.; Tang, Z.; Chen, K.; Wang, Z.; Cheng, G.; Chi, H.; Soteyome, T. A pH-sensitive film based on chitosan/gelatin and anthocyanin from *Zingiber striolatum* Diels for monitoring fish freshness. *Food Chem.-X* **2024**, *23*, 101639. [CrossRef]
- Nunes, C.; Coimbra, M.A.; Ferreira, P. Tailoring Functional Chitosan-Based Composites for Food Applications. *Chem. Rec.* **2018**, *18*, 1138–1149. [CrossRef]
- Mujtaba, M.; Salaberria, A.M.; Andres, M.A.; Kaya, M.; Gunyakti, A.; Labidi, J. Utilization of flax (*Linum usitatissimum*) cellulose nanocrystals as reinforcing material for chitosan films. *Int. J. Biol. Macromol.* **2017**, *104*, 944–952. [CrossRef]
- He, X.; Zhang, Z.; Xuan, X.; Tan, T.; Sun, J.; Wang, B.; Tian, Y.; Chen, H. Structure and properties of chitosan plasticized with hydrophobic short-chain fatty acid cosolvent. *Int. J. Biol. Macromol.* **2025**, *300*, 140250. [CrossRef]
- Wang, J.; Han, X.; Zhang, C.; Liu, K.; Duan, G. Source of Nanocellulose and Its Application in Nanocomposite Packaging Material: A Review. *Nanomaterials* **2022**, *12*, 3158. [CrossRef]
- Revathi, S.; Amanullah, M.; Al-Samghan, A.S.; Joseph, J.J.; Pazhanisamy, P.; Addich, M.; Gomathi, T. Sustainable heavy metal (Cr(VI) ion) remediation: Ternary blend approach with chitosan, carboxymethyl cellulose, and bioactive glass. *Int. J. Biol. Macromol.* **2024**, *278*, 134769. [CrossRef]
- Shi, X.; Kang, M.; Zhang, H.; Xu, J.; Wang, C.; Xu, Y.; Chang, C.; Ge, B.; Gao, S. Hydrogen Bond Cross-Linked Montmorillonite-Enhanced Biodegradable Carboxymethyl Cellulose/Guar Gum Composite Films. *Acs Appl. Polym. Mater.* **2024**, *6*, 10788–10797. [CrossRef]
- Yang, J.; Li, Y.; Liu, B.; Wang, K.; Li, H.; Peng, L. Carboxymethyl cellulose-based multifunctional film integrated with polyphenol-rich extract and carbon dots from coffee husk waste for active food packaging applications. *Food Chem.* **2024**, *448*, 139143. [CrossRef] [PubMed]
- Yue, C.; Wang, M.; Zhou, Z.; You, Y.; Wang, G.; Wu, D. Cellulose-based intelligent packaging films with antibacterial, UV-blocking, and biodegradable properties for shrimp freshness monitoring. *Chem. Eng. J.* **2024**, *488*, 150975. [CrossRef]
- Chen, H.; Meng, X.; Zhang, F.; Chen, J.; Ding, X.; Jian, T.; Niu, G.; Tong, B.; Gai, Y.; Zhao, H.; et al. Development of chitosan-carboxymethyl cellulose edible films loaded with blackberry anthocyanins and tea polyphenols and their application in beef preservation. *Food Hydrocoll.* **2025**, *164*, 111198. [CrossRef]
- Lan, W.; He, L.; Liu, Y. Preparation and Properties of Sodium Carboxymethyl Cellulose/Sodium Alginate/Chitosan Composite Film. *Coatings* **2018**, *8*, 291. [CrossRef]
- Oprea, M.; Voicu, S.I. Recent Advances in Applications of Cellulose Derivatives-Based Composite Films with Hydroxyapatite. *Materials* **2020**, *13*, 2481. [CrossRef]
- Hu, X.; Du, Y.; Tang, Y.; Wang, Q.; Feng, T.; Yang, J.; Kennedy, J.F. Solubility and property of chitin in NaOH/urea aqueous solution. *Carbohydr. Polym.* **2007**, *70*, 451–458. [CrossRef]
- Cai, J.; Zhang, L. Rapid dissolution of cellulose in LiOH/Urea and NaOH/Urea aqueous solutions. *Macromol. Biosci.* **2005**, *5*, 539–548. [CrossRef]

18. Yu, Y.; Xie, C. Preparation of a PVA/Chitosan/Glass Fiber Composite Film and the Performance in CO₂ Separation. *Films* **2023**, *13*, 36. [CrossRef]
19. Abu El-Soad, A.M.; Lazzara, G.; El-Magied, M.O.A.; Cavallaro, G.; Al-Otaibi, J.S.; Sayyed, M.I.; Kovaleva, E.G. Chitosan Functionalized with Carboxyl Groups as a Recyclable Biomaterial for the Adsorption of Cu (II) and Zn (II) Ions in Aqueous Media. *Int. J. Mol. Sci.* **2022**, *23*, 2396. [CrossRef]
20. Liu, S.; Li, L. Unique gelation of chitosan in an alkali/urea aqueous solution. *Polymer* **2018**, *141*, 124–131. [CrossRef]
21. Zhang, R.; Xie, J.; Yang, B.; Fu, F.; Tang, H.; Zhang, J.; Zhao, Y.; Zhang, Y.; Liu, L.; Zhu, Y.; et al. Self-assembly of chitosan and cellulose chains into a 3D porous polysaccharide alloy films: Co-dissolving, structure and biological properties. *Appl. Surf. Sci.* **2019**, *493*, 1032–1041. [CrossRef]
22. Duan, J.; Liang, X.; Cao, Y.; Wang, S.; Zhang, L. High Strength Chitosan Hydrogels with Biocompatibility via New Avenue Based on Constructing Nanofibrous Architecture. *Macromolecules* **2015**, *48*, 2706–2714. [CrossRef]
23. Zeng, J.; Ren, X.; Zhu, S.; Gao, Y. Cellulose nanocrystals from pomegranate peel: Isolation, characterization, and its reinforcement for chitosan film. *J. Mater. Sci.* **2022**, *57*, 11062–11076. [CrossRef]
24. Chen, C.; Deng, S.; Tian, H.; Yu, H.; Huang, J.; Lou, X.; Yuan, H. Enhanced fresh walnut preservation using chitosan films reinforced with cinnamon essential oil and bacterial cellulose pickering emulsion. *Food Hydrocoll.* **2025**, *165*, 111267. [CrossRef]
25. Mao, H.; Wei, C.; Gong, Y.; Wang, S.; Ding, W. Mechanical and Water-Resistant Properties of Eco-Friendly Chitosan Film Reinforced with Cellulose Nanocrystals. *Polymers* **2019**, *11*, 166. [CrossRef]
26. Almeida, E.V.R.; Frollini, E.; Castellan, A.; Coma, V. Chitosan, sisal cellulose, and biocomposite chitosan/sisal cellulose films prepared from thiourea/NaOH aqueous solution. *Carbohydr. Polym.* **2010**, *80*, 655–664. [CrossRef]
27. Morgado, D.L.; Frollini, E.; Castellan, A.; Rosa, D.S.; Coma, V. Biobased films prepared from NaOH/thiourea aqueous solution of chitosan and linter cellulose. *Cellulose* **2011**, *18*, 699–712. [CrossRef]
28. Tu, G.; Liu, X.; Li, Z.; Li, S.; Liu, J.; Li, W. Characterizing gelation kinetics of chitosan dissolved in an alkali/urea aqueous solution: Mechanisms accounting for the morphological development. *J. Membr. Sci.* **2021**, *635*, 119516. [CrossRef]
29. Pimonpan, K.; Chalalai, J.; Saroat, R.; Wirongrong, T.; Warinporn, K. Carboxymethyl cellulose from Young Palmyra palm fruit husk: Synthesis, characterization, and film properties. *Food Hydrocoll.* **2022**, *124 Pt A*, 107277. [CrossRef]
30. He, M.; Chen, H.; Zhang, X.; Wang, C.; Xu, C.; Xue, Y.; Wang, J.; Zhou, P.; Zhao, Q. Construction of novel cellulose/chitosan composite hydrogels and films and their applications. *Cellulose* **2018**, *25*, 1987–1996. [CrossRef]
31. Valizadeh, S.; Naseri, M.; Babaei, S.; Hosseini, S.M.H.; Imani, A. Development of bioactive composite films from chitosan and carboxymethyl cellulose using glutaraldehyde, cinnamon essential oil and oleic acid. *Int. J. Biol. Macromol.* **2019**, *134*, 604–612. [CrossRef] [PubMed]
32. Wang, W.; Hu, J.; Zhang, R.; Yan, C.; Cui, L.; Zhu, J. A pH-responsive carboxymethyl cellulose/chitosan hydrogel for adsorption and desorption of anionic and cationic dyes. *Cellulose* **2021**, *28*, 897–909. [CrossRef]
33. Ezati, P.; Khan, A.; Priyadarshi, R.; Bhattacharya, T.; Tammina, S.K.; Rhim, J.-W. Biopolymer-based UV protection functional films for food packaging. *Food Hydrocoll.* **2023**, *142*, 108771. [CrossRef]
34. Wang, L.; Li, Y.; Ye, L.; Zhi, C.; Zhang, T.; Miao, M. Development of starch-cellulose composite films with antimicrobial potential. *Int. J. Biol. Macromol.* **2024**, *276*, 133836. [CrossRef]
35. ASTM F2103-18; Standard Guide for Characterization and Testing of Chitosan Salts as Starting Materials Intended for Use in Biomedical and Tissue-Engineered Medical Product Applications. ASTM International: West Conshohocken, PA, USA, 2018.
36. GB/T 13022-1991; Plastics—Determination of Tensile Properties of Films. Standards Press of China: Beijing, China, 1991.
37. Muhammad Shahar, Y.; Isaiah Henry, I.; Bako, H.K.; Alnadari, F.; Bilal, M.; Rehman, F.; Zhu, J.; Zhou, T.; Zhao, Z.; Li, C. A novel carboxymethyl cellulose/gum xanthan and citric acid-based film that enhances the precision of blackcurrant anthocyanin-induced color detection for beef spoilage tracking. *Food Chem.* **2024**, *461*, 140905. [CrossRef]
38. Manjushree Nagaraj, G.; Saraswati, P.M.; D'Souza, O.J.; Eelager, M.P.; Kurabetta, L.K.; Chougale, R.B.; Kadapure, A.J.; Kumar, S.P. Fabrication of CuO nanoparticles embedded novel chitosan/hydroxypropyl cellulose bio-nanocomposites for active packaging of jamun fruit. *Food Hydrocoll.* **2024**, *152*, 109937. [CrossRef]
39. Wu, Z.; Huang, X.; Li, Y.-C.; Xiao, H.; Wang, X. Novel chitosan films with laponite immobilized Ag nanoparticles for active food packaging. *Carbohydr. Polym.* **2018**, *199*, 210–218. [CrossRef]
40. Zhang, Y.; Tang, Q.; Huang, K.; Xu, Z.; Feng, S.; Li, H.; Zou, Z. Developing strong and tough cellulose acetate/ZIF67 intelligent active films for shrimp freshness monitoring. *Carbohydr. Polym.* **2023**, *302*, 120375. [CrossRef]

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Article

Active Pectin/Carboxymethylcellulose Composite Films for Bread Packaging

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Abstract: A new active composite film intended for bread packaging is described here. The active film has the aim of prolonging bread's shelf life while avoiding the use of nanoparticles that, with very few exceptions, are a type of material not allowed by regulatory agencies like EFSA (European Food Safety Agency) and FDA (US Food and Drug Administration) in food contact materials. Moreover, the increasing consumer demand for natural and wholesome products, possibly "clean label", and packaged in natural, non-petroleum-based materials has been taken into consideration. Accordingly, precursor materials from renewable sources were used to prepare the active film: pectin from citrus peel (PEC) and carboxymethyl cellulose (CMC) were used as the matrix, with oleic acid (OA) as plasticizer. Moreover, the bread preservative calcium propionate (CaP) was used as the crosslinker, and also zeolite microparticles loaded with silver ions (AgZ) were added to the films as an additional antimold agent. This strategy allows us to avoid the addition to bread of the now commonly used preservatives ethanol and calcium propionate, moving the latter to the packaging. Permeance measurements revealed excellent barrier properties against O₂ and CO₂, while the typical high water vapor permeance of polysaccharide films was mitigated by the non-hydrophilic OA plasticizer. Moreover, the quantities of Ag⁺ and CaP released in bread are low and below the limits imposed by regulatory agencies. The antimold activity of the films is excellent, with *Aspergillus niger*, *Penicillium janthinellum*, and *wild-type Penicillium* molds reduction on bread in the 99.20–99.95% range for the films containing only CaP and in the 99.97–99.998% range for the films containing both CaP and AgZ. Finally, the rheological properties of the film-forming solutions were investigated, demonstrating their potential application as coatings on natural packaging materials for bread, such as paper.

Keywords: active packaging; zeolites; pectin; carboxymethyl cellulose; silver

1. Introduction

Despite the huge advantages of using polyolefin films as packaging for food, when the collection and recycling systems fail, they become a source of waste and pollution. In the European Union, the problem of plastic waste and pollution is gaining more attention. Recycling has received a huge boost [1], and various strategies have been implemented to reduce the production of petroleum-based plastic [2]. Moreover, although on a still uncertain time scale, it is important to note that natural oil reserves are destined to run out.

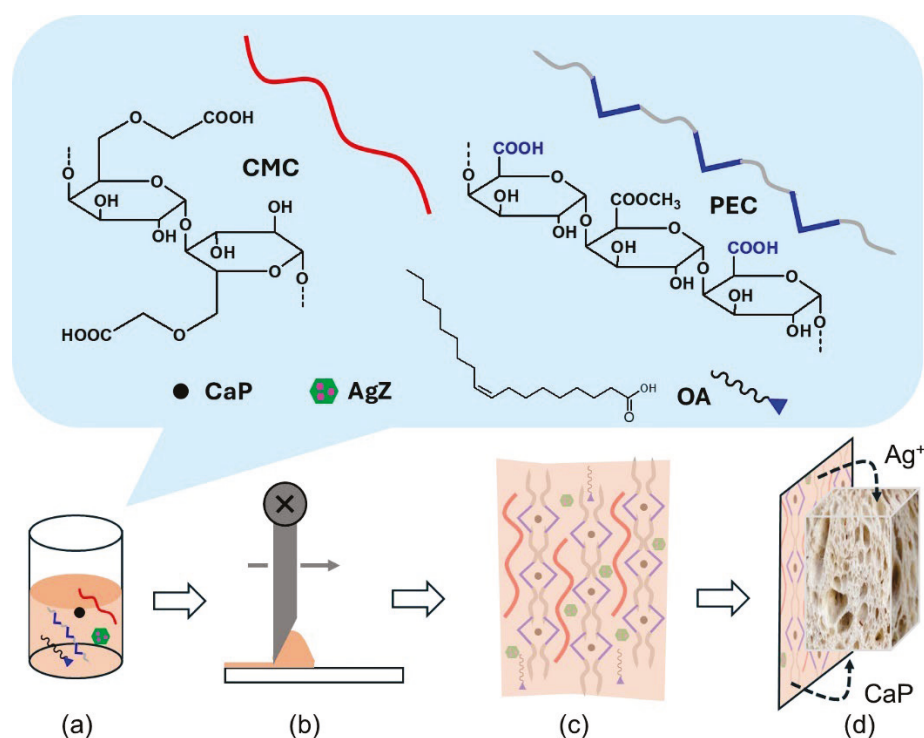
An obvious solution suitable both to mitigate the plastic waste and pollution problem inherent to food packaging, and to serve as an alternative for petroleum-derived packaging films, is to use polymers derived from renewable sources. Polysaccharides are among the best choices for producing biodegradable and/or compostable packaging [3]. Polysaccharides have many favorable features: they are non-toxic and biodegradable, they display good film-forming ability [3,4], they have good oxygen barrier properties, and they have acceptable mechanical properties when compared to petroleum-based plastics [5]. On the other hand, a drawback in the use of polysaccharide films for food packaging is their hydrophilicity [3,6], which has suggested their use as coatings rather than self-standing films, e.g., in paper-based or multilayer packaging [6,7].

Bread matrix has a relatively high moisture content and a water activity between 0.94 and 0.97, with a pH of about 6, so it is a favorable environment for the germination and growth of molds and bacteria [8–10]. While bread is perfectly sterile when it comes out of the oven, when it is cooled, sliced, packed, and wrapped, it undergoes microbial contamination. Polyethylene (PE) films are commonly used for bread packaging, with chemicals added to the packed bread to extend its shelf life. Calcium propionate (CaP) and/or potassium sorbate (KS) are added to bread during its preparation to inhibit the growth of microorganisms after baking [8,10]. Quantitative limitations hold depending on the country. In Europe, the maximum CaP allowed is 0.2% (*w/w*) and 0.3% (*w/w*) KS in prepacked rye bread, and 0.1% (*w/w*) for CaP in prepacked, unsliced bread [11]. While such percentage values seem small, it should be stressed that by eating a single bread portion, with a typical 50 g mass, a consumer ingests from 50 to 150 mg of preservatives. Another widely used preservative is ethanol, which is directly sprayed on bread before packing. An amount of ethanol ranging from 0.2% to 12% *w/w* is common, without restrictions in the EU [8], sometimes in combination with KS or CaP [12]. Physical sterilization methods are also possible, such as UV or IR irradiation and microwave heating, but they are very rarely used in bakeries because of their high cost and low efficiency [8,10].

High concentrations of chemical preservatives affect the taste and the sensory properties of bread [10], and the prolonged use of them may lead to fungal resistance [13]. In addition, the consumers demand for baked food has recently shifted towards natural and wholesome products, possibly “clean label”, i.e., without added chemical preservatives [10]. As regards bread, this presents a highly demanding challenge, because bread packed in PE [14] without preservatives has a shelf life of only a few days at room temperature [10]. Active packaging has been put forward to preserve food and to meet the demand of consumers towards “clean label” foods. Active materials are defined as materials intended to extend the shelf life or to maintain or improve the condition of packaged food by releasing or absorbing substances [15]. In particular, an extension of the shelf life of bread has been proposed by modifying the common PE packaging with embedded micro- or nanoparticles, either for the release of small, controlled quantities of active substances [8,14] or for the improvement of the oxygen barrier properties of the packaging to prevent food oxidation [14,16]. Active packaging has also been implemented in films for food packaging made of polysaccharides [17]. Interestingly, for the goals of the present research, only a few of the reported examples were studied for bread packaging [18]. Most researchers have proposed to embed silver nanoparticles (AgNPs) in the packaging film as the easiest way to develop active packaging with antimold and antibacterial action [19–21]. AgNPs are well known as an extremely active material against bacteria and molds, thanks to the sustained release of the Ag⁺ cation, which is the actual antimicrobial agent [22]. Unfortunately, AgNPs are usually not included by regulatory agencies in the substances allowed in food contact materials; see, e.g., EFSA in the EU [23,24]. Noticeably, the same regulatory agency excludes the use of nanoparticles of any material, with very few exceptions like titanium

nitride, ZnO, and nanoclays. Besides nanoparticles, microparticle-embedded natural antimicrobial agents, such as the extract of herbs and spices or essential oils, are also widely explored in the literature [8,14,16]. However, the already stressed disadvantage of food sensory properties modification limits the amount that can be added and consequently the antimicrobial capacity [8].

Pectin (PEC, formula in Scheme 1) is an anionic polysaccharide made mainly of D-galacturonic acid residues linked with α -1,4-bonds and defined as low methoxyl pectin when the carboxylic groups of uronic acid are partially methyl esterified (DE < 50%) [25]. Its films have a very high barrier to oxygen, reasonably good (i.e., low) water vapor transmission rates [7,26], and also exert an efficient barrier effect for oil and aroma preservation [25]. As a drawback, PEC is hydrophilic and has poor mechanical properties, since its films are brittle and rigid [7,26].



Scheme 1. Composition of the active film and stages from film preparation to its use, with (a–d) corresponding to film precursor solution (a), tape casting of the film with doctor blade technique (b), self-standing film with all embedded components (c), and contact of the film with bread and migration of the active components (d). CMC = Carboxy methylcellulose CaP = Calcium propionate, PEC = pectin, OA = oleic acid, AgZ = Ag⁺-loaded zeolites).

CMC is a water-soluble cellulose derivative made of glucopyranose residues linked by β -1,4 bonds. Thanks to the presence of a hydrophobic polysaccharide backbone and of many hydrophilic carboxyl groups, CMC is less hydrophilic than PEC [27]. Moreover, it has good mechanical properties, as its films are flexible, easily stretched, and ductile. PEC and CMC have already been used for the preparation of single-component biodegradable films [7,28–33] and as mixtures [25,27]. In most cases, glycerol was used as a plasticizer agent, improving the mechanical properties of the film but also exerting a negative influence on its barrier properties: an increase in the mesh size of the biopolymer network was observed, and, due to glycerol hydrophilicity, the water vapor transfer also increased [26]. Oleic acid (OA, Scheme 1) was studied as an alternative plasticizer for polysaccharide films, obtaining satisfactory mechanical properties and an improved barrier against water vapor [34].

Ca^{2+} is a cation capable of forming strong bridges between the carboxylic groups of the galacturonic chains of pectin when the pH is below the pK_a of the carboxyl groups, with the so-called egg-box model mechanism sketched in Scheme 1c, promoting pectin gelation [35]. Zeolites (Z) are microporous aluminosilicates that are low cost, non-toxic, naturally abundant, and with remarkable ion exchange capacity [36]. Moreover, the addition of Z can improve the mechanical [36,37] and barrier properties of self-standing films [36]. Z can be loaded with metal cations, including, significantly, Ag^+ [36–38]. In this regard, it must be mentioned that the limiting quantity of Ag^+ allowed in food by EFSA is 0.05 mg Ag/kg food [39].

In this paper, self-standing films are presented, made of PEC and CMC, with CaP as the crosslinking agent, OA as the plasticizer, and with embedded zeolites loaded with Ag^+ (AgZ). Such films are a new active packaging designed to allow the preservation of bread, avoiding the common practice of adding huge quantities of chemical preservatives, such as ethanol, CaP, or KS, in the bread. To this aim, the CaP antimold preservative was transferred from the bread to its packaging, and an Ag^+ -releasing agent, which is not nanosized, was incorporated into the same packaging. As sketched in Scheme 1b, the traditional solvent casting method [25] was avoided, and the film was prepared with a tape casting “doctor blade” approach that allows control of the thickness homogeneity and avoids the use of diluted film-forming solutions. The sum of all these features is a novelty in active packaging, especially considering the simultaneous double action of CaP and Ag^+ , Scheme 1d. The barrier, mechanical, and optical properties were evaluated on the self-standing films, along with the release of Ag^+ towards bread, in order to achieve values within the limits imposed by EFSA [39]. The antimold activity and shelf life experiments on bread were performed in order to demonstrate the effectiveness of the studied films with respect to traditional, non-active packaging. Finally, the viscosity of the film-forming solutions was determined to assess their applicability as coatings.

2. Results and Discussion

2.1. Film Formulation

Preliminary studies were carried out exploring a range of mass ratios of PEC, CMC, OA, and calcium salts for the preparation of the PEC/CMC self-standing films. The quantities of OA (0.30 g) and PEC (3.0 g) were kept constant, while the quantity of CMC was varied from 10% to 40% w/w (with respect to pectin), and the amount of Ca^{2+} from 0.75 mmol to 1.9 mmol. The mechanical properties of all the prepared films were determined and are reported in SI1. An increase in the tensile strength (TS) was observed on increasing the amount of CMC in the formulation: 34($\pm$ 8) MPa for 10% CMC, 38($\pm$ 18) MPa for 20% CMC, and 57($\pm$ 10) MPa for 30% CMC. The film with the maximum quantity of CMC (40% w/w) showed instead a decrease in the TS (40($\pm$ 13) MPa). Elongation at break (E%) was not affected by the different quantity of CMC (constant value $\sim$ 7–8%). Concerning the amount of crosslinker (Ca^{2+} salt), the rationale was to choose the maximum possible quantity to maintain good TS and E%. These preliminary studies revealed that the films with the best mechanical properties are prepared with 30% w/w of CMC and 1.9 mmol of Ca^{2+} , as higher quantities of Ca^{2+} crosslinker generated cracks in the films. All the studies described in the following sections were conducted with these weight proportions of the components, following the protocol described in detail in Section 3.2.1

2.2. Functional Properties of PEC/CMC Self-Standing Films

2.2.1. Mechanical Properties

TS, YM, and E% are critical parameters to be evaluated for the design of food packaging. Among all its functions, packaging also has to maintain its integrity during storage, distribution, processing, and handling [40].

TS indicates the maximum resistance of a film to breaking under tension. YM is correlated to TS and discloses the intrinsic stiffness of the films. The flexibility of the films is instead given by the value of E%. The mechanical properties of PEC/CMC films are displayed in Table 1. The thickness, TS, YM, and E% of Mater-bi® (MB) commercial sachets and of a low-density PE commercial film (LDPE) were also measured and included in Table 1. It has to be stressed that the comparison with the films prepared in this paper has limitations, as the preparation techniques of the examined commercial LDPE and MB films are unknown and most probably different from the tape casting method used in the present paper. However, MB is the most common biodegradable film currently produced and used (e.g., on the market for picking fruits and vegetables), and LDPE is commonly used for sliced bread packaging. The properties of these two films relevant to bread packaging have therefore been measured in this research to allow a reasonable quantitative comparison between the new films described here and the market standards.

Table 1. Mechanical properties of PEC/CMC films, MB, and LDPE.

Films	Thickness (μm)	Young's Modulus (GPa)	Tensile Strength (MPa)	Elongation at Break (%)
MB	12.6(± 0.5) ^a	0.80(± 0.03) ^{c,d}	26(± 1) ^{b,c}	198(± 28) ^c
LDPE	51(± 1) ^{c,d}	0.26(± 0.03) ^a	18(± 2) ^a	1206(± 121) ^d
PEC/CMC/OA	47(± 2) ^{b,c,d}	1.35(± 0.09) ^e	35(± 5) ^{b,c,d}	2.6(± 7) ^a
PEC/CMC/OA/CaCl ₂	54(± 7) ^d	0.56(± 0.05) ^b	51(± 7) ^{b,c,d}	7(± 1) ^b
PEC/CMC/OA/CaA	65(± 7) ^e	0.75(± 0.07) ^{b,c,d}	59(± 15) ^{c,d}	8(± 1) ^b
PEC/CMC/OA/CaA/Z	64(± 7) ^e	0.69(± 0.05) ^{b,c,d}	55(± 16) ^{c,d}	8(± 1) ^b
PEC/CMC/OA/CaA/AgZ	47(± 5) ^{b,c,d}	0.60(± 0.01) ^{b,c}	54(± 11) ^{b,c,d}	8(± 1) ^b
PEC/CMC/OA/CaP	43(± 4) ^{b,c}	0.85(± 0.06) ^d	63(± 15) ^d	8(± 2) ^b
PEC/CMC/OA/CaP/Z	40(± 5) ^b	0.70(± 0.01) ^{b,c,d}	50(± 16) ^{b,c,d}	9(± 3) ^b
PEC/CMC/OA/CaP/AgZ	50(± 4) ^{c,d}	0.60(± 0.01) ^{b,c}	54(± 11) ^{b,c,d}	8(± 1) ^b

Each value is the average of the measurements taken on five samples. Values are reported as mean (standard deviation). Different Roman letters correspond to the classification groups within the same column for the ANOVA Tukey's test ($p < 0.05$).

The examined MB is thinner (12.6(5) μm) but of comparable thickness with the LDPE film (51(1) μm). Using a total mass of solid precursors (PEC, CMC, Ca salts, OA, Z, or AgZ) ranging from 4.3 to 4.8 g (depending on the chosen Ca salt and on added/not added Z or AgZ), two films of approximately $12 \times 20 \text{ cm}^2$ were obtained with the “doctor blade” technique (see SI2). The thickness of the films prepared in this study, Table 1, is in the 40–65 μm range, depending on the composition. Moreover, the low standard deviation indicates that the “doctor blade” technique yields homogeneous and reproducible films, with a thickness almost identical to that of the LDPE used for bread packaging. PEC/CMC films are more resistant than the commercial MB and LDPE used for comparison, displaying a higher TS, a feature that is optimal for films intended for food packaging. Moreover, the addition of a crosslinker further enhances the TS, stepping from 35(± 5) MPa of PEC/CMC/OA to 50–70 MPa of the films with Ca^{2+} ions. On the other hand, the flexibility of LDPE and MB is, respectively, three and two orders of magnitude higher than that of our films, although Ca^{2+} also positively affects the E%, which increases from 2.6% (no Ca^{2+}) to 7–9%. The YM decreases significantly with the addition of Ca^{2+} , i.e., leading to less rigid films, with YM values comprised between those of MB and LDPE. The use of Ca^{2+}

as a crosslinker thus contributes positively to the mechanical properties of the films, as it improves their elasticity and strength while reducing the stiffness. Moreover, the literature shows that the mechanical properties of the films described in the present paper are close to those of other petroleum-based plastics of current use: e.g., TS has been reported in the 8–20, 30–40, and 48–72 MPa ranges for LDPE, PP, and PET, respectively [41]. As for zeolites (with or without silver), their addition does not significantly affect the mechanical properties of the films. Finally, comparison with pectin films without CMC (SI3, Table) highlights the already recognized brittleness of PEC-only films [7,26], which have much lower TS and E% values and a higher YM. The addition of CMC can thus be considered beneficial from a mechanical point of view.

2.2.2. Barrier Properties

Mass transport properties of films are crucial parameters to evaluate their performances and determine their effectiveness for food packaging. Water vapor, oxygen, and carbon dioxide can penetrate through the packaging film or, in the case of humid food like bread, leave the sachet during storage.

All this affects the microbial growth, the product texture, the quality of food (e.g., by oxidation), and sensory features [42]. It has been shown that staling, spoilage, and lipid oxidation of bread are promoted mainly by the presence of oxygen inside the packaging [8,14,42]. CO₂ could instead play a favorable role, as it can be added in the packaging sachet to suppress or limit microbial growth in bread [14,42]. Figure 1 displays the O₂ and CO₂ permeance of the composite PEC/CMC films and of LDPE and MB. The O₂ barrier performance of the PEC/CMC films is excellent. The O₂ permeance of all the PEC/CMC composite films with added Ca²⁺ salts is in the $1\text{--}9 \times 10^{-15} \text{ mol m}^{-2}\text{s}^{-1}\text{Pa}^{-1}$ range, which is four orders of magnitude lower than that of the commercial LDPE ($p\text{O}_2 = 1.90(\pm 9) \times 10^{-11} \text{ mol m}^{-2}\text{s}^{-1}\text{Pa}^{-1}$) and MB ($p\text{O}_2 = 4.0(\pm 7) \times 10^{-11} \text{ mol m}^{-2}\text{s}^{-1}\text{Pa}^{-1}$) used for comparison. This is not unexpected, as the literature reports high O₂ barrier values for PEC films, with reported permeabilities as low as $6.75 \times 10^{-15} \text{ mol m}^{-2}\text{s}^{-1}\text{Pa}^{-1}$ [43]. In the case of LDPE and MB, a structure without or with poor polar interactions and/or hydrogen bonds has affinity for the non-polar molecule dioxygen [44], thus promoting permeation. The O₂ barrier properties of biodegradable and non-biodegradable polymers at RH = 0% have been classified by Wu et al. [45]. According to this classification, all PEC/CMC films with CaA and CaP belong to the “very high barrier to oxygen” group (permeance $< 7.58 \times 10^{-15} \text{ mol m}^{-2}\text{s}^{-1}\text{Pa}^{-1}$), and the PEC/CMC film with CaCl₂ as crosslinker fits into the “high barrier” group (permeance between 7.58×10^{-15} and $7.58 \times 10^{-14} \text{ mol m}^{-2}\text{s}^{-1}\text{Pa}^{-1}$), while LDPE and MB are classified in the “poor barrier” group (permeance $> 7.58 \times 10^{-12} \text{ mol m}^{-2}\text{s}^{-1}\text{Pa}^{-1}$). Adding zeolites to PEC/CMC leads to an even lower O₂ permeance, an effect that is reasonably connected to the known increase in the tortuosity pathway of various gases when nano- or microparticles are added as fillers to polymeric films [46–48]. It has to be stressed that changing CaCl₂ with CaA or CaP has a beneficial effect (i.e., decrease) on O₂ permeance. As a result, the PEC/CMC/OA/CaP/Z film is the least permeable to oxygen ($p\text{O}_2 = 1.4 \times 10^{-15} \text{ mol m}^{-2}\text{s}^{-1}\text{Pa}^{-1}$). Interestingly, the addition of CMC enhances the O₂ barrier properties with respect to PEC-only films (SI5, Table), although cellulose derivatives are usually more permeable to the same gas [7,26]. Moreover, PEC-only films also show a decrease in O₂ permeance on changing the crosslinker from CaCl₂ to CaA and CaP. A rationalization of the differences in O₂ permeance can be put forward by observing the pH values of the film-forming solutions listed in Table 2 (see also SI6, Table) and the data in Figure 1 left (O₂ permeance). The overall trend observed here is that the higher the pH of the film-forming solution, the better the O₂ barrier properties of the obtained films.

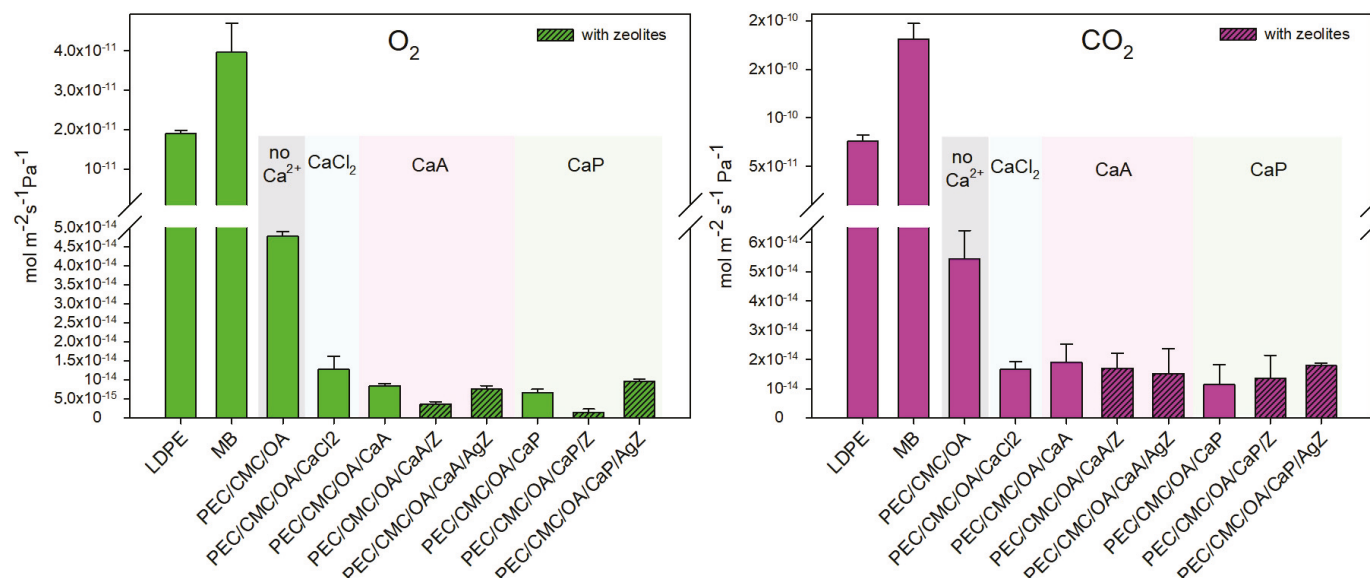


Figure 1. Oxygen (left) and carbon dioxide (right) permeance of composite films, compared with LDPE and MB. Bars for films containing zeolites are represented with a line pattern, and background colors guide the eye to the Ca^{2+} salt used as a crosslinker. All data were measured at 25 °C, RH = 0%. Each value is the average of five measurements. Error bars (standard deviation) are displayed in the graph. Numerical values and statistical analysis are reported in SI4.

Table 2. pH of the film-forming solutions.

Film-Forming Solutions	pH
PEC/CMC/OA	3.89(±4) ^b
PEC/CMC/OA/CaCl ₂	3.50(±1) ^a
PEC/CMC/OA/CaA	4.40(±8) ^c
PEC/CMC/OA/CaA Z	5.00(±2) ^d
PEC/CMC/OA/CaP	4.80(±3) ^d
PEC/CMC/OA/CaP Z	5.71(±7) ^e

Each value is the average of the measurements taken on three samples. Values are reported as mean (standard deviation). Different alphabetical letters in each column indicate significant ($p < 0.05$) differences between means using Tukey's test.

The pKa of PEC is 3.5 [49], and the pKa of CMC is 4.4 [50]. With a 3.5 pKa value, increasing the pH in the 3.5–5.00 range increases the % of deprotonated carboxylic groups of PEC (and CMC) leading to a more effective “egg-box” crosslinking due to the coordination of the COO^- groups of PEC to the Ca^{2+} cation. As a consequence, the structure of the film is denser, and it is more difficult for O_2 to penetrate and cross the films. The pH of the film-forming solutions decreases with the addition of CaCl_2 (3.89→3.50), due to the weak Lewis acid character of the Ca^{2+} cation but increases with CaA (4.40) and CaP (4.80), thanks to the weak base nature of the acetate and propionate anions. Also, the addition of zeolites helps to increase the pH, adding a contribution to lower O_2 permeance, besides their intrinsic role in increasing the tortuosity pattern of gas diffusion.

The nature of the permeants (their molecular volume and affinity to the polymer matrix) affects the mass transfer through the films. CO_2 is a molecule larger than O_2 ; it has a lower diffusivity coefficient but a higher solubility coefficient with respect to oxygen [41]. Figure 1 shows the CO_2 permeance of all composite films compared to that of LDPE and MB. Also, in this case, the permeance of CMC/PEC films is 3–4 orders of magnitude lower than that of LDPE and MB, and a significant decrease is observed with the presence of Ca^{2+} salts as crosslinkers compared to the PEC/CMC/OA film not crosslinked. However, in

the case of CO₂, there is no definite trend in changing the Ca²⁺ salt or adding zeolites, as the higher standard deviations found in all data lead to differences that are not significant ($p < 0.5$) from the statistical point of view (SI4, Table).

Water vapor permeance is a fundamental parameter in packaging applications, especially for a fresh, semi-humid food such as bread [18,42]. The water vapor permeance of the films was measured in semi-dry and humid conditions. The corresponding results are shown in Figure 2 and listed in detail in SI4. As a general observation, on passing from humid to semi-dry conditions, the water vapor permeance decreases of approximately one order of magnitude, i.e., from the 10^{−7} to the 10^{−8} mol m^{−2}s^{−1}Pa^{−1} range. The performance of all the examined films as a barrier to water vapor is not very satisfactory and is worse than that of MB. Only in semi-dry conditions are pH₂O for MB and PEC/CMC/OA/CaP similar (4.0(±2)10^{−8} mol m^{−2}s^{−1}Pa^{−1} and 3.53(±4)10^{−8} mol m^{−2}s^{−1}Pa^{−1}, respectively) and belong to the “medium barrier” group [45]. Comparison with the permeance found for LDPE (1.85(±5)10^{−9} and 1.39(5)10^{−9} mol m^{−2}s^{−1}Pa^{−1} in semi-dry and humid conditions, respectively) evidences the high permeance to water vapor of the films. This is obviously due to the hydrophilicity of polysaccharide-based materials [7] that is only partially mitigated in the present films by the use of OA as the plasticizer. When Z or AgZ are embedded in the films, we observe an increase in the water vapor permeance. The high affinity of Z for water [36] promotes adsorption and transport through the film.

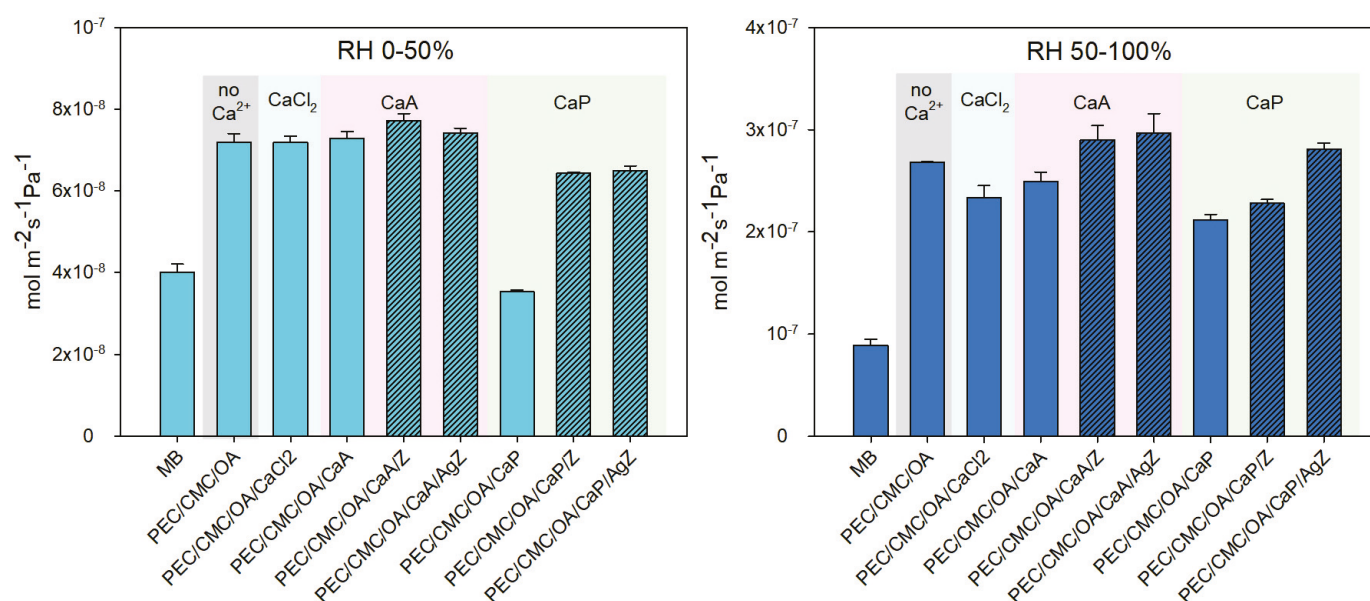


Figure 2. Left: water vapor permeance in semi-dry conditions (RH 0–50%). Right: water vapor permeance in humid conditions (RH 50–100%). Bars for films with zeolites are evidence with a lined pattern; background colors guide the eye to the Ca²⁺ salt used as a crosslinker. Data recorded at 25 °C. Each value is the average of four samples. Error bars (standard deviation) are displayed in the graph. Numerical values and statistical analysis are reported in SI4.

2.2.3. Morphological Characterization

Morphological and chemical analyses of the used zeolites were carried out by SEM-EDS. Figure 3 shows a homogenous grain size distribution before (A) and after (B) the treatment with silver nitrate. The grain dimensions are in the range of micrometers. The silver-loaded zeolites were prepared by suspending plain Z in an aqueous AgNO₃ solution, with 5% Ag mass with respect to Z.

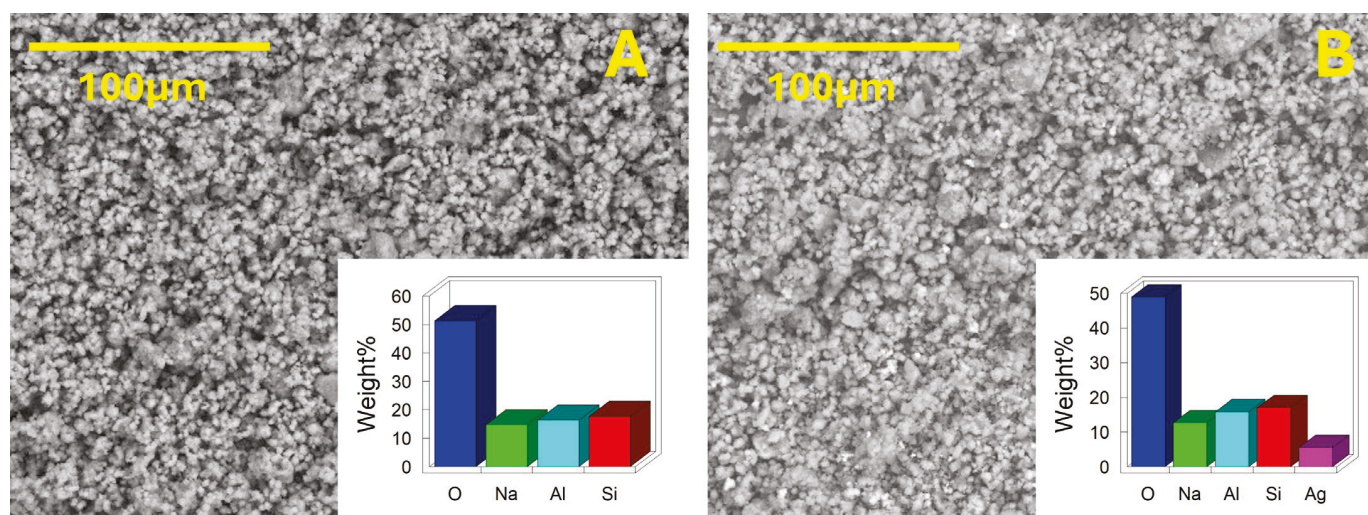


Figure 3. SEM-EDS image of zeolites (A) and silver-treated zeolite (B) powders; the insets show the % weight elemental composition, determined by SEM-EDS.

Zeolites are sodium aluminum silicates, and their SEM-EDS analysis shows the expected presence of O, Na, Al, and Si (Figure 3A, inset). In the case of AgZ, SEM-EDS analysis also displays the additional presence of Ag at $5.4(\pm 2)\%$ *w/w*. Control experiments carried out with 10% and 20% *w/w* Ag/Z solutions showed $10(\pm 1)\%$ and $19(1)\%$ *w/w* Ag, respectively, in AgZ at SEM-EDS analysis (SI7). This indicates that the used procedure gives AgZ solid materials with the same Ag/Z mass ratio as in solution over a large mass ratio range, allowing a fine tuning of the loaded Ag quantity.

The surface (1) and cross-section (2) of PEC/CMC films were studied using SEM. Images are shown in Figure 4 (see SI8 for larger images). The surfaces of the PEC/CMC/OA/CaCl₂, PEC/CMC/OA/CaA, and PEC/CMC/OA/CaP films that do not contain zeolites (A1, B1, and D1) are homogeneous except for a few defects, i.e., bubble-shaped voids. Such bubble-shaped voids are more evident in the cross-sections (Figure 4(A2,B2,D2)). While no difference is brought by the three different counter anions, the Ca²⁺ crosslinker is responsible for such voids. Ca²⁺ bridges the negative chains of pectin by -COO⁻ coordination, with the “egg-box” mechanism, and free volume is formed [51]. However, the O₂ transport experiments have demonstrated that such voids are non-communicating, as the addition of a Ca²⁺ crosslinker actually decreases the O₂ permeance. When zeolites are added (Figure 4(C1,E1)), microparticles are observed on the film’s surface. Cross-section imaging shows that the bubble-shaped voids disappear (Figure 4(C2,E2)). The zeolites are thus homogeneously dispersed in the films, exerting their known filler action [36], giving more homogeneous and compact materials with generally improved barrier properties (Figure 1), and slightly decreasing the stiffness of the films, as can be seen by comparing the YM of PEC/CMC/OA/CaA and PEC/CMC/OA/CaP with that of the same films containing Z or AgZ (Table 1). The SEM-EDS technique was used to determine the quantity of silver by mapping the area of the AgZ-containing films. The quantity of silver determined was $0.47(3)\%$ *w/w* for PEC/CMC/OA/CaA/AgZ and $0.49(3)\%$ wt for PEC/CMC/OA/CaP/AgZ.

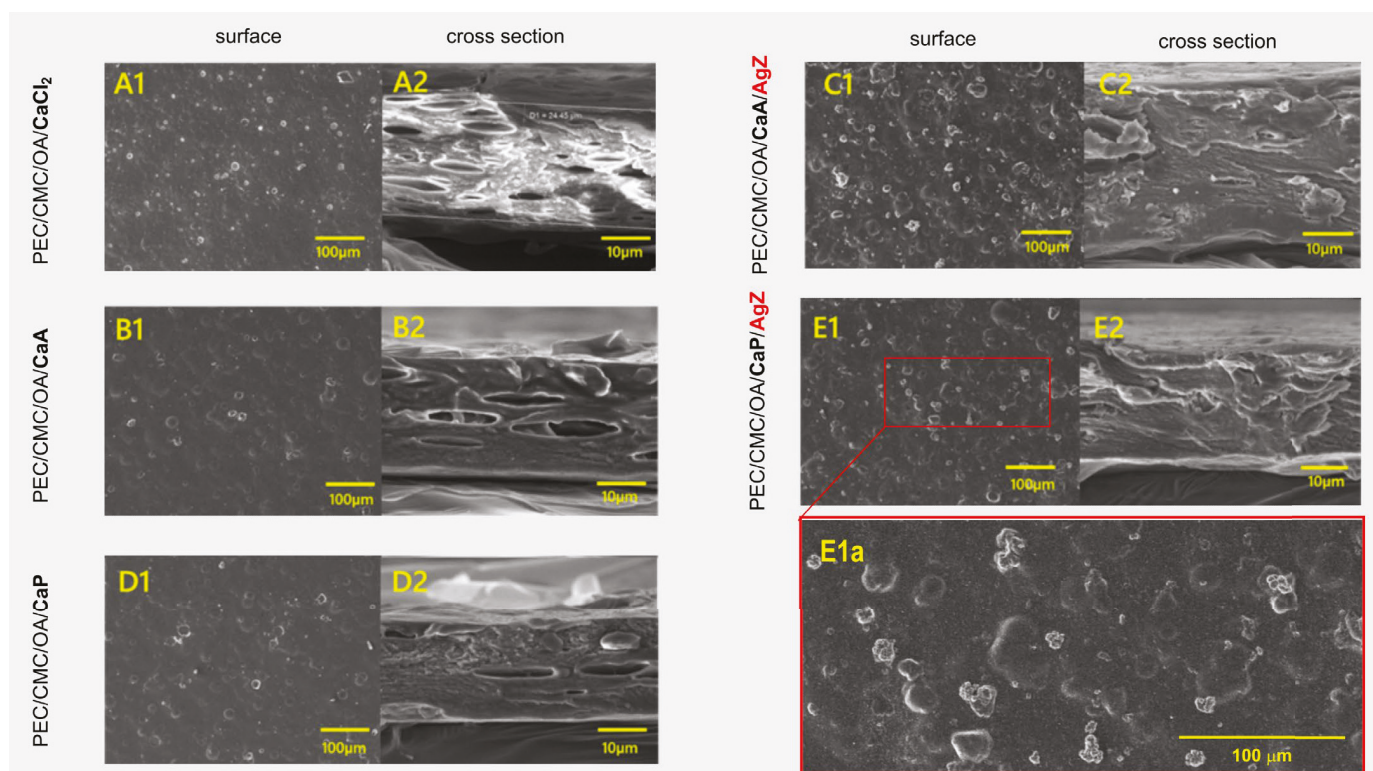


Figure 4. SEM image of the surface (1) and cross-section (2) of pectin/CMC films. (A1,A2) PEC/CMC/CMC/OA/CaCl₂; (B1,B2) PEC/CMC/CMC/OA/CaA; (C1,C2) PEC/CMC/CMC/OA/CaA/AgZ; (D1,D2) PEC/CMC/CMC/OA/CaP; (E1,E1a,E2) PEC/CMC/CMC/OA/CaP/AgZ. The film composition, the surface or cross-sectional typology, and the presence of AgZ are also indicated alongside the images, for the sake of clarity. (E1a) is a blowup of a section of (E1) (delimited by the red dashed rectangle) to evidence the presence of zeolite particles on the film surface. Larger images are available in SI8.

2.2.4. Antimold Activity

The target of this research was to obtain an active polysaccharide packaging film capable of prolonging the shelf life of bread. Accordingly, the antimold activity of the films was tested against the two most common molds formed in bread spoilage, *Penicillium janthinellum* and *Aspergillus niger* [8]. Moreover, a wild-type *Penicillium* mold was used for the same test. This fungal strain was isolated as an indoor contaminant of a grain mill storage [52]. Fungal suspensions were inoculated on cellulose acetate filter membranes and put in contact with PEC/CMC/OA/CaP and PEC/CMC/OA/CaP/AgZ films, as sketched in Figure 5 and described in the Methods section. The PEC/CMC/OA/CaCl₂ film, which does not contain antimold substances, was used as the control.

A total of 96 h was chosen as the contact time because fungi viability is typically noticeable after 3–4 days of incubation [53,54]. The antimold activity (ME, microbicidal effect) is reported in Table 3, and is expressed as follows:

$$ME = \log (N_c/N_d)$$

where N_c is the number of colony forming units (CFU) found with the control, and N_d the number of CFU found in the active films.

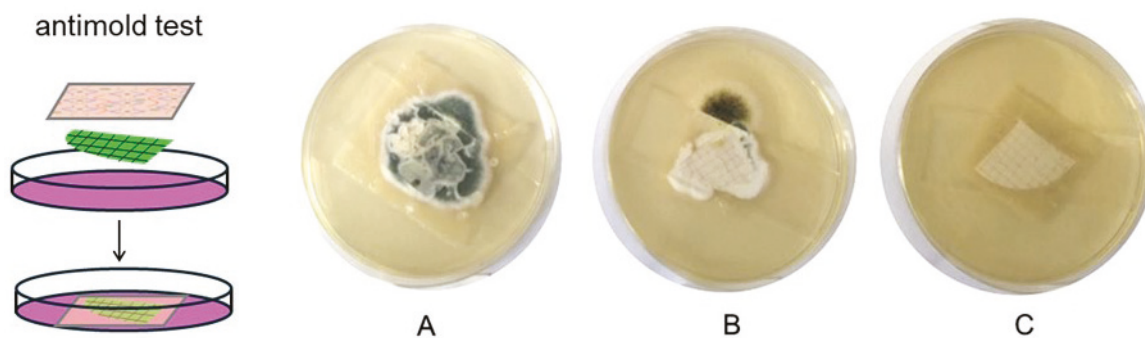


Figure 5. Left, sketch of the setup for the antimicrobial tests: a cellulose acetate membrane inoculated with molds (green, squared texture) is placed on a Petri dish with culture medium (purple) and covered with a film portion; (A–C) membranes (quarter of circle, white, squared texture) inoculated with *P. janthinellum* after 96 h in a Petri dish and coated with PEC/CMC/OA/CaCl₂ (A), PEC/CMC/OA/CaP (B), and PEC/CMC/OA/CaP/AgZ (C) films; a slide for microscopy was placed on top to assure contact.

Table 3. Antimold activity of PEC/CMC/OA/CaP and PEC/CMC/OA/CaP/AgZ against different fungal strains after 96 h of contact; each value is the average of three measurement repetitions, with standard deviation in parentheses.

Fungal Strain	PEC/CMC/OA/CaP ME	PEC/CMC/OA/CaP/AgZ ME
<i>Aspergillus niger</i>	3.36 (±0.34)	3.64 (0.23)
<i>Penicillium janthinellum</i>	2.7 (0.42)	4.71 (0.44)
<i>Penicillium</i> supp. Wild type	2.1 (0.56)	3.94 (0.62)

CaP is a known bread preservative [10], and Ag⁺ is a broad-spectrum antimicrobial whose antimold action has already been demonstrated [22,37]. On the basis of the film formulations, it can be calculated that the film pieces in contact with the inoculated membrane ($4 \times 4 \text{ cm}^2$, 130 mg mass) contain 10.1 mg CaP (PEC/CMC/OA/CaP film) and 9.5 mg CaP plus 0.40 mg Ag (PEC/CMC/OA/CaP/AgZ). As can be seen in Table 3, such small quantities are sufficient to secure a huge mold reduction after 96 h even with just CaP as an active agent (99.9%, 99.8%, and 99.2% reduction for *A. niger*, *P. janthinellum*, and *P. wild type*, respectively). When both CaP and AgZ are present in the film, the mold knockdown after 96 h increases to 99.97% for *A. niger*, reaching a remarkable 99.99% for the two examined *Penicillium* strains (99.998% for *P. janthinellum*). Figure 5 visually displays the appearance of membranes inoculated with *P. janthinellum* after 96 h in a Petri dish, covered with the PEC/CMC/OA/CaCl₂ control film (A), with the PEC/CMC/OA/CaP film (B), and with the PEC/CMC/OA/CaP/AgZ film (C). While in case A the *P. janthinellum* mold has flourished due to the absence of any antimold agent, in case B (film containing CaP) some mold contaminated the Petri dish and grew outside the film-protected area, with only mold traces visible at the edges of the underlying membrane. In case C, the double action of CaP and Ag⁺ kept the membrane perfectly clean and free of mold.

Finally, to qualitatively evaluate the performance of PEC/CMC/OA/CaP and PEC/CMC/OA/CaP/AgZ films as active packaging for bread, shelf life experiments were carried out on freshly produced bread (baked and supplied by Millbo srl, Trecate, Italy) that contained no preservatives. The bread was sliced in $3 \times 3 \times 1.6 \text{ cm}^3$ portions. After baking and during the slicing process, the bread was manipulated in an average, non-sterile environment for ~2 h before packaging. Each bread portion was then enveloped in our films and, for comparison, also in LDPE. We enveloped 30 portions of bread for each type of film, using $10 \times 6 \text{ cm}$ film sections that were externally wrapped in LDPE to

avoid further environmental contamination. As shown in Figure 6, such a setup allowed the direct visual examination of the bread surface to search for mold traces. We carried out such visual inspections each day since time 0.

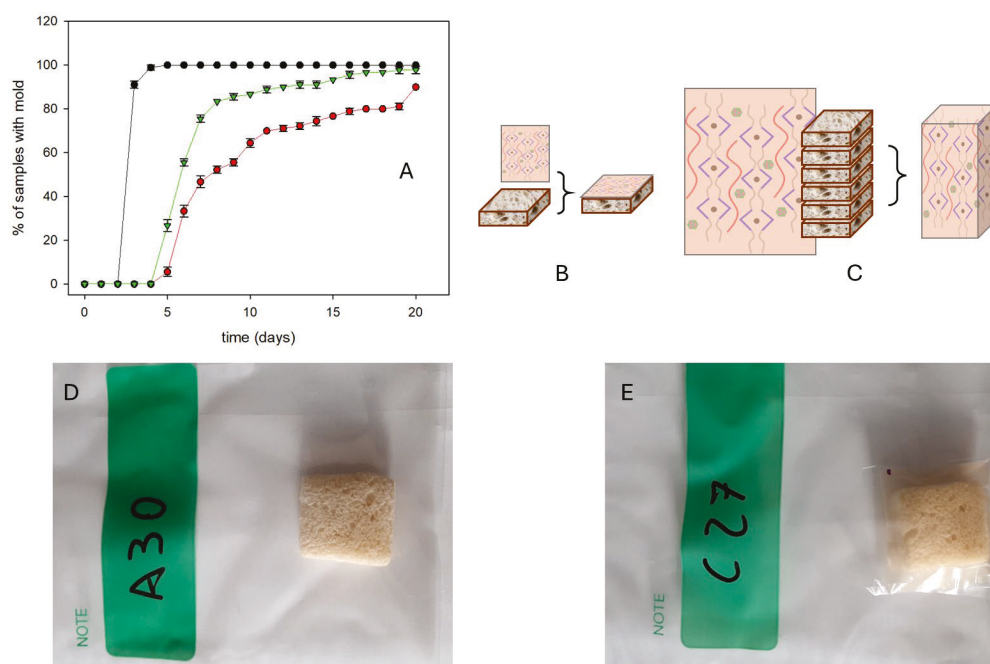


Figure 6. (A) Shelf life experiment on bread without preservatives, conducted through visual inspection of samples showing mold traces over time (days) (see the inset for the film used as packaging) (average values on three experiments); (B) setup for Ag⁺ release from the film to a single bread slice; (C) same setup for a stack of six bread slices; (D,E) photographs of bread samples (3 × 3 × 1.6 cm) for the shelf life experiments: a bread slice in the LDPE envelope (D) and a bread slice wrapped in a PEG/CMC/OA/CaP film inside the LDPE envelope (E).

Figure 6A displays the percentage of samples with mold over the total vs. time (days) and the average values for three repetitions. While with the non-active LDPE packaging, mold is developed on 90% of the samples after 2 days and on 100% after 3 days, no mold is visible for the first 3 days with the PEC/CMC/OA/CaP/AgZ and PEC/CMC/OA/CaP films. Moreover, the rate of contamination is lower for the PEC/CMC/OA/CaP/AgZ film than for the PEC/CMC/OA/CaP film, consistent with the ME data listed in Table 3.

2.2.5. Release of Active Species to Bread

A common sliced bread package available in European stores contains 400 g of bread and has 9 × 9 × 22 cm³ dimensions. Considering the maximum permitted levels of CaP defined by EC regulations (0.2% *w/w* in bread [11]), such an amount of bread is allowed to contain up to 800 mg CaP. The total surface of the packaging film in contact with bread is <900 cm². If one supposes to use the PEC/CMC/OA/CaP films for the same sachet, the total quantity of CaP contained in a 900 cm² film area would be 0.57 g, i.e., even lower than the quantity allowed in the bread. This simple consideration would suggest by itself the safe use of the PEC/CMC/OA/CaP films for bread packaging. However, in view of using this material for bread packaging, it is important to show that the quantity of CaP transferred from the packaging to bread is indeed very low, as this would avoid the ingestion of large CaP quantities by the consumers. An attempt was made to determine the CaP released to 5.0 g bread slices put in contact for 7 days with the PEC/CMC/OA/CaP films. A published method was used [55]; see the Methods section for details. The released CaP was lower than the LOD reported for the method. This does not allow us to determine

the exact quantity of CaP released to bread, but it allows us to state that such quantity is lower than 2.0 mg for each bread slice. Noticeably, the maximum EC allowed quantity would be 10 mg in the same sample. On the other hand, the release of Ag⁺ to bread from the PEC/CMC/OA/Ca/AgZ film has to be assessed accurately, as the allowed quantities for this cation in bread are particularly low (e.g., <0.05 mg/kg of bread according to EFSA [39]). The Ag⁺ film-to-bread release test was carried out in two different setups. The first setup is the same used for CaP release (Figure 6B): a 5 g slice of bread ($3 \times 4 \times 1.6 \text{ cm}^3$) was topped with a $3 \times 4 \text{ cm}^2$ portion of the PEC/CMC/CaP/AgZ film (100 mg, containing 0.31 mg Ag). The second setup (Figure 6C) had the aim to mimic a real sliced bread package: six bread pieces ($3 \times 4 \times 1.6 \text{ cm}^3$ each, total mass 30.0 g) were enveloped in the PEC/CMC/CaP/AgZ film, with a total contact surface of 158.4 cm^2 (a film area containing 4.1 mg Ag). Both types of samples were further enveloped in aluminum foil and kept in the dark under controlled 100% humidity, and after 1, 3, and 7 days, the bread was mineralized. ICP-OES analysis followed, and the quantity of silver released to bread was determined. In each test condition, see Table 4, the quantities of Ag⁺ released after 1, 3, and 7 days are very similar, indicating that the film-to-bread Ag⁺ transfer is complete within 24 h of contact. In the case of the stack of bread slices, Figure 6C, the lower and more variable values are to be attributed to the loose contact between the bread and the film envelope, this actually mimicking real shelf conditions. Except for the small exceedance of setup B at 3 days of contact, all the found values are reassuringly equal to or lower than the 0.05 mg/kg limit established by EFSA [39]. While this result is satisfactory, it must also be stressed that the quantity of Ag in the films could be easily fine-tuned to lower values by adjusting either the Ag mass percentage in AgZ or the amount of AgZ added to the film-forming solutions.

Table 4. Release of silver from PEC/CMC/OA/CaP/AgZ composite film.

Days of Contact	B: Small Samples, 5 g of Bread mg Ag/kg Bread	C: Piled Samples, 30 g of Bread mg Ag/kg Bread
1	0.05(±0.01)	0.043(±0.004)
3	0.06(±0.02)	0.030(±0.005)
7	0.05(±0.01)	0.03(±0.01)

Each value is the average of the measurements taken on three samples. Values are reported as mean ± standard deviation.

2.2.6. Further Characterization in View of Use as Films for Packaging

In the FT-IR spectra of the films, the PEC features dominate all the spectra, as PEC is the main component. Neat PEC has a broad absorption around 3300 cm^{-1} (stretching vibration of -OH groups), a three-peak band at 2927 cm^{-1} (stretching vibration of methyl and methylene groups), a band at 1739 cm^{-1} (C=O stretching of the ester group) and a larger one at 1618 cm^{-1} (vibration of the carboxylic acid and carboxylate anion), plus a complex fingerprint envelope at lower wavenumbers [25,27,31]. Such spectral features are maintained in all the prepared films. The 1739 cm^{-1} band decreases in intensity due to the partial hydrolysis of PEC esters during the film preparation, and in the carboxylic acids/carboxylate bands envelope the contribution of -COOH decreases, while the carboxylate band increases (apparent shift to $1618\text{--}1583 \text{ cm}^{-1}$), mostly due to the PEC-COO- groups coordinated to Ca²⁺ [31]. Slight variations in this spectrum range are observed in the various film formulations due to the diverse contributions of OA, of the acetate and propionate anions, and of the different pH during the synthesis. All spectra are shown in Figure 7C.

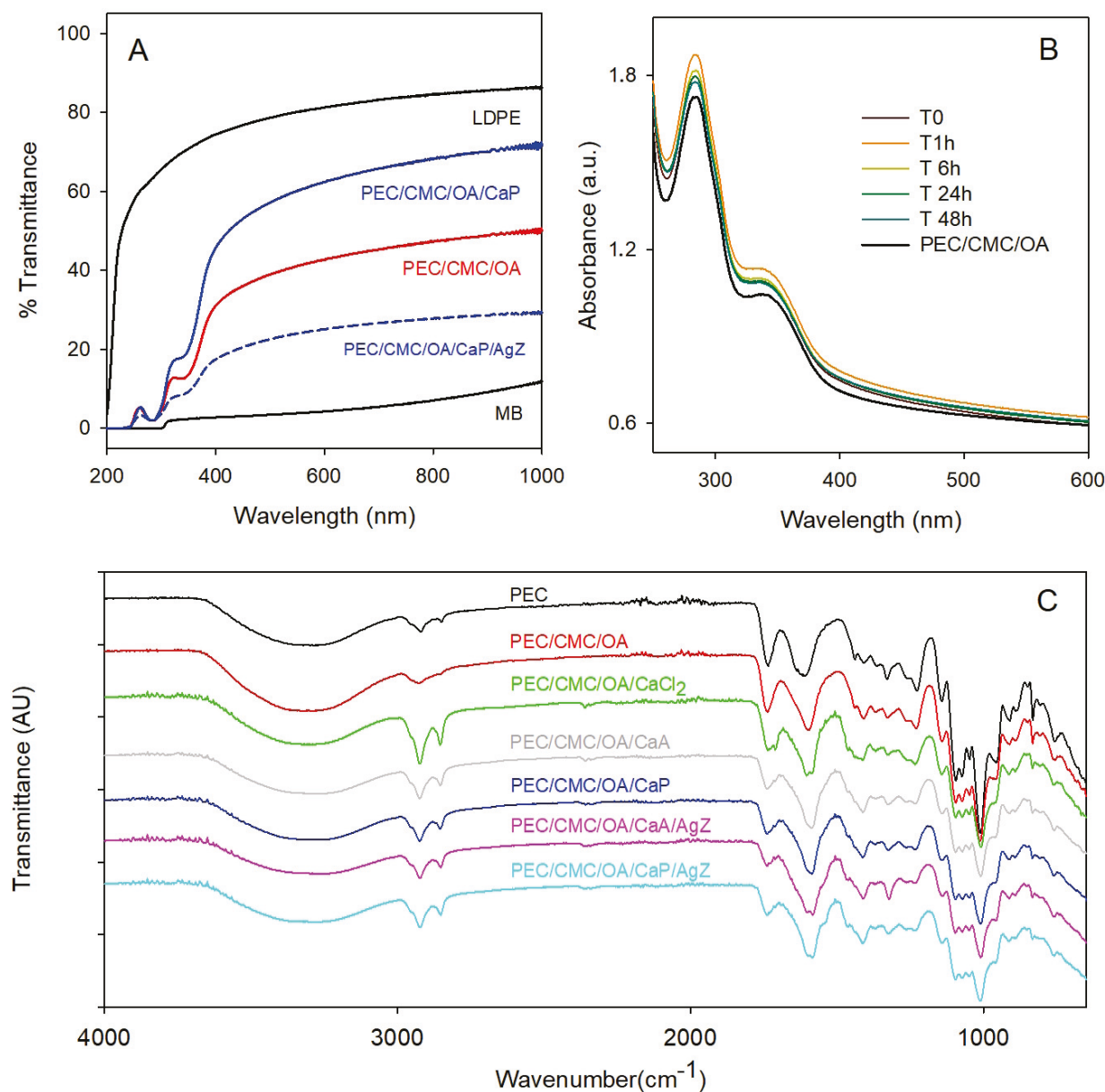


Figure 7. (A) Transmission spectra of representative PEC/CMC films, compared with those of LDPE and MB (see the panel for spectra attribution). (B) Absorption spectra vs. time of the PEC/CMC/OA/CaP/AgZ under exposure to environmental light; the absorption spectrum of PEC/CMC/OA (black line) is included for comparison. (C) FT-IR spectra.

Transparency is an additional parameter to be evaluated in food packaging, depending on the packaged food [56]. Also, the UV screen ability of a film must be considered, since UV light may induce photo-oxidation of food or damage photosensitive substances [57]. The transmission spectra of all the composite films were recorded from 250 to 1100 nm (SI9A). The representative spectra of PEC/CMC/OA/CaP and of PEC/CMC/OA/CaP/AgZ are displayed in Figure 7A, together with the transmission spectra of the non-crosslinked PEC/CMC/OA film and of the MB and LDPE films. Transparency is related to the amount of visible light that crosses a film, and it is evaluated by the % transmittance at 550 nm [56]. The crosslinked PEC/CMC/OA/CaP film is more transparent than its non-crosslinked version, PEC/CMC/OA, with T at 550 nm = 52(1)% and 11.3(4)%, respectively, while the addition of Z contributes to the opacity of a given formulation, decreasing T to 21.6(5)% for PEC/CMC/OA/CaP/AgZ. However, as it was already mentioned in describing the shelf life experiment, the transparency in the visible range is enough to actually see the

enveloped product and to appreciate its color and texture features. On the other hand, the LDPE transparency is by far superior (T at 550 nm = 80%), while that of the opaque MB is extremely poor (T at 550 nm = 4%). UV screen ability is evaluated by the % transmittance at 280 nm. All the PEC/CMC films are UV-blocking, with a transmittance < 3% at this wavelength, a value one order of magnitude lower than that of the UV-semitransparent LDPE (62.7%) and in agreement with what was already found for pectin-based films [7]. UV-vis absorption spectra were also recorded from 250 to 1100 nm to check the characteristic peaks of pectin and, in the AgZ-containing film, also the eventual formation of nanoparticles during environmental light exposition (promoted by the Ag^+ to $\text{Ag}(0)$ photoinduced reduction). The two characteristic bands of pectin are centered at 283 nm and 341 nm, due to $n \rightarrow p$ transitions in carbonyl groups of carboxylate or ester moieties [58], as can be seen in Figure 7B for the representative PEC/CMC/OA film (SI9B for all spectra). The spectrum of the PEC/CMC/OA/CaP/AgZ film (spectrum at T0 in Figure 7B) is identical to that of PEC/CMC/OA. The typical LSPR band of silver nanoparticles, centered at 390–400 nm [22], is not present, and it did not form during 48 h of exposition to environmental light (T1 h–T48 h spectra, Figure 7B).

2.3. Functional Properties of PEC/CMC Film-Forming Solutions Used as Coatings

In the previous sections it has been demonstrated that the PEC/CMC/OA/CaP and PEC/CMC/OA/CaP/AgZ films have brilliant antimold activity and excellent O_2 and CO_2 barrier ability. On the other hand, their permeance to water vapor is high. While the use of such materials as self-standing films can be imagined to increase the shelf life of fresh bread for short periods (days range), it seems less feasible for long-term packaging, e.g., in the case of sliced bread. Considering its matrix of polysaccharides and cellulose, which are biodegradable, the possibility of using our films as a coupled component for paper packaging was investigated from the perspective of increasing the food preservation capacity of this popular material while maintaining its environment-friendly nature. Polysaccharide-based film-forming solutions have been found suitable as coaters for paper [6,7]. Rheological properties are the crucial parameters to choose the correct technique of coating. By investigating the relationship between apparent viscosity and shear rate, we found that for all the PEC/CMC-based films, the apparent viscosity of their film-forming solutions decreases as the shear rate increases, with a shear-thinning behavior (SI10, A). Spraying, brushing, and roller methods require low viscosity values (<0.5 Pa s) at high shear rates ($>1000 \text{ s}^{-1}$) [59]. These would not be the methods of choice for most of the PEC/CMC films, as only PEC/CMC/OA/ CaCl_2 and PEC/CMC/OA/CaA/Z fit this parameter. Dip-coating necessitates viscosity values $> 10 \text{ Pa s}$ when low shear rates are applied (10 s^{-1}) [59]. All the PEC/CMC films are suitable for this method. The viscous properties of the PEC/CMC film-forming solutions were measured at different shear rates, from 0 s^{-1} to 200 s^{-1} and then back from 200 s^{-1} to 0 s^{-1} at 25(1) °C with a rotational rheometer, and the experimental data were fitted with the Ostwald de Waele model (SI10, B). The results for the model parameters are shown in Table 5. An R^2 value near 1 was found in all cases, indicating that the Ostwald de Waele model could be confidently used to describe the rheological properties of all the PEC/CMC film-forming solutions. The flow behavior index (n) correlates with the concentration of crosslinker ions, decreasing with the addition of Ca^{2+} ions and implying an increase in pseudoplasticity [60]. Furthermore, while neat PEC films without Ca^{2+} have $n = 0.940(3)$, the addition of CMC decreases n to 0.774(4) in PEC/CMC/OA. The K value corresponds to the consistency of the fluids, with higher K values meaning higher viscosity [60]. Also, in this case (Table 5), the parameter strongly depends on the presence of crosslinker calcium ions, increasing by an order of magnitude from the PEC/CMC/OA film-forming solution, $K = 2.01(7)$, to $K = 20(2)$ in the

case of PEC/CMC/OA/CaCl₂. On changing calcium salts to CaA and CaP and on adding zeolites, the K value further increases, an effect that is connected to the increase in pH in the film-forming solutions (Table 2) that in turn increases the efficiency of the crosslinking between PEC carboxylates with the “egg-box” mechanism, as we have already observed for the barrier effect towards gases.

Table 5. Fitting parameters of the Ostwald de Waele model for pectin film-forming solutions and thixotropy values.

Film-Forming Solutions	Ostwald de Waele Model Fitting Parameters			Thixotropy (ΔA Pa/s)
	K	N	R ²	
PEC/CMC/OA	2.01(± 7) ^a	0.744(± 4) ^d	0.99(± 1)	566(± 45) ^a
PEC/CMC/OA/CaCl ₂	20(± 2) ^b	0.45(± 2) ^c	0.9991(± 3)	3780(± 1221) ^b
PEC/CMC/OA/CaA	31(± 3) ^c	0.44(± 1) ^{b,c}	0.9988(± 5)	3812(± 691) ^b
PEC/CMC/OA/CaA/Z	57(± 3) ^e	0.308(± 7) ^a	0.996(± 2)	5878(± 488) ^c
PEC/CMC/OA/CaP	40(± 1) ^d	0.422(± 4) ^b	0.9989(± 2)	4595(± 294) ^{b,c}
PEC/CMC/OA/CaP/Z	35.1(± 6) ^c	0.413(± 2) ^b	0.9992(± 5)	3993(± 232) ^b

Each value is the average of the measurements taken on four samples. Values are reported as mean (standard deviation). Different alphabetical letters in each column indicate significant ($p < 0.05$) differences between means using Tukey’s test.

By calculating the surface area of the hysteresis loop in the shear stress vs. shear rate profiles (SI10, Figure S2B), the thixotropic properties of the film-forming solutions were also determined and added to Table 5. Thixotropy is the property of certain gels of becoming thinner when a constant force is applied, with the viscosity fully recovering the initial state in an appropriate period of time after reduction of the applied force [61,62]. It influences the way in which paints level out, and, when high, it ensures a sufficient and consistent wet layer thickness for coating applications [61,62]. Table 5 shows that thixotropy increases with the addition of crosslinking Ca²⁺ ions, stepping from 566(45) Pa/s for PEC/CMC/OA to 3780(1221)Pa/s for PEC/CMC/OA/CaCl₂. A further thixotropy increase is observed with the Ca²⁺ salts having a weak base as counter anion (CaA and CaP). As observed for the fluid consistency, this trend is connected to the increased interactions between the polymer chains of pectin due to Ca²⁺ coordination [60]. All the collected data point towards the possibility of using the film-forming solutions of our PEC/CMC/OA/CaP and PEC/CMC/OA/CaP/AgZ as coatings with dip-coating methods.

3. Materials and Methods

3.1. Materials

Pectin from citrus peel (PEC, galacturonic acid $\geq 74.0\%$ dried basis, methoxy group $\geq 6.7\%$), carboxymethylcellulose (CMC, average $M_w \sim 250,000$, degree of substitution 0.7), oleic acid (OA, cis, purity $\geq 90\%$), zeolites (Z, molecular sieves 4A, powder, ~ 325 mesh particle size), calcium acetate monohydrate (CaA, CAS 114460-21-8, purity $\geq 99\%$), and calcium propionate (CaP, CAS 4075-81-4, purity $\geq 97\%$) were all purchased from Sigma-Aldrich, Milan, Italy. Silver nitrate was purchased from Fluka (Milan, Italy), and calcium chloride was purchased from Merck (Milan, Italy). Commercially available low-density polyethylene (LDPE) films were obtained from Conad (Pavia, Italy) bread packaging. Commercially available MaterBi (MB) was purchased in store.

3.2. Methods

3.2.1. Experimental Preparations

Silver-loaded zeolites (AgZ). A total of 0.140 g (0.824 mmol) of AgNO₃ were dissolved in 100 mL of bi-distilled water at room temperature (RT), and 1.8 g of Z were added to the mixture. The blend was stirred in the dark for 16 h at RT to allow Ag⁺ loading by ion

exchange. The AgZ was then filtered and washed with 3×15 mL bi-distilled water and dried overnight at 80 °C. They contained 4.9% *w/w* Ag with respect to Z mass.

Film preparation. Two solutions were prepared: (i) 3.0 g PEC was dissolved into 50 mL of water at 80 °C under magnetic stirring; (ii) 0.90 g CMC was dissolved in 25 mL of water at 70 °C and kept under magnetic stirring until complete dissolution. A total of 0.30 g of OA was added to the solution of PEC. The two solutions were mixed at 75 °C under magnetic stirring. The following additives were then added:

- (a) Ca^{2+} salts: Calcium chloride (CaCl_2), calcium acetate monohydrate (CaA), or calcium propionate (CaP) were used as crosslinkers. A total of 1.9 mmol was dissolved in 25 mL of water and added to the PEC/CMC solution under vigorous stirring at 75 °C. After 30 min of stirring, the solution was allowed to cool at RT.
- (b) AgZ or plain Z in a 10% *w/w* proportion with respect to PEC was suspended in 1 mL of water and added at RT.

Precursor solutions for film formation were also prepared with Ca^{2+} salts but without the addition of Z or AgZ. To obtain polymeric films with a precise and homogeneous thickness, we used a tape casting method with a “doctor blade” manual instrument built in our laboratories. The blade was regulated at a 1200 μm height from the base. With the described quantities, 2 sheets of 12×20 cm^2 were prepared. After tape casting, the films were kept in an oven at 40 °C for 18 h. The average final thickness of films was $50(\pm 5)$ μm .

3.2.2. Characterization of PEC/CMC Films

Thickness. The thickness of the films was measured by using a micrometer Thickness Gauge FD-100 with a resolution of 1 μm (Hans Schimdt & Co. GmbH; D-84478, Waldkraiburg, Germany), averaging five measurements on different points of the same film.

Mechanical properties. The mechanical behavior of the films was evaluated by uniaxial tensile test under ambient conditions ($T = 25$ °C, $\text{RH} = 50\%$) using the TA.HD+ Texture Analyzer testing machine (Stable Micro Systems, Godalming, UK), equipped with a 5 kg load cell. From six to nine specimens (1×3 cm^2) were cut for each film and then clamped on an A/TG tensile grip probe. An initial distance of 1 cm was set between the grips. The upper grip was raised at a constant speed of 0.5 mm/s up to a distance of 10 mm. Young’s modulus (YM) (GPa), tensile strength (TS) (MPa), and elongation at break (E%) (%) were determined from the stress–strain curves.

Barrier properties to oxygen and carbon dioxide. The permeance of the films to oxygen and carbon dioxide ($P_{\text{O}_2} - P_{\text{CO}_2}$) was assessed by a manometric method using a GTP-c permeameter (Brugger Feinmechanik GmbH, Munich, Germany) at 25 °C under 0% relative humidity conditions. The system was outgassed under a primary vacuum before testing. At time zero, one side of the film was flushed with the gas (flow $27 \text{ cm}^3 \cdot \text{min}^{-1}$), and the increase in pressure on the other side was recorded over time. The partial pressure differential was 1000 hPa. The experiments were performed in quadruplicate. The permeance (expressed in $\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \cdot \text{Pa}^{-1}$) was determined from the steady state according to Equation (1), written as follows:

$$P = \frac{\Delta n}{A \cdot \Delta t \cdot \Delta p} \quad (1)$$

where P is permeance, Δn is the average molar quantity associated with the gas transfer (mol), A is the surface area of the film (m^2), Δt is the time (s), and Δp is the pressure difference between the two sides of the film (Pa).

Barrier properties to water vapor. The permeance of the films to water vapor ($P_{\text{H}_2\text{O}}$) was determined according to ASTM E96 [63]. This gravimetric method was conducted at 25 °C under two different relative humidity conditions: semi-dry (0–50% RH) and humid (50–100% RH). For this purpose, permeability cells (3.4 cm inner diameter) were

used, with the film held between two silicon O-rings at the top and placed in a climatic chamber at 25 °C and 50% RH. To obtain the semi-dry condition, previously dried silica gel was put inside the permeability cell to reach a relative humidity close to 0%. For the humid condition, distilled water was placed in the cells to maintain the relative humidity close to 100%. The cell weight was measured once a day using an analytical balance with an accuracy of 0.1 mg (Sartorius Analytical balance; Sartorius Lab Instruments GmbH & Co. KG—Goettingen, Germany). The permeance to water vapor was determined from the steady state of the transfer according to Equation (1). The analyses were carried out in quadruplicate.

Interaction with light. UV-vis absorption and transmission spectra were recorded on a Varian Cary 6000 spectrophotometer with a dedicated sample holder for films. Transmittance and absorption spectra were recorded between 200 nm and 1100 nm. The evaluation of stability under environmental light was conducted by the exposure of the films to the ambient light of the laboratory (sun and neon), and the absorption spectra were collected after 1, 6, 24, and 48 h. Transparency of the films was given as the percentage of transmittance at 550 nm, and UV screening ability by the percentage at 280 nm. The values obtained were divided by the exact thickness of the measured sample and multiplied by 50 µm for normalization.

Determination of the CaP transfer to bread. Four 5.0 g samples of commercial bread (slices of $3 \times 4 \times 1.6 \text{ cm}^3$) were kept in contact for 7 days with a $3 \times 4 \text{ cm}^2$ portion of the PEC/CMC/OA/CaP film (100 mg mass, containing 7.8 mg CaP). The bread samples were soaked in 5.0 mL of water each for two hours, then the solutions were filtered, and the CaP content was analyzed according to a published colorimetric method, used for the quantitative determination of CaP contained in bread [55], by treating each solution with 250 µL of $\text{NH}_4\text{Fe}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$ solution in water (0.8 g/10 mL). No sample gave detectable CaP. The reported method has an LOD of 0.4 mg/mL, so it can be stated that the CaP quantity transferred to bread was <2.0 mg.

Determination of silver release to bread. A total of 100 mg portions ($3 \times 4 \text{ cm}^2$) of AgZ-containing film were put in contact with a $3 \times 4 \times 1.6 \text{ cm}$ slice of commercially available bread (5.0 g, Pan Bauletto Bianco, Mulino Bianco, Barilla, Italy) and enveloped in aluminum foil. The samples were kept in a glass bowl with RH 100% at RT and analyzed after 1, 3, and 7 days. At the chosen time, the bread was mineralized (1:5 H_2O_2 - HNO_3 at 100 °C for 30 min in a total volume of 10 mL), and 1 mL of the obtained solution was diluted to 5 mL with bidistilled water. The Ag content of the sample was analyzed by ICP-OES on a Perkin Elmer Optima 3300 DV instrument (Milano, Italy).

In the attempt to mimic a pack of sliced bread, larger portions of each film ($12 \times 20 \text{ cm}^2$, 2.0 g) were used to envelop six piled-up $3 \times 4 \times 1.6 \text{ cm}^3$ slices of commercial bread (5.0 g each, 30.0 g total mass; Pan Bauletto Bianco, Mulino Bianco, Firenze, Italy). The pack was in turn enveloped in aluminum foil and kept in a glass bowl with RH 100% at RT for 1, 3, and 7 days. After these times, all six slices of bread were mineralized together (1:5 H_2O_2 - HNO_3 at 100 °C for 30 min, 50 mL total volume), and 5.0 mL of the obtained solution were diluted to 25 mL and the Ag content was analyzed by ICP-OES on a Perkin Elmer Optima 3300 DV instrument.

Morphology characterization and quantification of silver. Morphology characterization was carried out by scanning electron microscopy (SEM). For samples containing Z and AgZ, ~5 mg of each sample were directly attached onto the stub by using carbon tape and then sputtered with carbon and imaged with a Scanning Electron Microscope (SEM) Zeiss EVO MA10 (Carl Zeiss, Oberkochen, Germany). The SEM-EDS analysis of Z and AgZ was obtained with the same instrument, coupled with Xmax microprobe (Oxford, UK). The images were acquired at high voltage (20 kV), in high vacuum, at room temperature, and

at different magnifications. The SEM images of the films were achieved with a Tescan Mira XMU-FEG SEM instrument (Brno, Czech Republic) on films ($1 \times 1 \text{ cm}^2$) attached to the stub with carbon tape and then sputtered with carbon (thickness $\leq 10 \text{ nm}$). The films were also analyzed by SEM-EDS as previously described.

Antimicrobial activity test. The antimicrobial activity of the films containing either CaP or CaP and AgZ was evaluated against three fungal strains: *Aspergillus niger* ATCC 16404, *Penicillium janthinellum* ATCC 20312, and *Penicillium* spp. wild type. Fungal suspensions were filtered with an initial inoculum of approximately $1\text{--}2 \times 10^5 \text{ CFU/mL}$ (colony forming unit/mL) on filter membranes of cellulose acetate with a porosity of $0.22 \mu\text{m}$. The membranes were then deposited on Petri plates containing a culture medium/agar suitable for the growth of microorganisms selected. The filter membranes were covered with films containing CaP or CaP and AgZ films for 96 h. Similarly, plates were prepared containing membrane filters covered with a control film, prepared with CaCl_2 as the crosslinker and containing no AgZ or CaP. After the contact time, the filter membranes were recovered and washed by suspending them in sterile water with a standardized method [64]. After washing, dilutions of the microbial suspensions were made to determine the microbial content with subsequent sowing in a plate and calculation of the microbicidal effect (ME). This was calculated for each test organism and contact time according to the following equation:

$$\text{ME} = \log(N_c/N_d)$$

where N_c is the number of CFU of the microbial suspension after contact with the control film, and N_d is the number of CFU of the microbial suspension after contact with the CaP or CaP and AgZ film.

Fourier-transform infrared spectroscopy (FTIR). FTIR analysis was carried out with a Bruker IFS 28 (Milano, Italy) equipped with a DTGS detector and working in a spectral range of $4000\text{--}600 \text{ cm}^{-1}$, with a resolution of 2 cm^{-1} . A total of 32 scans were collected to obtain a high signal-to-noise ratio.

Shelf life evaluation of bread slices. The bread used for this experiment was supplied by Millbo s.r.l. (Trecate, Italy) and was prepared without any type of preservatives.

- A total of 30 slices of bread ($3 \times 3 \text{ cm}^2$ each) were packed in common LDPE film closed with a heat sealer.
- A total of 30 slices of bread ($3 \times 3 \text{ cm}^2$ each) were packed and sealed in a CaP film and then further packed in an external LDPE bag.
- A total of 30 slices of bread ($3 \times 3 \text{ cm}^2$) were packed in a CaP + AgZ film and then further packed in an external LDPE bag. All the bags were maintained in the dark at a controlled temperature (25°C), and visual evaluation of the presence of molds was conducted twice a day. The shelf life experiment was repeated three times.

Thermal analysis. The evolution of the thermal events of the films was studied by Differential Scanning Calorimetry (DSC) using a Q20 calorimeter (T.A. Instruments, New Castle, DE, USA). Film samples were weighed ($3\text{--}5 \text{ mg}$) and sealed into aluminum pans (T-zero, T.A. Instruments, New Castle, DE, USA) before being subjected to a double heating-cooling cycle at $10^\circ\text{C}\cdot\text{min}^{-1}$ under N_2 atmosphere (flow rate = $25 \text{ mL}\cdot\text{min}^{-1}$), from 0 to 300°C .

3.3. Characterization of the Film-Forming Solutions

The pH of the film-forming solutions was measured using a pH meter (Instruments pH-meter (pH 50 model) with a Thermo Scientific Orion 91022 BNWP (Monza, Italy) combined glass electrode). Electrode calibration was carried out before measurements,

with solutions buffered at pH = 4, pH = 7, and pH = 10. The samples were equilibrated to ambient conditions ($T \approx 25\text{ }^{\circ}\text{C}$) before analysis. The analysis was performed in triplicate.

Rheological properties. A rotational rheometer (HAAKETM RotoViscoTM 1; Thermo Fisher Scientific, Boston, MA, USA) was used to determine the rheological properties of the solutions at $25 \pm 1\text{ }^{\circ}\text{C}$. For the analysis, a Z34DIN sensor with a rotor No. 222-1499 (radius = 17.000 mm, length 51 mm, clearance bottom 7.2 mm) and a cup No. 222-1498 (radius = 18.44 mm), which makes a gap of 1.44 mm between the rotor and the cup. The rheological properties were measured at different shear rates from 0 s^{-1} to 200 s^{-1} . The measurements were performed in triplicate. The experimental data of all the solutions' flow curves were analyzed using the Ostwald de Waele model fitting, written as follows:

$$\tau = \kappa \dot{\gamma}^n \quad (2)$$

where τ is the shear stress (Pa), $\dot{\gamma}$ is the shear rate (s^{-1}), κ is the consistency index ($\text{Pa}\cdot\text{s}^n$), and n is the flow behavior index (dimensionless).

The apparent viscosity of the film-forming solutions was extrapolated using the κ and n constants that were previously calculated to predict the apparent viscosity of the solutions at 1000 s^{-1} . The values of apparent viscosity at 10 s^{-1} and 1000 s^{-1} were chosen for the comparison of the different solutions.

3.4. Statistical Analysis

Statistical analysis of the data was performed using IBM (Armonk, NY, USA) SPSS statistics 20 software. The data were ranked, and statistical differences were evaluated using a one-way analysis of variance (ANOVA) and Tukey's multiple comparison tests. In all cases, a p -value < 0.05 was considered significant.

4. Conclusions

PEC and CMC are biodegradable polymers obtained from renewable sources. In this paper it has been shown that new self-standing films based on a mixture of the two polymers can be obtained with enhanced preservative features using OA as a non-hydrophilic plasticizer, CaP as the crosslinker, and AgZ as an Ag^+ releasing additive. The combination of these components leads to a satisfactory mechanical behavior of the films and to excellent gas barrier properties for O_2 and CO_2 . Comparison with the gas barrier properties of the market-standard films LDPE (currently used for bread packaging) and MB (a commonly used material for biodegradable sachets) may have some limitations—due to the presumably different preparation techniques of the two commercial films—but impressively lower (3–4 orders of magnitude) CO_2 and O_2 permeance values are found anyway. It has also been found that due to the hydrophilic nature of PEC and CMC, the water vapor barrier exerted by the films remains instead low, i.e., far from that of LDPE and comparable to that of MB. From the preservation point of view, the films prepared with CaP as the crosslinker exert a drastic anti-mold effect, both on mold lines inoculated on a membrane and on real bread samples. Significantly, such an effect is obtained by avoiding the addition of the CaP preservative in the bread and moving it to the packaging, this allowing only trace quantities ($<0.04\%$ w/w) to be released during contact. The preservative properties of this active packaging, PEC/CMC/OA/CaP, are further improved by adding a source of the antimicrobial Ag^+ ions that are released in very small traces from the films. This is an innovation: while Ag^+ release from active packaging to food is commonly obtained using AgNP, in this research nanoparticles have been intentionally avoided, as they are generally not allowed as food contact materials by regulatory agencies. AgZ were instead embedded in the films, i.e., micrometric zeolite powders were used, loaded with Ag^+ cations. Moreover, it has been experimentally verified that the Ag^+ released from PEC/CMC/OA/CaP/AgZ to bread

is lower than 0.05 mg/Kg of bread, which is the quantity allowed by EU regulation [39]. Finally, it has been found that the PEC/CMC/OA/CaP and PEC/CMC/OA/CaP/AgZ films are excellent UV filters while they are transparent in the visible, allowing visual examination of the enveloped food.

While the two PEC/CMC/OA/CaP and PEC/CMC/OA/CaP/AgZ films are not competitive with the currently used LDPE packaging for bread, if a very long shelf life is needed (e.g., weeks, typically for sliced bread), their renewable feedstock origin, their biodegradable nature, their composition, and features well within the limits imposed by regulatory agencies make these films good candidates for a new generation of packaging material, with the elimination of high-dosage preservatives inside bread. As a matter of fact, they prevent mold formation, prolong the shelf life of bread, and comply with the consumer demands for clean-label foods (i.e., with no added preservatives) and more sustainable packaging.

5. Patents

An Italian patent was granted based on the work described in this paper: Pallavicini, P.; Doveri, L.R. *Film per la conservazione di alimenti*, 102021000032831 (granted 2024)—an international patent was filed (PCT/IB2022/062808, currently under examination).

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/molecules30112257/s1>: mechanical properties of PEC/CMC/AO/CaCl₂ films as a function of CMC and Ca²⁺ quantities; “Doctor blade” instrument and film photos; mechanical properties of the films without CMC; barrier properties (O₂, CO₂, and water vapor); pH of the film-forming solutions (no CMC added); SEM-EDS analysis; SEM imaging of the films; FT-IR spectra; transmission and absorption spectra; rheological properties of the film-forming solutions.

Author Contributions: Conceptualization: P.P., L.D. and T.K.; methodology: P.G. and G.D.; investigation, L.D., P.G., C.M. and M.U.; resources, N.S.; data curation, P.P. and L.D.; writing—original draft preparation, L.D.; writing—review and editing, P.P., T.K. and Y.A.D.F.; supervision, N.S.; funding acquisition, P.P. and C.M. All authors have read and agreed to the published version of the manuscript.

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References

1. European Union Regulation (EU). 2025/40 of the European Parliament of the Council of 17 January 2025 on packaging and packaging waste. *Off. J. Eur. Union*. **2025**, *L40*, 22–42.
2. European Commission. *Strategy for Plastics in a Circular Economy*; European Commission: Brussels, Belgium, 2018; Volume 30, pp. 577–580.

3. Nechita, P.; Roman, M. Review on Polysaccharides Used in Coatings for Food Packaging Papers. *Coatings* **2020**, *10*, 566. [CrossRef]
4. Kumar, N.; Neeraj. Polysaccharide-based component and their relevance in edible film/coating: A review. *Nutr. Food Sci.* **2019**, *49*, 793–823. [CrossRef]
5. Garavand, F.; Rouhi, M.; Razavi, S.H.; Cacciotti, I.; Mohammadi, R. Improving the integrity of natural biopolymer films used in food packaging by crosslinking approach: A review. *Int. J. Biol. Macromol.* **2017**, *104*, 687–707. [CrossRef]
6. Kunam, P.K.; Ramakanth, D.; Akhila, K.; Gaikwad, K.K. Bio-based materials for barrier coatings on paper packaging. *Biomass Convers. Biorefinery* **2022**, *14*, 12637–12652. [CrossRef]
7. Ureña, M.; Phùng, T.T.-T.; Gerometta, M.; de Siqueira Oliveira, L.; Chanut, J.; Domenek, S.; Dole, P.; Roudaut, G.; Lagorce, A.; Karbowiak, T. Potential of polysaccharides for food packaging applications. Part 1/2: An experimental review of the functional properties of polysaccharide coatings. *Food Hydrocoll.* **2023**, *144*, 108955. [CrossRef]
8. Cauvain, S.P. *Bread Making*; Woodhead Publishing Limited: Cambridge, UK, 2003; ISBN 978-1-85573-553-8.
9. Melini, V.; Melini, F. Strategies to Extend Bread and GF Bread Shelf-Life: From Sourdough to Antimicrobial Active Packaging and Nanotechnology. *Fermentation* **2018**, *4*, 9. [CrossRef]
10. Axel, C.; Zannini, E.; Arendt, E.K. Mold spoilage of bread and its biopreservation: A review of current strategies for bread shelf life extension. *Crit. Rev. Food Sci. Nutr.* **2017**, *57*, 3528–3542. [CrossRef]
11. European Parliament and the Concil of the European Union Regulation (EC). No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. *Off. J. Eur. Union.* **2008**, *L354*, 16–33.
12. Dao, T.; Dantigny, P. Control of food spoilage fungi by ethanol. *Food Control* **2011**, *22*, 360–368. [CrossRef]
13. Levinskaite, L. Susceptibility of food-contaminating *Penicillium* genus fungi to some preservatives and disinfectants. *Ann. Agric. Environ. Med.* **2012**, *19*, 85–89.
14. Pasqualone, A. Bread Packaging: Features and Functions. In *Flour and Breads and their Fortification in Health and Disease Prevention*; Academic Press: Cambridge, MA, USA, 2019; pp. 211–222. ISBN 9780128146392.
15. European Commission Regulation (EC). No 450/2009 of on active and intelligent materials and articles intended to come into contact with food. *Off. J. Eur. Union.* **2009**, *L135*, 3–11.
16. Jideani, V.A.; Vogt, K. Antimicrobial Packaging for Extending the Shelf Life of Bread—A Review. *Crit. Rev. Food Sci. Nutr.* **2016**, *56*, 1313–1324. [CrossRef]
17. Zhao, Y.; An, J.; Su, H.; Li, B.; Liang, D.; Huang, C. Antimicrobial food packaging integrating polysaccharide-based substrates with green antimicrobial agents: A sustainable path. *Food Res. Int.* **2022**, *155*, 111096. [CrossRef]
18. Noshirvani, N.; Ghanbarzadeh, B.; Rezaei Mokarram, R.; Hashemi, M. Novel active packaging based on carboxymethyl cellulose-chitosan-ZnO NPs nanocomposite for increasing the shelf life of bread. *Food Packag. Shelf Life* **2017**, *11*, 106–114. [CrossRef]
19. Istiqola, A.; Syafiuddin, A. A review of silver nanoparticles in food packaging technologies: Regulation, methods, properties, migration, and future challenges. *J. Chin. Chem. Soc.* **2020**, *67*, 1942–1956. [CrossRef]
20. Adeyemi, J.O.; Fawole, O.A. Metal-Based Nanoparticles in Food Packaging and Coating Technologies: A Review. *Biomolecules* **2023**, *13*, 1092. [CrossRef]
21. Couto, C.; Almeida, A. Metallic Nanoparticles in the Food Sector: A Mini-Review. *Foods* **2022**, *11*, 402. [CrossRef]
22. Pallavicini, P.; Taglietti, A.; Dacarro, G.; Antonio Diaz-Fernandez, Y.; Galli, M.; Grisoli, P.; Patrini, M.; Santucci De Magistris, G.; Zaroni, R. Self-assembled monolayers of silver nanoparticles firmly grafted on glass surfaces: Low Ag⁺ release for an efficient antibacterial activity. *J. Colloid. Interface Sci.* **2010**, *350*, 110–116. [CrossRef]
23. European Commission Regulation (EU). No 10/2011 of 14 January 2011. *Off. J. Eur. Union.* **2011**, *L10*, 1–89.
24. European Commission Regulation (EU). 2016/1416 of 24 August 2016 amending and correcting Regulation (EU) No 10/2011 on plastic materials and articles intended to come into contact with food. *Off. J. Eur. Union.* **2016**, *L230*, 22–42.
25. Šešlija, S.; Nešić, A.; Škorić, M.L.; Krušić, M.K.; Santagata, G.; Malinconico, M. Pectin/Carboxymethylcellulose Films as a Potential Food Packaging Material. *Macromol. Symp.* **2018**, *378*, 1600163. [CrossRef]
26. Phùng, T.T.-T.; Gerometta, M.; Chanut, J.; Raise, A.; Ureña, M.; Dupont, S.; Beney, L.; Karbowiak, T. Comprehensive approach to the protection and controlled release of extremely oxygen sensitive probiotics using edible polysaccharide-based coatings. *Int. J. Biol. Macromol.* **2022**, *218*, 706–719. [CrossRef]
27. Yu, W.-X.; Wang, Z.-W.; Hu, C.-Y.; Wang, L. Properties of low methoxyl pectin-carboxymethyl cellulose based on montmorillonite nanocomposite films. *Int. J. Food Sci. Technol.* **2014**, *49*, 2592–2601. [CrossRef]
28. Yaradoddi, J.S.; Banapurmath, N.R.; Ganachari, S.V.; Soudagar, M.E.M.; Mubarak, N.M.; Hallad, S.; Hugar, S.; Fayaz, H. Biodegradable carboxymethyl cellulose based material for sustainable packaging application. *Sci. Rep.* **2020**, *10*, 21960. [CrossRef]
29. Roy, S.; Rhim, J. Carboxymethyl cellulose-based antioxidant and antimicrobial active packaging film incorporated with curcumin and zinc oxide. *Int. J. Biol. Macromol.* **2020**, *148*, 666–676. [CrossRef]
30. Peighambardoust, S.J.; Zahed-Karkaj, S.; Peighambardoust, S.H.; Ebrahimi, Y.; Peressini, D. Characterization of carboxymethyl cellulose-based active films incorporating non-modified and Ag or Cu-modified Cloisite 30B and montmorillonite nanoclays. *Iran. Polym. J.* **2020**, *29*, 1087–1097. [CrossRef]

31. Ren, W.; Qiang, T.; Chen, L. Recyclable and biodegradable pectin-based film with high mechanical strength. *Food Hydrocoll.* **2022**, *129*, 107643. [CrossRef]
32. Mellinas, C.; Ramos, M.; Jiménez, A.; Garrigós, M.C. Recent Trends in the Use of Pectin from Agro-Waste Residues as a Natural-Based Biopolymer for Food Packaging Applications. *Materials* **2020**, *13*, 673. [CrossRef]
33. Ureña, M.; Fournier, P.; Phùng, T.T.-T.; Lagorce, A.; Karbowiak, T. Potential of polysaccharides for food packaging applications. Part 2/2: An experimental review of the effect of aging conditions on the functional properties of polysaccharide films. *Food Hydrocoll.* **2023**, *144*, 108954. [CrossRef]
34. Vlacha, M.; Giannakas, A.; Katapodis, P.; Stamatis, H.; Ladavos, A.; Barkoula, N.-M. On the efficiency of oleic acid as plasticizer of chitosan/clay nanocomposites and its role on thermo-mechanical, barrier and antimicrobial properties—Comparison with glycerol. *Food Hydrocoll.* **2016**, *57*, 10–19. [CrossRef]
35. da Silva, M.A.; Bierhalz, A.C.K.; Kieckbusch, T.G. Alginate and pectin composite films crosslinked with Ca²⁺ ions: Effect of the plasticizer concentration. *Carbohydr. Polym.* **2009**, *77*, 736–742. [CrossRef]
36. Villa, C.C.; Valencia, G.A.; López Córdoba, A.; Ortega-Toro, R.; Ahmed, S.; Gutiérrez, T.J. Zeolites for food applications: A review. *Food Biosci.* **2022**, *46*, 101577. [CrossRef]
37. Youssef, H.F.; El-Naggar, M.E.; Fouda, F.K.; Youssef, A.M. Antimicrobial packaging film based on biodegradable CMC/PVA-zeolite doped with noble metal cations. *Food Packag. Shelf Life* **2019**, *22*, 100378. [CrossRef]
38. Fernández, A.; Soriano, E.; Hernández-Muñoz, P.; Gavara, R. Migration of Antimicrobial Silver from Composites of Polylactide with Silver Zeolites. *J. Food Sci.* **2010**, *75*, E186–E193. [CrossRef]
39. EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF). Scientific Opinion on the safety evaluation of the substance silver zeolite A (silver zinc sodium ammonium alumino silicate), silver content 2–5%, for use in food contact materials. *EFSA J.* **2011**, *9*, 1999. [CrossRef]
40. Jafarzadeh, S.; Jafari, S.M. Impact of metal nanoparticles on the mechanical, barrier, optical and thermal properties of biodegradable food packaging materials. *Crit. Rev. Food Sci. Nutr.* **2021**, *61*, 2640–2658. [CrossRef]
41. Robertson, G.L. Legislative and Safety Aspects of Food Packaging. In *Food Packaging*; CRC Press: Boca Raton, FL, USA, 2016; pp. 637–674. ISBN 9781439862421.
42. Giacinti Baschetti, M.; Minelli, M. Test methods for the characterization of gas and vapor permeability in polymers for food packaging application: A review. *Polym. Test.* **2020**, *89*, 106606. [CrossRef]
43. Ngo, T.M.P.; Nguyen, T.H.; Dang, T.M.Q.; Tran, T.X.; Rachtanapun, P. Characteristics and antimicrobial properties of active edible films based on pectin and nanochitosan. *Int. J. Mol. Sci.* **2020**, *21*, 2224. [CrossRef]
44. Wang, Q.; Chen, W.; Zhu, W.; McClements, D.J.; Liu, X.; Liu, F. A review of multilayer and composite films and coatings for active biodegradable packaging. *Npj Sci. Food* **2022**, *6*, 18. [CrossRef]
45. Wu, F.; Misra, M.; Mohanty, A.K. Challenges and new opportunities on barrier performance of biodegradable polymers for sustainable packaging. *Prog. Polym. Sci.* **2021**, *117*, 101395. [CrossRef]
46. Long, J.; Zhang, W.; Zhao, M.; Ruan, C.Q. The reduce of water vapor permeability of polysaccharide-based films in food packaging: A comprehensive review. *Carbohydr. Polym.* **2023**, *321*, 121267. [CrossRef]
47. Dharini, V.; Periyar Selvam, S.; Jayaramudu, J.; Sadiku Emmanuel, R. Functional properties of clay nanofillers used in the biopolymer-based composite films for active food packaging applications-Review. *Appl. Clay Sci.* **2022**, *226*, 106555. [CrossRef]
48. Sadegh-Hassani, F.; Mohammadi Nafchi, A. Preparation and characterization of bionanocomposite films based on potato starch/halloysite nanoclay. *Int. J. Biol. Macromol.* **2014**, *67*, 458–462. [CrossRef]
49. Yang, X.; Nisar, T.; Liang, D.; Hou, Y.; Sun, L.; Guo, Y. Low methoxyl pectin gelation under alkaline conditions and its rheological properties: Using NaOH as a pH regulator. *Food Hydrocoll.* **2018**, *79*, 560–571. [CrossRef]
50. Lopez, C.G.; Richtering, W. Oscillatory rheology of carboxymethyl cellulose gels: Influence of concentration and pH. *Carbohydr. Polym.* **2021**, *267*, 118117. [CrossRef]
51. Russo, R.; Malinconico, M.; Santagata, G. Effect of Cross-Linking with Calcium Ions on the Physical Properties of Alginate Films. *Biomacromolecules* **2007**, *8*, 3193–3197. [CrossRef]
52. Dacarro, C.; Grisoli, P.; Del Frate, G.; Villani, S.; Grignani, E.; Cottica, D. Micro-organisms and dust exposure in an Italian grain mill. *J. Appl. Microbiol.* **2005**, *98*, 163–171. [CrossRef]
53. Varga, J.; Kocsubé, S.; Tóth, B.; Frisvad, J.C.; Perrone, G.; Susca, A.; Meijer, M.; Samson, R.A. *Aspergillus brasiliensis* sp. nov., a biseriata black Aspergillus species with world-wide distribution. *Int. J. Syst. Evol. Microbiol.* **2007**, *57*, 1925–1932. [CrossRef]
54. Perugini, P.; Bonetti, M.; Guerini, M.; Musitelli, G.; Grisoli, P. A New In Vitro Model to Evaluate Anti-Adhesive Effect against Fungal Nail Infections. *Appl. Sci.* **2021**, *11*, 1977. [CrossRef]
55. Phechkrajang, C.M.; Yooyong, S. Fast and simple method for semiquantitative determination of calcium propionate in bread samples. *J. Food Drug Anal.* **2017**, *25*, 254–259. [CrossRef] [PubMed]
56. Guzman-Puyol, S.; Benítez, J.J.; Heredia-Guerrero, J.A. Transparency of polymeric food packaging materials. *Food Res. Int.* **2022**, *161*, 111792. [CrossRef]

57. Intawiwat, N.; Myhre, E.; Øysaed, H.; Jamtvedt, S.H.; Pettersen, M.K. Packaging materials with tailor made light transmission properties for food protection. *Polym. Eng. Sci.* **2012**, *52*, 2015–2024. [CrossRef]
58. Kowalonek, J.; Kaczmarek, H. Studies of pectin/polyvinylpyrrolidone blends exposed to ultraviolet radiation. *Eur. Polym. J.* **2010**, *46*, 345–353. [CrossRef]
59. Fluidan Automate Viscosity Control in Manufacture of Paint. Available online: <https://fluidan.com/manufacturing-of-paint/> (accessed on 12 May 2025).
60. Mierczyńska, J.; Cybulska, J.; Sołowiej, B.; Zdunek, A. Effect of Ca²⁺, Fe²⁺ and Mg²⁺ on rheological properties of new food matrix made of modified cell wall polysaccharides from apple. *Carbohydr. Polym.* **2015**, *133*, 547–555. [CrossRef]
61. Cheng, D.C.-H. Thixotropy. *Int. J. Cosmet. Sci.* **1987**, *9*, 151–191. [CrossRef]
62. Mewis, J.; Wagner, N.J. Thixotropy. *Adv. Colloid. Interface Sci.* **2009**, *147–148*, 214–227. [CrossRef]
63. ASTM E96-E96M:2016; Standard Test Method for Water Vapor Transmission of Materials. Annual Book of ASTM Standards. American Society for Testing and Materials: Philadelphia, PA, USA, 2016.
64. Grisoli, P.; De Vita, L.; Milanese, C.; Taglietti, A.; Diaz Fernandez, Y.; Bouzin, M.; D’Alfonso, L.; Sironi, L.; Rossi, S.; Vigani, B.; et al. PVA Films with Mixed Silver Nanoparticles and Gold Nanostars for Intrinsic and Photothermal Antibacterial Action. *Nanomaterials* **2021**, *11*, 1387. [CrossRef]

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Article

Laser-Powered Homogeneous Pyrolysis (LPHP) of Lignin Dispersed into Gas Phase

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Abstract: The gas-phase delivery of lignin into the hot zone of cw-CO₂ laser-powered homogeneous pyrolysis (LPHP) reactor under “wall-less” conditions led to the breakdown of lignin macromolecules into neutral oligomers and paramagnetic fragments deposited onto the reactor cell walls. The formation of PAHs was observed during the defragmentation of lignin, accelerated with increased laser power. Remarkably, no phenolic compounds were detected among lignin fragments—intermediate radicals and neutral oligomers. It is concluded that the PAH and soot-like conjugated particulates are formed in the hot zone of the LPHP reactor, resembling the high-temperature combustion processes. The key role of the resonantly stabilized radicals in the formation of low-molecular-weight PAHs is outlined. An alternative pathway is proposed for the generation of PAH involving the formation of cyclopentadienyl radical precursors (CPDa) that are adsorbed onto or trapped within lignin macromolecules.

Keywords: PAH; lignin pyrolysis; laser reactor; depolymerization; soot; radicals

1. Introduction

Understanding and monitoring the production of bio-oils by fast biomass pyrolysis is an efficient way to optimize the involved chemical processes and improve their subsequent refinement. To bypass intractable secondary processes during the solid-phase pyrolysis of lignin, we have recently introduced a straightforward approach involving the initial gasification of lignin macromolecules. Lignin dissolved in a solution of acetone/water (9:1 ratio) was pulverized into a gas phase in a contactless, tubular reactor [1,2]. The overall goal of the experiments was to limit the side effects typically accompanying the pyrolysis of solid-state lignin in conventional reactors. Depending on the delivery modes, two different types of reactors were utilized: (1) Continuous Droplet Evaporation (CDE) and (2) Constant Output Atomization (CA) reactors. Lignin was transported through the isothermal zone by either a syringe pump (CDE reactor) or a TSI atomizer (CA reactor). The results revealed new features for the degradation of hydrolytic lignin (HL) dispersed into the gas phase compared to those in the conventional batch reactors. In particular, the formation of intermediate oligomers in both reactors was shown to occur, involving only trace amounts of phenolic compounds [1,2].

Some preliminary results on the pyrolysis of lignin in the gas phase using a “wall-less”, cw-IR CO₂ laser-powered homogeneous pyrolysis (LPHP) non-isothermal reactor

were briefly reported elsewhere [3,4]. The temperature distribution in the LPHP reactor was monitored by thermocouple measurements validated by the method of “chemical thermometer” and COMSOL Multiphysics simulations [3,4]. Large differences were found between the gas-phase depolymerization of lignin in the LPHP reactor and conventional reactors, yet much similarity was seen with CDE and CA reactors. The gas-phase delivery of lignin into the hot zone of the LPHP reactor under “wall-less” conditions led to the breakdown of lignin into neutral and paramagnetic fragments that deposited onto the cell walls [3]. Because of the steep/narrow temperature zone in the center of the LPHP reactor, these fragments readily leave the hot zone and quench/stabilize in the cold areas of the reactor, primarily on the cold walls.

No phenolics were detected in the LPHP reactor; instead, the formation of polycyclic aromatic hydrocarbons (PAHs) was observed, and the yields of PAHs accelerated with increasing laser power. Note that the accumulation of PAHs in the conventional reactors, as detailed in [5], tends to increase with temperature. However, a critical decrease is observed in the yields of PAHs containing two fused aromatic rings. This insight is essential for regulating the composition of PAH mixtures based on temperature variations.

Understanding the underlying chemistry of PAHs formation observed virtually in any biomass conversion and combustion process, refs. [6,7] presents a significant challenge [8]. While PAHs are typically perceived as toxic and unwanted byproducts of biomass pyrolysis, they could serve as biomass-derived precursors for valuable carbon-based materials, including carbon fibers [9], films [10], hydrogels [11], etc. Additionally, the inclusion of small quantities of low-molecular-weight PAHs as additives in diesel fuel enhances the physicochemical properties of diesel surrogate fuel blends, leading to improvements in both energy density and viscosity [12,13]. Note that a diesel surrogate blend is a simplified fuel mixture tailored to replicate diesel’s combustion and physical properties [14]. Generally, diesel fuel is composed of $C_{10}\sim C_{24}$ hydrocarbons, including 50–65% alkanes (mostly n-alkanes), 20–30% cycloalkanes, and 10–30% aromatics [15]. For instance, the addition of methyl naphthalene in a concentration of 13 to 21% to 3, 5, and 7 components of a diesel surrogate fuel blend, for instance, accurately reproduces actual engine combustion characteristics [14].

The goal of this work is to explore the applicability of the laser-powered pyrolysis of lignin in well-known combustion-related processes, leading to the formation of low-molecular-weight (LMW) aromatics and “younger” soot macromolecules within the hot zone of the LPHP reactor. In addition to the homogeneous reaction channels, an alternative “heterogeneous” pathway for PAH formation at elevated temperatures is also proposed, involving reactions of cyclopentadienyl (CPDa) radicals adsorbed onto HL macromolecules.

2. Results and Discussion

Due to the complexity of the direct temperature measurements, computational numerical modeling was undertaken to simulate and identify the temperature distribution in the IR countercurrent reactor, as shown in Figure 1.

COMSOL Multiphysics (v5.3a, COMSOL Inc, Burlington, MA, USA), a finite element analysis (FEA) software package, was used to solve the ordinary differential equations (ODEs) and partial differential equations (PDEs) involved in the laser light absorption and transition into heat transfer in the fluid and fluid dynamic models, using a procedure modified in our previous work [4]. The initial phase of computational temperature measurements, along with the experimental validation for a similar system, can be found elsewhere [3].

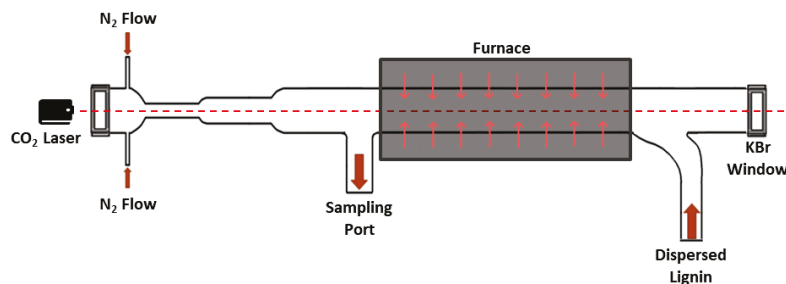


Figure 1. Schematic of the IR countercurrent LPHP reactor.

2.1. Temperature Distribution in LPHP Countercurrent Reactor

The temperature profiles inside the LPHP reactor based on COMSOL Multiphysics simulation are mapped in Figure 2.

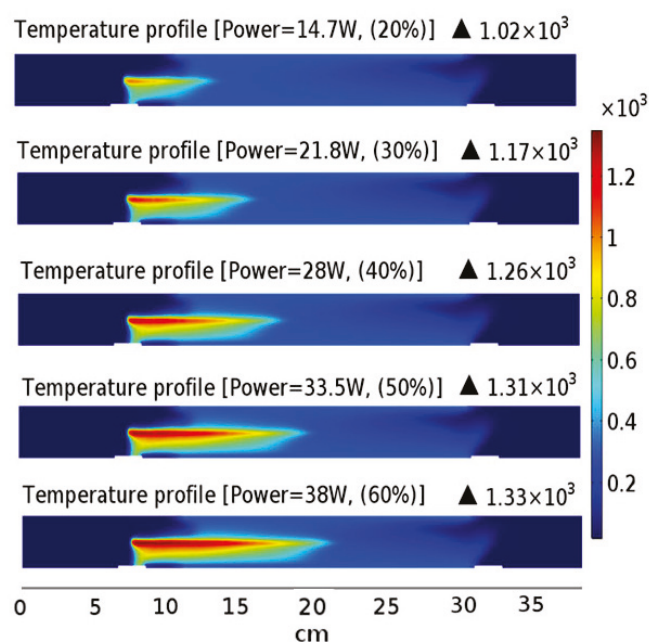


Figure 2. COMSOL prediction of the “hot zone” temperature shown on a cross-sectional plane along axial direction (Z coordinate) at 14.7, 21.8, 28, 33.5, 38 W laser power with 2% SF₆ in N₂ gas mixture under atmospheric conditions.

For these calculations, three types of mesh (coarse, normal, and fine mesh) were studied with respect to the efficiency of simulation and accuracy of the results. As expected, by elevating the laser power, the temperature of the hot zone increases and propagates over a longer distance at the onset of absorption. The laser beam has a 2.5 mm diameter where the maximum radial temperature is calculated.

Considering a volume with temperature above 400 °C as the reacting zone for each laser power, the calculated residence time of the lignin in the hot zone, for a 3 L/min carrier gas flowrate, can be evaluated to range from 40 ms to 160 ms, corresponding to the 14.7 W to 38 W laser power, respectively. The high maximum temperature shown in Figure 2 for each laser power can compensate for such a short residence time to achieve reasonable conversion rates. Note, the TSI 3076 Constant Output atomizer generates extremely fine aerosols with a number-mean droplet diameter of 0.30 µm from the lignin solution, ideal to be rapidly heated to the temperature achieved in the hot zone. Moreover, the steep temperature profile achieved in this reactor and fast cooling of pyrolysis products provide

great conditions for obtaining the primary products or preserving intermediates emerging from lignin pyrolysis.

2.2. Condensable Products from Countercurrent LPHP Reactor

The GC-MS (Agilent 6890Nbraker) results from the countercurrent LPHP reactor are represented in Figure 3 (the product identifications are provided in Tables S1–S4, Supplementary Materials) at different laser powers from 14.7 to 38 W. The products condensed in the cold trap were extracted with acetone at the end of the experiment and analyzed via GC-MS.

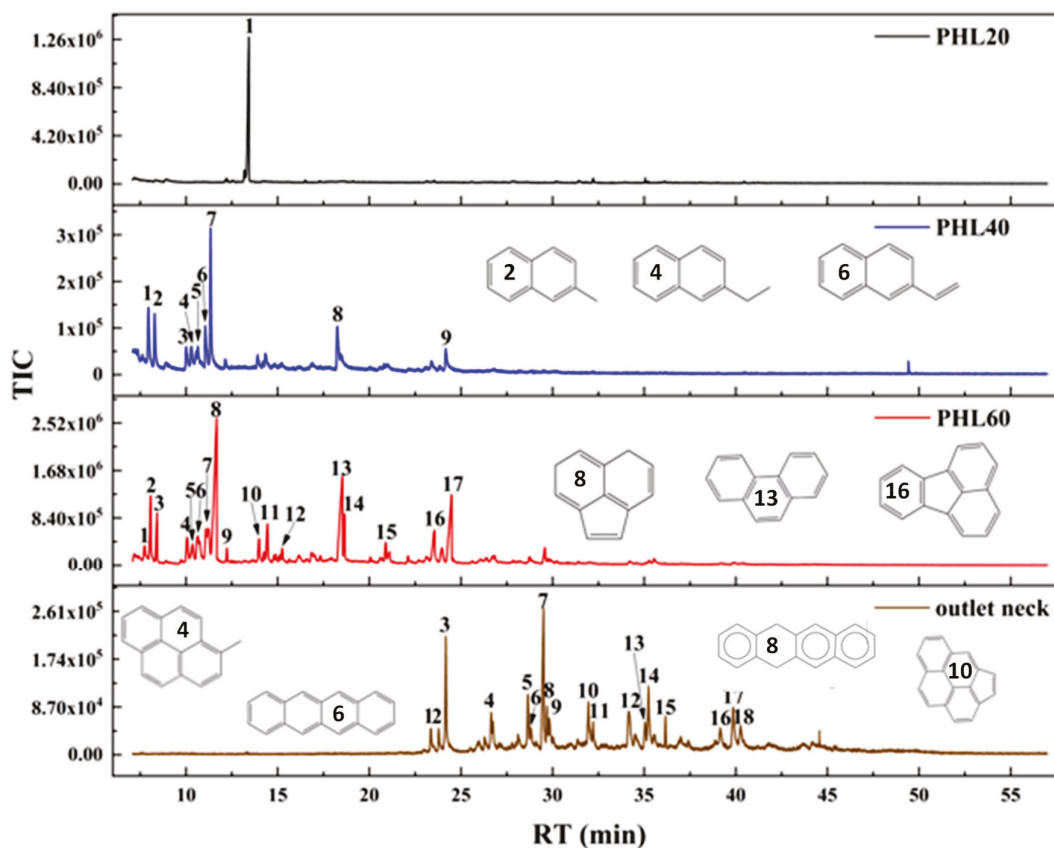


Figure 3. GC-MS chromatograms of the products of the homogeneous pyrolysis of lignin in a counter-current LPHP reactor (Figure 1) at various laser powers and locations in the reactor. HL mass transfer rate $\sim 2.5 \mu\text{g/s}$, residence time 100 ms, outside reactor temperature 300°C . The product identifications are shown in Tables S1–S4 (Supplementary Materials). The laser power assignments PHL 20, 40, and 60 stand for pyrolyzed hydrolytic lignin (PHL) at laser power of 20% (14.7 W), 40% (28 W), and 60% (38 W). #1 compound at PHL20 is guaiacol, which most probably formed from vinyl guaiacol; the latter one is an intrinsic compound in HL. TIC refers to Total Ion Count, and RT denotes retention time in minutes.

Surprisingly, no phenolics (phenols, guaiacols, syringols, etc.) were detected in noticeable amounts. Different fused aromatic rings containing PAHs were detected with increasing laser power at ascending order of aromatic rings in PAHs, Figure 3, (Tables S1–S4, Supplementary Materials). Notably, the PAHs were not detected below 22 W laser power.

The formation of PAHs was also identified by FTIR (Bruker Tensor 27, Ettlingen, Germany) analysis of trapped products from the sampling port of the LPHP reactor; the frequency at 726 cm^{-1} is a characteristic vibration mode for the C–H wags in an aromatic ring in fused ring systems, as shown in Figure 4, at PHL 40 and PHL 60; refer to the

abbreviations in Figure 4 caption. These peaks, however, are missing in the FTIR spectra of deposited products on the outlet neck and top wall of the reactor, as shown in Figure 4.

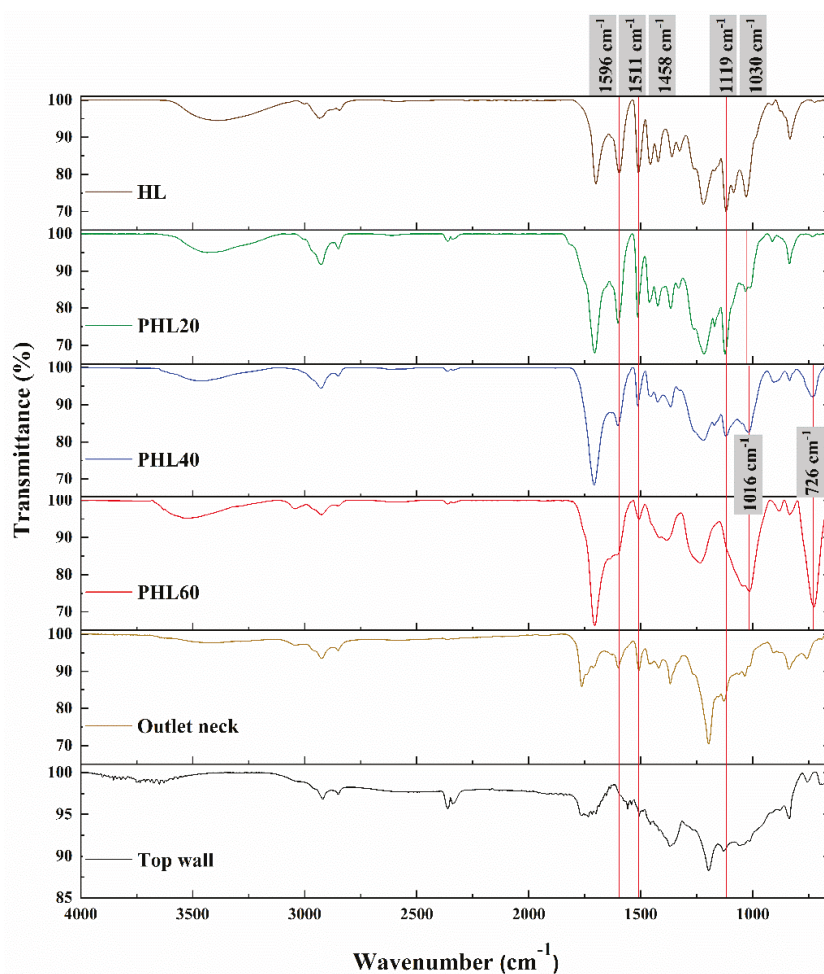


Figure 4. FTIR spectra of the condensed products in the trap from lignin homogeneous pyrolysis in the LPHP reactor at laser power of 20% (14.7 W), 40% (28 W), and 60% (38 W), and the deposited products on the outlet neck and top wall. PHL—pyrolyzed HL. The laser power assignments PHL 20, 40, and 60 stand for pyrolyzed hydrolytic lignin (PHL) at laser power of 20% (14.7W), 40% (28 W), and 60% (38 W).

In other words, the emergence of the strong peak at 726 cm^{-1} (spectra at PHL 40 and PHL 60, Figure 4), due to the C–H wags for an aromatic ring with 3, 4, and 5 adjacent hydrogen atoms, indicates the formation of fused ring systems, such as substituted naphthalene, anthracene, or phenanthrene. These compounds were abundant among products trapped during pyrolysis at high laser power.

A detailed peak identification of the FTIR spectra is presented in Table 1. It is obvious that as the energy of the laser increases, the major aromatic structural units in the lignin macromolecules, labeled as the most characteristic bands at 1596 and 1511 cm^{-1} , and 1119 and 1030 cm^{-1} for C=C and C–H vibration in the skeletal aromatic ring, break down, as shown in Figure 4.

Table 1. FTIR characteristics of the analyzed samples.

Wavenumber (cm ^{−1})	Peak Assignment
3394	O–H stretching in hydroxyl groups in phenolic and aliphatic structures [16,17]
2936	C–H stretching in methoxy, methyl, and methylene [16]
2846	C=O stretching in p-substituted aryl ketones [16]
1700	C=C stretching in aromatic ring (S) [16]
1596	C=C stretching in skeletal aromatic ring (S > G) [17,18]
1511	C=C stretching in skeletal aromatic ring (G > S) [17,18]
1458	C–H bending in methoxy and methylene [17]
1422	C–H in-plane deformation combined with skeletal aromatic ring [17]
1367	O–H in-plane bending [18]; O–H in phenol, aliphatic C–H stretch in CH ₃ [17]
1326	C–H bending in aromatic ring (S or condensed G) [17]
1220	Aryl–O of aryl–OH and aryl–OCH ₃ [17]
1119	C–H bending in aromatic ring (S > condensed G) [17]
1085	C–O deformation in secondary alcohols and aliphatic ethers [17]
1030	C–O stretch in O–CH ₃ and C–OH [18]
915	C–H out-of-plane deformation of aromatic ring [17]
834	C–H out-of-plane in H unit and C _{2,6} of S unit [17]
726	C–H wags for an aromatic fused ring [18]

Demethoxylation constitutes another phenomenon that is intensified when the temperature is increased, as observed from the FTIR spectra. Bands at 1458, 1085, and 1030 cm^{−1} gradually disappeared, showing the complete removal of the methoxy functional groups from the substituted aromatic rings in both trapped and deposited products. Similar behavior was reported during conventional pyrolytic char formation of lignin [18]. These results, along with the results indicating the formation of soot particulates detected by FTIR from the outlet neck and top wall of the LPHP reactor (Figure 4), are consonant with literature data on conventional pyrolysis of lignin and cellulose at high temperatures [18,19].

GPC measurements described below were performed to further analyze the content and behavior of trapped products not detectable by GC-MS.

2.3. Molecular Weight Distribution of the Lignin Homogeneous Pyrolysis Products

2.3.1. GPC Analysis

To better understand the nature of the hydrolytic lignin gas-phase depolymerization process during homogeneous pyrolysis in the LPHP reactor, gel permeation chromatography (GPC) was employed to examine the molecular weight distribution of the products. The results are shown in Figure 5 for initial lignin (HL) in comparison with depolymerized lignin (PHL) at various laser powers.

These GPC spectra show the various fractions of the hydrolytic lignin and how they change as the lignin structure is deconstructed during depolymerization. As the temperature of the pyrolysis increases, the summits of these fractions move toward lower molecular weights while the new peaks, indicating the new fragments, are also formed and subsequently intensified. Even though the GPC is the most common method to determine molecular weight distribution among many researchers, this technique has a major drawback. Obtained by this method, Mw values are relative to other compounds such as polystyrene. Thus, one cannot draw a definite conclusion based on the values reported in Table 2. The weight-average molecular weights (M_w), the number-average molecular weights (M_n), the polydispersity indexes (PDI) of the initial lignin, and the generated products after pyrolysis are provided for comparison purposes.

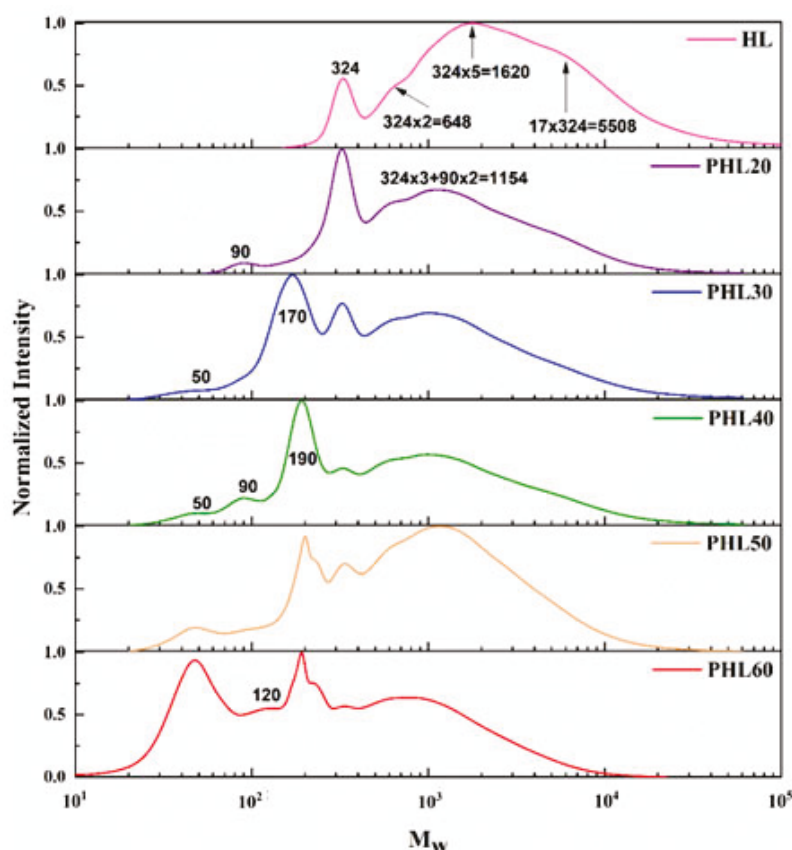


Figure 5. GPC spectra for initial HL vs. products from HL pyrolysis (collected in the trap) at various laser powers in LPHP reactor: 20% (14.7W), 30% (21.8W), 40% (28 W), 50% (33.5W), 60% (38 W). The laser power assignments PHL 20, 30, 40, 50, 60 stand for pyrolyzed hydrolytic lignin (PHL) at laser power of 20% (14.7W), 30% (21.8W), 40% (28 W), 50% (33.5W), 60% (38 W).

Table 2. The weight-average (M_w) and number-average (M_n) molecular weights of lignin and pyrolyzed lignin.

	HL	PHL20	PHL30	PHL40	PHL50	PHL60
M_w (g/mol)	5591	2298	1985	1947	1601	763
M_n (g/mol)	1397	614	325	309	354	113
PDI	4.0	3.7	6.1	6.3	5.1	6.7

The molecular weight values of the intact lignin sample, M_n and M_w of 1397 and 5591 g/mol, respectively, are significantly higher than those for the rest of the pyrolyzed sample, indicating more efficiency of the homogeneous pyrolysis in the gas phase in the depolymerization of hydrolytic lignin. It is also evident that by increasing the laser power, these values reduce gradually as a result of the increased pyrolysis temperature.

Furthermore, the polydispersity index (PDI) of the intact lignin is lower than that of all the pyrolyzed samples except the PHL20 sample, which was pyrolyzed at 20% of the maximum laser power. Collectively, these results suggest that the lignin macromolecules are broken down into smaller fragments and spread over a broader range of molecular weight values, as shown in Figure 5.

This observation contrasts with previous reports in the literature regarding the effect of temperature on the molecular weight of pyrolytic lignin [20–22]. Based on the reviewed studies, an increasing trend in the molecular weight distribution, as determined by GPC, was observed with higher pyrolysis temperatures for kraft lignin, organosolv lignin, spruce organosolv lignin, wheat straw organosolv lignin, and milled pine wood lignin. Ragauskas

and Ben, for instance, observed that the molecular weights are increased with increasing pyrolysis temperature for both fast and slow pyrolysis oils produced from pine wood [20]. Kersten et al. explained this phenomenon in terms of the interplay of chemistry and mass transport [21]. They reported heavier bio-oil recovery during pyrolysis, regardless of the lignin type, by increasing temperature due to the higher escape rate of heavier components from the reacting region and the low partial pressure.

In fact, lignin macromolecules undergo fragmentation during homogeneous gas-phase processes in the LPHP reactor, seemingly indicating that products in other reactors are involved in the secondary repolymerization reactions, which increase the Mw of the final products. It is pertinent to mention that both ESI-MS and GPC results on the primary pyrolysis products of organosolv lignin at temperatures between 360 and 700 °C show a negligible effect of the pyrolysis temperature on the molar weight distribution using an original vacuum screen heater [22].

2.3.2. The Radical Character of the Decomposition of Lignin in LPHP Reactors

A dramatic new environment for the pyrolysis of HL in the LPHP reactor, excluding direct contact between volatiles and residue chars, leads to the principal simplification of the primary decomposition processes of the lignin macromolecules. As a result, the major fraction of the pyrolysis products (~80%, w) represents a mixture of the initial intact HL and its depolymerized fragments—oligomers and oligomer radicals—as described in the current work and mentioned in refs [2,3]. The converted lignin (~10–15%, w) deposited on the cold areas, outside of the reactor, shows a high content of radicals, Figure 6. The low *g* values of the detected radicals from the neck of the countercurrent reactor equal to 2.0029 and 2.0028 confirms the removal of most of the lignin functional groups containing polar groups by the formation of “naked” soot-like conjugated substances; a notable change in the *g* values of the intrinsic radicals (2.0043, initial lignin, inset, Figure 6) occurs.

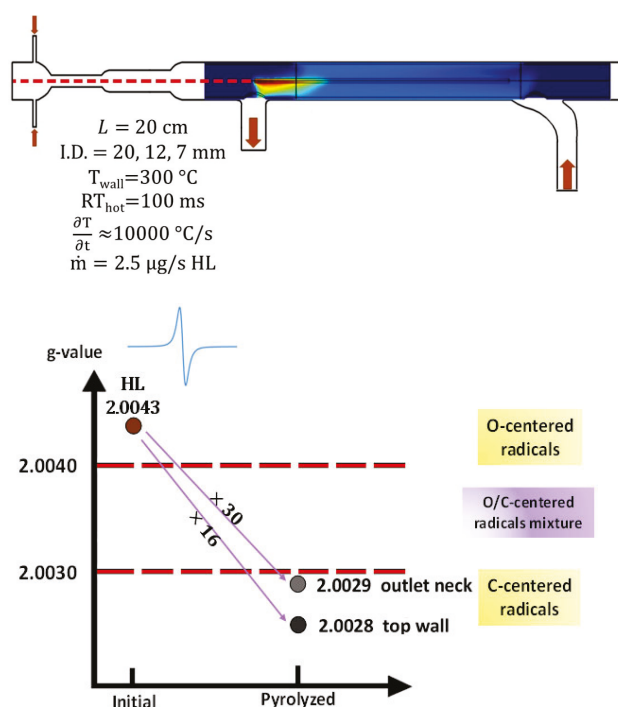


Figure 6. Brown deposits (*g*-value of 2.0029) on the reactor outlet neck with x16 higher EPR signal intensity than that in initial HL. Black deposits (*g*-value of 2.0028) on the reactor top wall with x30 higher intensity (at 28W of laser power) than in HL. The *g*-values 2.0030 and 2.0040 on the Y-axis are arbitrary marker values for C-, O-centered radicals, respectively, as well as a mixture of these radicals between these values.

Generally, the presence of the macro-radicals provides strong binding conditions to develop the initial clusters of PAHs [23].

2.4. Formation Mechanism of PAHs from HL Gas-Phase Pyrolysis in LPHP Reactor

2.4.1. Combustion-Related Homogeneous Channels for Formation of PAHs:

The formation of PAHs and soot particulates (Figures 3 and 4) from lignin gas-phase decomposition in the LPHP reactor can be explained by the traditional multizone soot particulate formation mechanism in hydrocarbon flames. In the pre-flame zone, the fuel or combustible precursors are vaporized at temperatures ranging from ambient up to about 1200 °C. In the flame zone, where the temperature exceeds 1200 °C, the fuel species undergo complete combustion; this zone is associated with the formation of soot and other organic pollutants. The molecular growth of PAHs and soot inception occurs in the post-flame zone with decreased temperatures ranging from 1200 to 600 °C. Below 600 °C, the soot inception/formation rate is exceedingly low.

The possible mechanisms relevant to the growth of aromatics and PAHs/soot particulate formation have been actively disputed in the literature over the last three decades. The current primary focus is the generation of the first few aromatic rings out of small aliphatic compounds. This is perceived by many to be a rate-limiting stage in the reaction sequence leading to larger aromatics, and it is commonly accepted that the formation of the first aromatic ring is the kinetic bottleneck in the soot formation chain [23–25]. Subsequent growth of PAHs from a single aromatic species can proceed via a repetitive sequence of hydrogen abstraction and acetylene addition reactions (seminal HACA mechanism) [23–25].

Various mechanisms of the formation of PAHs at high temperatures are also largely discussed in the literature [5,26–29]. In particular, an aryl–aryl combination [30], phenyl or methyl addition/cyclization [31], and others (see detailed discussion in Supplementary Materials, Section S4) have also been reported.

The formation of naphthalene [3] and its derivatives directly observed in the LPHP reactor, as shown in Figure 3, suggests the importance of its formation channels (reaction (S1) and reaction (S3) in Supplementary Materials, Section S4).



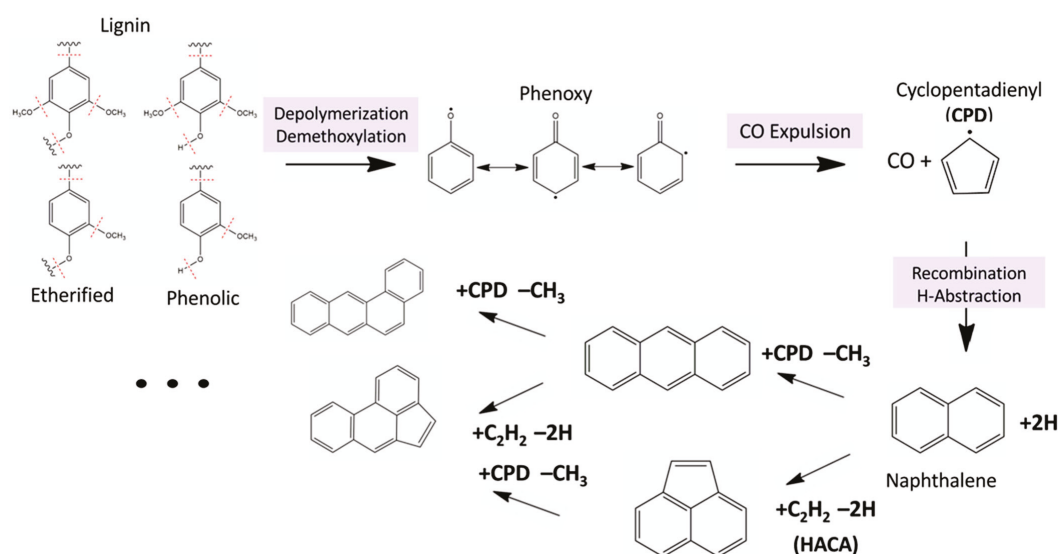
This involves a key, cyclopentadienyl, C_5H_5 (CPD) [32,33], and naphthalenyl radicals [34].

The formation of CPD radicals from pyrolysis/oxidation reactions of numerous lignin contracted models (aromatics) such as catechol, hydroquinone, and phenol has been shown in our early publications [35–37], and more recently [38], it has been suggested to occur during the pyrolysis of *p*-coumaryl alcohol at elevated temperatures, >700 °C, as shown in Figure S3. The expulsion of CO from phenoxy rings is typically considered to lead to the formation of cyclopentadienyl and hydroxy-cyclopentadienyl radicals, particularly from the pyrolysis of catechol, hydroquinone [39], guaiacol [40], and para-coumaryl alcohol [38]. Further recombination of such radicals forms naphthalene, indene, hydroxy indene, and other derivatives, as experimentally established in isothermal reactors [35–37].

The role of the resonance-stabilized cyclopentadienyl and other aromatic radicals in soot formation has also been indirectly demonstrated recently during the pyrolysis/oxidative pyrolysis of 1-methylnaphthalene at ~1100 °C in a two-section tubular combustion reactor [41]. It should be noted that the CPD radicals are more stable than phenoxy radicals at such high temperatures (>700–1000 °C) [36]. Accordingly, the ratio $[\text{CPD}]/[\text{PhO}] = 2.0$ at 700 °C was suggested to increase to 88.4 at 1000 °C in a numerical modeling study using a known scheme of phenol pyrolysis reactions analyzed in detail in the same publication [36]. The formation of derivatized CPD radicals from lignin key models and monomers, particularly para-coumaryl alcohol at elevated temperatures, has been

justified theoretically by Asatryan et al. based on the first-principles analysis of reaction mechanisms [42] (detailed in the Supplementary Materials, Figure S4).

Thus, due to the flash heating at elevated temperatures and intense de-functionalization of lignin macromolecule (intermediate oligomers), the formation and further reactions of CPD radicals can occur as described in Scheme 1 (and Figure S5). In other words, the naphthalene formation initiates the generation and growth of myriads of low-molecular-weight (LMW) PAHs (Figure 3), both benzenoid- (phenanthrene, pyrene, etc.) and cyclopentane-based fused (acenaphthylene, fluoranthene, etc.) PAH structures, through both HACA mechanisms and/or addition of the new cyclopentadienyl radicals, [24,43–45], as summarized in Figure S5.



Scheme 1. Mechanistic homogeneous pathway of PAHs formation during high-temperature gas-phase pyrolysis of lignin.

A detailed analysis of the formation of soot particulates from LPHP using ESI MS and LDI MS techniques is underway, while similar results from the CA reactor on the detection of soot particulates up to 2000–2500 g/mol after the pyrolysis of lignin dispersed into the gas phase in isothermal conditions have already been reported [2].

2.4.2. A “Heterogenous” Mechanism for Formation of PAHs

As it was mentioned above, we did not detect any phenolics from co-current and counter-current LPHP reactors in the entire range of laser power from 20 to 38W. Therefore, it could be concluded that phenolics do not form in these conditions (decomposition reactions, particularly CO expulsion from a phenoxy-type radical being dominant over hydrogen abstraction reactions, which lead to phenolics), and they rapidly convert into PAHs and soot particulates at elevated temperatures, as soon as they are formed (≥ 900 – 1000 °C, Figure 2), as discussed in Section 2.4.1 above.

An alternative possibility is a heterogeneous formation of PAHs that also involves CPD radicals. The phenoxy radicals released from the destruction of lignin macromolecules (Scheme 1) stay trapped between polar lignin sub-units, and further expulsion of CO from the phenoxy unit leads to the formation of adsorbed CPD radicals (CPDa) on the lignin macromolecule. Consequently, the surface reactions of CPDa, due to their known higher mobility (see Supplementary Materials, Section S6), can produce PAHs via a scenario described in Scheme 1 (Figure S5).

One could assume a similar mobility of the adsorbed and stabilized CPDa radicals on the surface of the HL macromolecule (as discussed in Supplementary Materials, Section S6),

perhaps to a lesser extent due to the polar nature of the lignin branches; the formation of naphthalene, according to reaction (S1), can be envisioned at elevated temperatures.

Evidence on formation of the immobilized CPD radicals into the lignin lattice: Evidence of embedded CPD radicals (CPDa) on the lignin matrix has been provided in our early pyroprobe fast fractional pyrolysis experiments on lignin [46]. The products released at different temperatures in the fast flow of nitrogen gas were collected on a Cambridge filter (CF) at room temperature (Figure S6) and subjected to EPR analysis, as shown in Figure 7a. A broad, structureless EPR signal with $g = 2.0051$ observed at 300 °C gradually evolves into a well-resolved, complex EPR spectrum as the pyrolysis temperature increases to 600 °C. The EPR spectra seem to be a superposition of EPR signals of several paramagnetic species. A further increase in the temperature to 700 °C leads to the distortion of the well-resolved spectrum at 600 °C with $g = 2.0061$ at the center to generate another signal with a lower $g = 2.0039$. A trend can be traced in conversion into the single line at temperatures higher than 700 °C. A benchmark EPR line at $g = 1.9932$ from the top spectra in Figure 7a is compared with the reference EPR spectrum of pure CPD generated from the vacuum pyrolysis of tricarbonylcyclopentadienylmanganese (Figure S3 [38,47]), as shown by the black line in Figure 7b. The line at $g = 1.9932$ of the CPD radical does not overlay with any other EPR lines of an organic radical generated from the vacuum pyrolysis of a number of lignin model compounds to provide evidence of the presence of CPD radicals [36]. Importantly, it exactly fits with the same line for the radicals generated from the vacuum pyrolysis of *p*-coumaryl alcohol (green line in Figure 7b) and identified as traces of CPD after the annihilation of the radical mixture [38], as shown in Figure S3. Therefore, the immobilization of CPD radicals on a lignin matrix appears feasible, and the subsequent reactions of these adsorbed radicals, as discussed above, may be highly relevant.

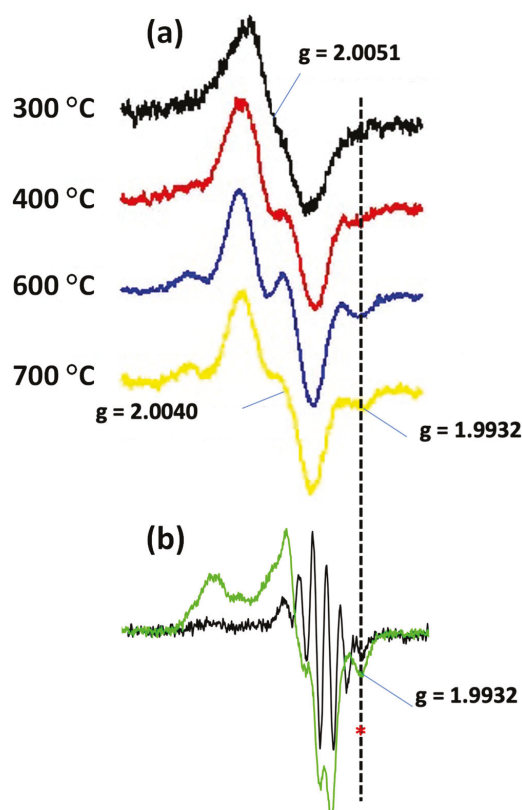


Figure 7. (a) Top four EPR spectra were detected at room temperature from lignin pyrolysis in pyroprobe at 300 °C (black), 400 °C (red), 600 °C (blue), and 700 °C (yellow) set temperatures.

Radicals were accumulated in the Cambridge filter. (b) The two spectra below present a reference EPR spectrum from vacuum pyrolysis of tricarbonylcyclopentadienylmanganese (black line, reaction (S1) in Figure S3) overlaid with the radicals EPR spectrum detected from the vacuum pyrolysis of *p*-coumaryl alcohol at elevated temperatures (>700 °C); both spectra were detected at liquid N₂ temperature. The red asterisk shows the reference line from the CPD radical at $g = 1.9932$.

In summary, the reaction sequences presented in Scheme 1 (Figure S5) up to the generation of LMW PAHs may occur both homogeneously in the gas phase and heterogeneously on the surface of the macromolecules of HL.

3. Materials and Methods

3.1. Materials

Hydrolytic lignin (HL) is produced by isolating lignin from lignocellulosic biomass during hydrolysis [48]. It primarily consists of complex aromatic polymers derived from phenylpropanoid monomers. More specifically, HL is rich in lignin structures consisting of guaiacyl (G), syringyl (S), and *p*-hydroxyphenyl (H) units. These units are interconnected through various linkages, such as β -O-4, β - β , and α -O-4 bonds, thus forming a highly cross-linked three-dimensional network.

HL was purchased from Sigma-Aldrich (St. Louis, MO, USA) (a currently discontinued product, 37-107-6). This product mostly contains low-molecular-weight oligomers (<500 Da), i.e., dimers containing four oxygen atoms (C₁₈H₁₈O₄) and trimers with six oxygen atoms (C₂₇H₂₆O₆), with a weight percent ratio of C/H/O that equals 76:6:18 [49]. Lignin was fractionated by molecular sieves from mesh #60–120, and the fraction of #100–120 was used in this study ($125\ \mu\text{m} \leq \text{nominal size} \leq 149\ \mu\text{m}$). The hydrolytic lignin was well dissolved using a vortex stirrer followed by 30 min of sonication in an acetone/water (9:1 volume ratio) mixture. This solution was then atomized in the gas phase by a Constant Output TSI 3076 atomizer into the pyrolysis reactor (refer [1,2] for details).

3.2. IR Laser-Powered Homogeneous Pyrolysis (LPHP) Reactor

The general features of the LPHP reactor, which facilitate the homogeneous pyrolysis of organic compounds, are described in detail elsewhere [3,4]. The schematic of the IR LPHP reactor, which is irradiated from the side window by an IR CO₂ laser, is represented in Figure S1. A continuous-wave cw-CO₂ laser source was used at the P (20) line of the 00^o1 $\rightarrow$ 10^o0 transition (10.59 μm) with a maximum output of 40 W (Synrad Firestar CO₂ laser, FSV40KWD, Manufacturer: Synrad, Inc., Mukilteo, WA, USA). A set of highly transparent KBr disks to the CO₂ laser irradiation (10.6 μm) was used as an entry window for the laser reactor cell. The reactor is a Pyrex glass tube (i.d. = 20 mm, length = 10 cm) fitted from both sides with KBr windows. To avoid the destructive effect of moisture on the hygroscopic window material and the deposition of expected heavy intermediates from HL pyrolysis on the surfaces of both windows, they were protected by direct flow of N₂ through the reaction cell close to the windows, as shown in Figure S1. The laser energy is absorbed by the sensitizer gas SF₆, which is mixed with the initial flow of the lignin dispersed into N₂ carrier gas and rapidly transferred to the ambient gas and reactants. An important characteristic of SF₆ is its general non-reactivity, i.e., it does not pyrolyze significantly below 1350 °C [50]. The secondary tar-forming reactions are limited in the LPHP reactor because of the sharp temperature drop on the border of the hot volume reaction zone and the corresponding drastic decrease in the chain propagation reaction rates [3]. In other words, the extremely pronounced temperature drop from the central axis of the LPHP reactor toward the surrounding areas has a quite different effect on the various reaction steps in comparison with a conventionally heated tube reactor, as shown in a number of earlier publications [50–56].

LPHP Countercurrent Reactor

The countercurrent design reactor was placed in an isothermal reactor, as shown in Figure 1 (refer to LPHP co-current reactor in the Supplementary Materials, an inset in Figure S2); the wall temperature was maintained at 300 °C to avoid condensation/deposition on the cold walls of reactor. Most deposits were easily collected from the neck of the sampling port, and, at the same time, analysis of the condensable gases trapped at −196 °C could be undertaken. This reactor is made from Pyrex with a 20 cm length of heated wall using an electric furnace. The unique countercurrent design with various tube sizes (I.D.= 6, 11, and 20 mm), and the side injection of the N₂ gas ensured the confinement of the reactive hot zone in the target zone. Dispersed hydrolytic lignin in the gas phase was provided by a commercially available TSI 3076 Constant Output atomizer (Shoreview, MN, USA) operated at a 3–5 L/min flowrate, and 2% SF₆ was provided in the N₂ gas mixture.

Due to the complexity of the direct temperature measurements, computational numerical modeling was undertaken to simulate and identify the temperature distribution in the reactor. COMSOL Multiphysics (v5.3a, COMSOL Inc., Burlington, MA, USA), a finite element analysis (FEA) software package, was used to solve the ordinary differential equations (ODEs) and partial differential equations (PDEs) involved in the laser light absorption and transition into heat transfer in the fluid and fluid dynamic models, using a procedure modified in our previous work [4]. The initial phase of computational temperature measurements, along with the experimental validation for a similar system, can be found elsewhere [3].

The details of the results obtained from the standard analytical techniques, namely GC-MS, EPR, FTIR, and GPC, are presented in the Supplementary Materials.

4. Conclusions

The fast pyrolysis of hydrolytic lignin (HL) dispersed in the gas phase was performed in an IR CO₂ laser-powered homogeneous pyrolysis (LPHP) reactor. Lignin breaks down to fragments and forms GC-MS-detectable PAHs (MW < 300 Da). The phenolic compounds were not observed among products at higher than ~20 W non-focused laser power conditions. GPC analysis revealed a partial fragmentation of HL (at ~10–15% conversion) deposited on the cold area of the LPHP reactor. PAHs and soot-like substances were dominant components of the deposits at higher than 20–22W laser powers. The dominant fraction of lignin (~80%) transported through the hot zone consists of the decomposed neutral fragments, oligomer radicals, and non-reacted intact macromolecules of lignin.

A traditional combustion chemistry mechanism relevant to the soot formation in hydrocarbon flames at elevated temperatures was applied to explain the formation of PAHs under laser irradiation, involving the formation and reactions of CPD radicals. An alternative, “heterogenous” pathway for the generation of PAHs (parallel to the homogeneous pathways) is also proposed, involving reactions of adsorbed on HL macromolecules CPD radicals.

Non-catalytic gas-phase deconstruction methods, such as the homogeneous pyrolysis of lignin dispersed in the gas phase, as explored in the current paper, could become more practically significant as potential biodiesel surrogates if they achieve high yields and selectivity toward low-molecular-weight PAHs.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/molecules30102215/s1>, Figure S1: Schematic diagram of lignin laser-powered homogeneous pyrolysis (LPHP) flow reactor; Figure S2: FTIR data of the pyrolyzed lignin samples from LPHP reactors collected from the different locations of the reactor (labeled locations 1, 2, and 3 in the inset picture). The pyrolysis conditions were as follows: IR laser power

~22 W, main flow through atomizer 1 L/min, auxiliary flows 350 mi/min, SF6 content in main flow ~3% in (v/v); Table S1(a,b): The integral intensity of characteristic IR absorption bonds of OH (alcoholic and phenolic), C-H (stretching aliphatic), and CH₃ (methoxy) for initial HL and pyrolyzed lignin sampled from locations 1, 2, and 3 (inset in Figure 1); The ratio of integral intensity of characteristic IR absorption bonds of OH (alcoholic and phenolic), C-H (stretching aliphatic), and CH₃ (methoxy) toward aromatic skeletal mode in lignin (1513 cm⁻¹), depending on pyrolyzed lignin sampled from locations 1, 2, and 3 (inset in Figure 1); Tables S1–S4: Compounds identification at different laser power; Figure S3: Comparison of CPD reference EPR spectrum from pyrolysis of tricarbonylcyclopentadienylmanganese (black line, reaction 1) with traces of CPD radicals detected from pyrolysis of *p*-coumaryl alcohol at elevated temperatures (>700 °C); Figure S4: Formation of substituted cyclopentadienyl (CPD) radical from decomposition of *p*-coumaryl alcohol based on M06-2X/6-31G(d,p) DFT level calculations; Figure S5: Stepwise formation of small, GC-detectable PAHs (as an example) through HACA and cyclopentadienyl radical (CPD) addition (and CH₃ removal) in LPHP reactor; Figure S6: Pyroprobe 1000 for fast pyrolysis of hydrolytic lignin. References [57–77] are cited in the Supplementary Materials.

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References

1. Barekati-Goudarzi, M.; Boldor, D.; Marculescu, C.; Khachatryan, L. Peculiarities of Pyrolysis of Hydrolytic Lignin in Dispersed Gas Phase and in Solid State. *Energy Fuels* **2017**, *31*, 12156–12167. [CrossRef]
2. Barekati-Goudarzi, M.; Boldor, D.; Khachatryan, L.; Lynn, B.; Kalinoski, R.; Shi, J. Heterogeneous and Homogeneous Components in Gas-Phase Pyrolysis of Hydrolytic Lignin. *ACS Sustain. Chem. Eng.* **2020**, *8*, 12891–12901. [CrossRef]
3. Khachatryan, L.; Barekati-Goudarzi, M.; Kekejian, D.; Aguilar, G.; Asatryan, R.; Stanley, G.G.; Boldor, D. Pyrolysis of Lignin in Gas-Phase Isothermal and cw-CO₂ Laser Powered Non-Isothermal Reactors. *Energy Fuels* **2018**, *32*, 12597–12606. [CrossRef]
4. Kekejian, D.; Khachatryan, L.; Barekati-Goudarzi, M.; Boldor, D. Implication of COMSOL to Laser Powered Non-Isothermal Reactors for Pyrolysis in the Gas Phase. In Proceedings of the COMSOL Conference, Boston, MA, USA, 3–5 October 2018.
5. Zhou, H.; Wu, C.; Onwudili, J.A.; Meng, A.; Zhang, Y.; Williams, P.T. Polycyclic Aromatic Hydrocarbon Formation from the Pyrolysis/Gasification of Lignin at Different Reaction Conditions. *Energy Fuels* **2014**, *28*, 6371–6379. [CrossRef]
6. McGrath, T.; Sharma, R.; Hajaligol, M. An experimental investigation into the formation of polycyclic-aromatic hydrocarbons (PAH) from pyrolysis of biomass materials. *Fuel* **2001**, *80*, 1787–1797. [CrossRef]
7. Fabbri, D.; Adamiano, A.; Torri, C. GC-MS determination of polycyclic aromatic hydrocarbons evolved from pyrolysis of biomass. *Anal. Bioanal. Chem.* **2010**, *397*, 309–317. [CrossRef]
8. Kozliak, E.I.; Kubatova, A.; Artemyeva, A.A.; Nagel, E.; Zhang, C.; Rajappagowda, R.B.; Srinirnova, A.L. Thermal Liquefaction of Lignin to Aromatics: Efficiency, Selectivity, and Product Analysis. *ACS Sustain. Chem. Eng.* **2016**, *4*, 5106–5122. [CrossRef]
9. Valero-Romero, M.J.; García-Mateos, F.J.; Kapteijn, F.; Rodríguez-Mirasol, J.; Cordero, T. Fischer-Tropsch synthesis over lignin-derived cobalt-containing porous carbon fiber catalysts. *Appl. Catal. B-Environ.* **2023**, *321*, 122078. [CrossRef]

10. Shen, Q.; Zhong, L. Lignin-based carbon films and controllable pore size and properties. *Mater. Sci. Eng. A-Struct.* **2007**, *445*, 731–735. [CrossRef]
11. Li, X.H.; You, X.Y.; Wang, X.L.; Kang, J.; Zhang, H.J. Advanced Lignin-Based Hydrogels with Superior Stiffness, Toughness, and Sensing Capabilities. *Adv. Funct. Mater.* **2025**, *35*, 2415744. [CrossRef]
12. Westerholm, R.N.; Alsberg, T.E.; Frommelin, A.B.; Strandell, M.E.; Rannug, U.; Winkvist, L.; Grigoriadis, V.; Egeback, K.E. Effect of Fuel Polycyclic Aromatic Hydrocarbon Content on the Emissions of Polycyclic Aromatic-Hydrocarbons and Other Mutagenic Substances from a Gasoline-Fueled Automobile. *Environ. Sci. Technol.* **1988**, *22*, 925–930. [CrossRef] [PubMed]
13. de Souza, C.V.; Corrêa, S.M. Polycyclic aromatic hydrocarbons in diesel emission, diesel fuel and lubricant oil. *Fuel* **2016**, *185*, 925–931. [CrossRef]
14. Qian, Y.; Yu, L.; Li, Z.L.; Zhang, Y.H.; Xu, L.L.; Zhou, Q.Y.; Han, D.; Lu, X.C. A new methodology for diesel surrogate fuel formulation: Bridging fuel fundamental properties and real engine combustion characteristics. *Energy* **2018**, *148*, 424–447. [CrossRef]
15. Guibet, J.C.; Faure-Birchem, E. *Fuels and Engines: Technology, Energy, Environment*; Éditions Technip: Paris, France, 1999.
16. Todorciuc, T.; Capraru, A.-M.; Kratochvilova, I.; Popa, V.I. Characterization of non-wood lignin and its hydroxymethylated derivatives by spectroscopy and self-assembling investigations. *Surfaces* **2009**, *43*, 399–408.
17. Faix, O. Classification of Lignins from Different Botanical Origins by FT-IR Spectroscopy. *Holzforschung* **1991**, *45*, 21. [CrossRef]
18. Sharma, R.K.; Wooten, J.B.; Baliga, V.L.; Lin, X.; Chan, W.G.; Hajaligol, M.R. Characterization of chars from pyrolysis of lignin. *Fuel* **2004**, *83*, 1469–1482. [CrossRef]
19. Boon, J.; Bobeldijk Pastorova, I.; Botto, R.E.; Arisz, P. Structural studies on cellulose pyrolysis and cellulose chars by PYMS, PYGCMS, FTIR, NMR and by wet chemical techniques. *Biomass Bioenergy* **1994**, *7*, 25–32. [CrossRef]
20. Ben, H.; Ragauskas, A.J. Comparison for the compositions of fast and slow pyrolysis oils by NMR characterization. *Bioresour. Technol.* **2013**, *147*, 577–584. [CrossRef]
21. Marathe, P.S.; Westerhof, R.J.M.; Kersten, S.R.A. Fast pyrolysis of lignins with different molecular weight: Experiments and modelling. *Applied Energy* **2019**, *236*, 1125–1137. [CrossRef]
22. Zhou, S.; Garcia-Perez, M.; Pecha, B.; Kersten, S.R.A.; McDonald, A.G.; Westerhof, R.J.M. Effect of the Fast Pyrolysis Temperature on the Primary and Secondary Products of Lignin. *Energy Fuels* **2013**, *27*, 5867–5877. [CrossRef]
23. Wang, H. Formation of nascent soot and other condensed-phase materials in flames. *Proc. Combust. Inst.* **2011**, *33*, 41–67. [CrossRef]
24. Frenklach, M.; Gardiner, W.C.; Stein, S.E.; Clary, D.W.; Yuan, T. Mechanism of Soot Formation in Acetylene-Oxygen Mixtures. *Combust. Sci.* **1986**, *50*, 79–115. [CrossRef]
25. Frenklach, M.; Singh, R.I.; Mebel, A.M. On the low-temperature limit of HACA. *Proc. Combust. Inst.* **2019**, *37*, 969–976. [CrossRef]
26. Saggese, C.; Frassoldati, A.; Cuoci, A.; Faravelli, T.; Ranzi, E. A wide range kinetic modeling study of pyrolysis and oxidation of benzene. *Combust. Flame* **2013**, *160*, 1168–1190. [CrossRef]
27. Ledesma, E.B.; Marsh, N.D.; Sandrowitz, A.K.; Wornat, M.J. Global kinetic rate parameters for the formation of polycyclic aromatic hydrocarbons from the pyrolysis of catechol, a model compound representative of solid fuel moieties. *Energy Fuels* **2002**, *16*, 1331–1336. [CrossRef]
28. Mastral, A.M.; Callen, M.S. A review on polycyclic aromatic hydrocarbon (PAH) emissions from energy generation. *Environ. Sci. Technol.* **2000**, *34*, 3051–3057. [CrossRef]
29. Akazawa, M.; Kojima, Y.; Kato, Y. Formation mechanism of polycyclic compounds from phenols by fast pyrolysis. *EC Agric.* **2015**, *1*, 67–85.
30. Sarofim, A.F.; Longwell, J.P.; Wornat, M.J.; Mukherjee, J. (Eds.) *Soot Formation in Combustion*; Springer: Berlin, Germany, 1994.
31. Shukla, B.; Koshi, M. Comparative study on the growth mechanisms of PAHs. *Combust Flame* **2011**, *158*, 369–375. [CrossRef]
32. Lu, M.; Mulholland, J.A. Aromatic hydrocarbon growth from indene. *Chemosphere* **2001**, *42*, 623. [CrossRef]
33. Yang, B.; Hu, B.; Koylu, U.O. Mean soot volume fractions in turbulent hydrocarbon flames: A comparison of sampling and laser measurements. *Combust. Sci.* **2005**, *177*, 1603–1626. [CrossRef]
34. Chu, T.C.; Smith, M.C.; Yang, J.; Liu, M.J.; Green, W.H. Theoretical study on the HACA chemistry of naphthalenyl radicals and acetylene: The formation of C₁₂H₈, C₁₄H₈, and C₁₄H₁₀ species. *Int. J. Chem. Kinet.* **2020**, *52*, 752–768. [CrossRef]
35. Khachatryan, L.; Adoukpe, J.; Maskos, Z.; Dellinger, B. Formation of Cyclopentadienyl Radical from the Gas-Phase Pyrolysis of Hydroquinone, Catechol, and Phenol. *Environ. Sci. Technol.* **2006**, *40*, 5071–5076. [CrossRef] [PubMed]
36. Adunkpe, J.; Khachatryan, L.; Dellinger, B.; Ghosh, M. Radicals from the Atmospheric Pressure Pyrolysis and Oxidative Pyrolysis of Hydroquinone, Catechol and Phenol. *Energy Fuel* **2009**, *23*, 1551–1554. [CrossRef]
37. Khachatryan, L.; Xu, M.X.; Wu, A.J.; Pechagin, M.; Asatryan, R. Radicals and Molecular Products from the Gas-Phase Pyrolysis of Lignin Model Compounds. Cinnamyl Alcohol. *J. Anal. Appl. Pyrolysis* **2016**, *121*, 75–83. [CrossRef] [PubMed]
38. Xu, M.X.; Khachatryan, L.; Baev, A.; Asatryan, R. Radicals from the Gas-Phase Pyrolysis of Lignin Model Compounds. p-Coumaryl Alcohol. *RSC Adv.* **2016**, *6*, 62399–62405. [CrossRef]

39. Adoukpe, J.; Aina, M.; Mama, D.; Sinsin, B. Gas Chromatography Mass Spectrometry Identification of Labile Radicals Formed during Pyrolysis of Catechol, Hydroquinone, and Phenol through Neutral Pyrolysis Product Mass Analysis. *Int. Sch. Res. Not.* **2013**, *2013*, 930573. [CrossRef]
40. Custodis, V.B.F.; Hemberger, P.; Ma, Z.Q.; van Bokhoven, J.A. Mechanism of Fast Pyrolysis of Lignin: Studying Model Compounds. *J. Phys. Chem. B* **2014**, *118*, 8524–8531. [CrossRef]
41. Herring, P.; Khachatryan, L.; Lomnicki, S.; Dellinger, B. Paramagnetic centers in particulate formed from the oxidative pyrolysis of 1-methylnaphthalene in the presence of Fe(III)(2)O-3 nanoparticles. *Combust. Flame* **2013**, *160*, 2996–3003. [CrossRef]
42. Asatryan, R.; Bennadji, H.; Bozzelli, J.W.; Ruckenstein, E.; Khachatryan, L. Molecular Products and Fundamentally Based Reaction Pathways in the Gas-Phase Pyrolysis of the Lignin Model Compound p-Coumaryl Alcohol. *J. Phys. Chem. A* **2017**, *121*, 3352–3371. [CrossRef]
43. Shukla, B.; Koshi, M. A novel route for PAH growth in HACA based mechanisms. *Combust Flame* **2012**, *159*, 3589–3596. [CrossRef]
44. Bockhorn, H.; Fetting, F.; Wenz, H. Investigation of the Formation of High Molecular Hydrocarbons and Soot in Premixed Hydrocarbon-Oxygen Flames. *Ber. Bunsenges. Phys. Chem.* **1983**, *87*, 1067–1073. [CrossRef]
45. Frenklach, M.; Warnatz, J. Detailed modeling of PAH profiles in a sooting low-pressure acetylene flame. *Combust. Sci.* **1987**, *51*, 265–283. [CrossRef]
46. Khachatryan, L.; Mascos, Z.; Dellinger, B. The Tar and Tar Radicals from Lignin Pyrolysis. *Free. Radic. Biol. Med.* **2014**, *76*, S140. [CrossRef]
47. Khachatryan, L.; Adoukpe, J.; Dellinger, B. Formation of Phenoxy and cyclopentadienyl radicals from the gas-phase pyrolysis of phenol. *J. Phys. Chem. A* **2008**, *112*, 481–487. [CrossRef]
48. Yuan, Y.F.; Jiang, B.; Chen, H.; Wu, W.J.; Wu, S.F.; Jin, Y.C.; Xiao, H.N. Recent advances in understanding the effects of lignin structural characteristics on enzymatic hydrolysis. *Biotechnol. Biofuels* **2021**, *14*, 205. [CrossRef]
49. Smith, E.A.; Lee, Y.J. Petroleomic Analysis of Bio-oils from the Fast Pyrolysis of Biomass: Laser Desorption Ionization–Linear Ion Trap–Orbitrap Mass Spectrometry Approach. *Energy Fuels* **2010**, *24*, 5190–5198. [CrossRef]
50. Shaub, W.M.; Bauer, S.H. Laser-Powered Homogeneous Pyrolysis. *Int. J. Chem. Kinet.* **1975**, *7*, 509–529. [CrossRef]
51. Kubat, P.; Pola, J. Spatial Temperature Distribution in Cw Co2-Laser Photosensitized Reactions. *Collect. Czech. Chem. C.* **1984**, *49*, 1354–1359. [CrossRef]
52. Molin, Y.N.; Panfilov, V.N.; Petrov, A.K. *Infrared Photochemistry*; Novosibirsk Izd. Nauka: Novosibirsk, Russia, 1985.
53. Sukiasyan, A.A.; Khachatryan, L.A.; Il'in, S.D. Measuring of the kinetic parameters of homogeneous decomposition of Azomethane under CO₂-laser irradiation. *Arm. Khim. Zhur.* **1988**, *41*, 104.
54. Russell, D.K. Infrared-Laser Powered Homogeneous Pyrolysis. *Chem. Soc. Rev.* **1990**, *19*, 407–437. [CrossRef]
55. Mantashyan, A.A. Peculiarities of the slow combustion of a hydrocarbon in a “wall-less” reactor with laser heating. *Combust Flame* **1998**, *112*, 261–265. [CrossRef]
56. Swihart, M.T.; Carr, R.W. Pulsed laser powered homogeneous pyrolysis for reaction kinetics studies: Probe laser measurement of reaction time and temperature. *Int. J. Chem. Kinet.* **1996**, *28*, 817–828. [CrossRef]
57. Liu, E.; Das, L.; Zhao, B.; Crocker, M.; Shi, J. Impact of Dilute Sulfuric Acid, Ammonium Hydroxide, and Ionic Liquid Pretreatments on the Fractionation and Characterization of Engineered Switchgrass. *Bioenergy Res.* **2017**, *10*, 1079–1093. [CrossRef]
58. Faix, O. Investigation of Lignin Polymer Models (Dhps) by Ftir Spectroscopy. *Holzforschung* **1986**, *40*, 273–280. [CrossRef]
59. Yang, H.P.; Yan, R.; Chen, H.P.; Lee, D.H.; Zheng, C.G. Characteristics of hemicellulose, cellulose and lignin pyrolysis. *Fuel* **2007**, *86*, 1781–1788. [CrossRef]
60. Clapp, R.; deFur, P.; Silbergeld, E.; Washburn, P. EPA on the right track. *Environ. Sci. Technol.* **1995**, *29*, 29–30. [CrossRef]
61. Kislov, V.; Islamova, N.; Kolker, A.; Lin, S.; Mebel, A. Hydrogen Abstraction Acetylene Addition and Diels–Alder Mechanisms of PAH Formation: A Detailed Study Using First Principles Calculations. *J. Chem. Theory Comput.* **2005**, *1*, 908–924. [CrossRef]
62. Hansen, N.; Schenk, M.; Moshhammer, K.; Kohse-Hoinghaus, K. Investigating repetitive reaction pathways for the formation of polycyclic aromatic hydrocarbons in combustion processes. *Proc. Combust. Flame* **2017**, *180*, 250–261. [CrossRef]
63. Frenklach, M. Reaction mechanism of soot formation in flames. *Phys. Chem. Chem. Phys.* **2002**, *4*, 2028–2037. [CrossRef]
64. Frenklach, M.; Schuetz, C.A.; Ping, J. Migration mechanism of aromatic-edge growth. *Proc. Combust. Inst.* **2005**, *30*, 1389–1396. [CrossRef]
65. Kholghy, M.R.; Eaves, N.A.; Veshkini, A.; Thomson, M.J. The role of reactive PAH dimerization in reducing soot nucleation reversibility. *Proc. Combust. Inst.* **2019**, *37*, 1003–1011. [CrossRef]
66. Herdman, J.D.; Miller, J.H. Intermolecular potential calculations for polynuclear aromatic hydrocarbon clusters. *J. Phys. Chem. A* **2008**, *112*, 6249–6256. [CrossRef] [PubMed]
67. Whitesides, R.; Kollias, A.C.; Domin, D.; Lester, W.A.; Frenklach, M. Graphene layer growth: Collision of migrating five-member rings. *Proc. Combust. Inst.* **2007**, *31*, 539–546. [CrossRef]
68. Cavallotti, C.; Mancarella, S.; Rota, R.; Carra, S. Conversion of C5 into C6 cyclic species through the formation of C7 intermediates. *J. Phys. Chem. A* **2007**, *111*, 3959–3969. [CrossRef]

69. Agafonov, G.L.; Naydenova, I.; Vlasov, P.A.; Warnatz, J. Detailed Kinetic Modeling of Soot Formation in Shok Tube Pyrolysis and Oxidation of Toluene and N-Heptane. *Proc. Combust. Inst.* **2007**, *31*, 575–583. [CrossRef]
70. Violi, A.; Izvekov, S. Soot Primary Particle Formation from Multiscale Coarse-Grained Molecular Dynamics Simulation. *Proc. Combust. Inst.* **2007**, *31*, 529–537. [CrossRef]
71. Sinha, S.; Rahman, R.K.; Raj, A. On the role of resonantly stabilized radicals in polycyclic aromatic hydrocarbon (PAH) formation: Pyrene and fluoranthene formation from benzyl-indenyl addition. *Phys. Chem. Chem. Phys.* **2017**, *19*, 19262–19278. [CrossRef]
72. Johansson, K.O.; Head-Gordon, M.P.; Schrader, P.E.; Wilson, K.R.; Michelsen, H.A. Resonance-stabilized hydrocarbon-radical chain reactions may explain soot inception and growth. *Science* **2018**, *361*, 997–1000. [CrossRef]
73. Marinov, N.M.; Pitz, M.J.; Westbrook, C.K.; Vincitore, A.M.; Castaldi, M.J.; Senkan, S.M. Aromatic and polycyclic aromatic hydrocarbon formation in a laminar premixed n-butane flame. *Combust. Flame* **1998**, *114*, 192. [CrossRef]
74. Ihm, H.; White, J.M. Stepwise Dissociation of Thermally Activated Phenol on Pt(III). *J. Phys. Chem. B* **2000**, *104*, 6202–6211. [CrossRef]
75. Netzer, F.P. Low-Temperature Polymerization of Condensed Cyclopentadiene Induced by Uv Irradiation. *Chem. Phys. Lett.* **1988**, *146*, 566–569. [CrossRef]
76. Welipitiya, D.; Dowben, P.A.; Zhang, J.D.; Pai, W.W.; Wendelken, J.F. The adsorption and desorption of ferrocene on Ag(100). *Surf. Sci.* **1996**, *367*, 20–32. [CrossRef]
77. Pai, W.W.; Zhang, Z.Y.; Zhang, J.D.; Wendelken, J.F. Direct visualization in manipulation of stable molecular radicals at room temperature. *Surf. Sci.* **1997**, *393*, L106–L112. [CrossRef]

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Article

Preparation and Modification of Sucrose-Based Non-Isocyanate Polyurethane Adhesives for Plywood Bonding

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Abstract: The production of non-isocyanate polyurethane (NIPU) resins using recyclable biomass materials and no isocyanates as a substitute for traditional polyurethane (PU) materials has become a research focus in the polyurethane industry. The development of such NIPU resins for application as wood adhesives has also emerged as an interesting new research topic. In this study, sucrose was used to react with dimethyl carbonate, and then polymerized with an amine to prepare sucrose-based non-isocyanate polyurethane (SNIPU) adhesives and evaluate their suitability for use in plywood. Four amines, namely polyethylene amine (PEI) of molecular weight (MW) 10,000, PEI of MW 1800, diethylenetriamine, and hexanediamine were tested in the preparation of SNIPU adhesives to determine a more suitable amine showing optimal adhesion performance. The effect of the amount of the amine added on adhesive properties was further investigated. The results showed that the SNIPU adhesive prepared with PEI-10000 as amine presents a good bonding performance. The SNIPU prepared with a PEI-10000 content of 45% (*w/w* on sucrose) presented the highest bonding strength. The dry strength, 24 h cold water (23 °C) wet strength, and 3 h hot water (63 °C and 93 °C) wet strengths of its bonded plywood were 1.26 MPa, 0.90 MPa, 0.84 MPa, and 0.80 MPa, respectively. Furthermore, the addition of 13% (*w/w* on SNIPU adhesive) of ethylene glycol diglycidyl ether (EGDE) as a modifier showed a significant decrease of 20 °C of the curing temperature of the SNIPU adhesive.

Keywords: biomass adhesive; NIPU; sucrose; bonding strength

1. Introduction

Polyurethanes (PU) are high-performance polymers formed by polycondensation of polyols and isocyanates. Due to their excellent mechanical properties and high plasticity, they are widely used in the wood-based panels industry, including coatings and adhesives [1,2]. However, isocyanates, a raw material used in PU, are highly toxic and volatile. To avoid their potential dangers, research into environmentally friendly and high-performance polyurethanes (PU) has become a significant trend. Consequently, non-isocyanate polyurethane (NIPU) wood adhesives that do not use isocyanates as raw materials have become a focal point of research. Currently, the reaction of cyclic carbonates with aliphatic primary amines has become one of the mainstream methods for synthesizing NIPUs due to its safety and environmental benefits. Furthermore, in the context of green

and sustainable development, utilizing natural renewable resources through the reaction of cyclic carbonates and aliphatic primary amines to prepare sustainable NIPU adhesives holds a significant research interest [3–7].

Natural biomass materials are widely regarded as good substitutes for non-renewable and toxic substances due to their broad availability, low cost, and lack of toxicity [8–10]. There are reports of using natural tannin as a raw material to prepare a tannin-based non-isocyanate adhesive [11]. However, tannin raw extract is a macromolecular polyphenol containing pectin carbohydrates, which normally results in an excessively high viscosity of the tannin-based non-isocyanate adhesive [12]. Additionally, a lignin-based non-isocyanate polyurethane resin prepolymer with both branched and straight chain architectures was developed utilizing lignin as raw material [13]. Lignin presents fewer reaction sites and lower reactivity due to the many substituents on its aromatic rings of lignin. This results in a poor adhesive performance when used in the production of particleboard. Due to these disadvantages, its use for wood gluing when alone is not ideal. Thus, to find a chemical biomass feedstock with more reactive sites is a worthwhile endeavor.

Sucrose is a smaller size carbohydrate with eight hydroxyl groups that can directly serve as reaction sites for adhesive preparation. Consequently, sucrose is gradually being used as a biomass raw material in the production of wood adhesives [14–16]. Additionally, sucrose is a commercially and widely available raw material also found in the leaves, flowers, stems, seeds, and fruits of plants, with particularly high concentrations in sugarcane, sugar beets, and maple sap [17,18]. Due to its abundant availability and low cost, sucrose is extensively used in the beverage, food, and chemical industries [19–22]. In previous work, good wood bonding sucrose-based non-isocyanate polyurethanes were developed by reacting sucrose with dimethyl carbonate and hexamethylenediamine. However, hexamethylenediamine has a relatively low molecular weight with only two amine groups resulting in a relatively low level of condensation resulting in a relatively higher curing temperature and longer curing time than is useful for wood panel adhesives. Moreover, unreacted hexamethylene diamine might volatilize during hot pressing, posing a hazard to the environment and human health [23]. Therefore, to address these issues, selecting a high-molecular-weight amine with a suitably higher degree of polymerization as a raw material for preparing sucrose-based NIPU adhesives is an interesting research direction.

Polyethyleneimine (PEI) is a water-soluble polymer comprising a large number of repeating structural units and a substantial number of amino groups [24]. The ultra-high branching of PEI is conducive to enhanced reactivity and higher levels of crosslinking [25]. PEI has polar (amino) and hydrophobic groups that enable crosslinking with a number of compounds. Because of this, PEI presents advantages in the preparation of adhesives and can thus also be employed in wood adhesives [26,27].

Thus, in the research work presented here, a novel sucrose-based NIPU adhesive was prepared from sucrose, dimethyl carbonate (DMC), and PEI-10000 as the primary raw materials. Unlike conventional polyurethane adhesives, this approach completely avoids the use of toxic isocyanates, making it more environmentally friendly and sustainable. The impact of incorporating diverse polyamine compounds and varying quantities of PEI-10000 on the adhesive's functionality was systematically examined, and the synthesis procedure was refined to enhance its performance. Moreover, the addition of EGDE as an additional cross-linker has been strategically introduced, leading to further optimization of the bonded panels' hot-pressing conditions. This study not only provides a sustainable alternative to traditional adhesives but also offers new insights into the molecular design and processing optimization of non-isocyanate polyurethanes, addressing critical challenges in bio-based adhesive technology.

2. Results and Discussions

2.1. Effect of Polyamine Type on SNIPU Adhesives

Figure 1a shows the shear strength test results of SNIPU adhesives prepared with different polyamines. The plywood bonded with the SNIPU1 adhesive synthesized from PEI-10000 showed the best bonding performance. Its dry strength, 24 h cold water (23 °C) wet strength, and 3 h hot water (63 °C and 93 °C) wet strengths were 1.26 MPa, 0.90 MPa, 0.84 MPa, and 0.80 MPa, respectively, compliant with the national standard GB/T 9846-2015 [28] (dashed line in Figure 1a). However, the other three adhesives did not show a good bond strength, and in particular a poor wet shear strength. Water and moisture resistance are critical parameters that determine whether the adhesive can be used in practice as they affect plywood durability [29]. Figure 1b shows the water resistance of cured adhesives test results of SNIPU adhesives synthesized from different types of amines and indicates that the two PEIs present better water resistance of cured adhesives of lower-molecular-weight linear diamines and triamines. The SNIPU1 adhesive synthesized from PEI-10000 achieved a water resistance of cured adhesives of over 90%, demonstrating good water resistance of cured adhesives, which suggests that reactions with macromolecular amines increase the polymerization degree, thus enhancing the water resistance of cured adhesives. This also supports the test results shown in Figure 1a, indicating that it has superior water resistance of cured adhesives compared to adhesives synthesized from other amines.

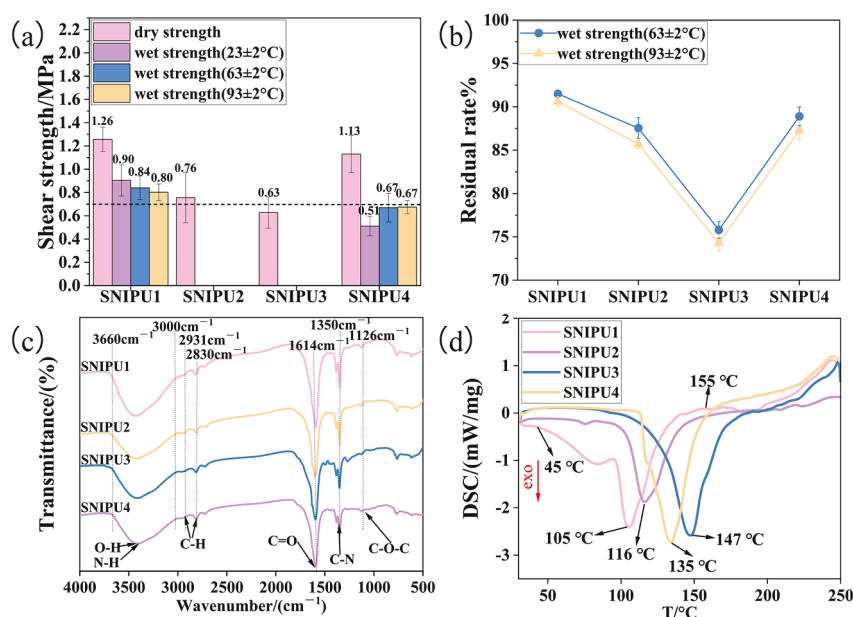


Figure 1. (a) Shear strength of SNIPU adhesives. (b) The water resistance of cured adhesives test results of SNIPU adhesives. (c) FT-IR curves of SNIPU adhesives. (d) DSC curves of SNIPU adhesives.

Figure 1c shows the FTIR spectra characterizing the chemical structures of the different SNIPU adhesives. The absorption bands in the region 3000 cm⁻¹–3660 cm⁻¹ are generated by O-H and N-H stretching vibrations; the peaks 2830 cm⁻¹ and 2931 cm⁻¹ are generated by the stretching vibrations of C-H; the 1614 cm⁻¹ peak is attributed to the absorption peak from the C=O stretching vibration. A distinct absorption peak at 1350 cm⁻¹ is a C-N stretching vibration; the peak at 1126 cm⁻¹ is the characteristic absorption peak of C-O-C in polyether groups [30,31]. The stretching vibration of C=O at the peak of 1614 cm⁻¹ is indicative of the production of the carbamate structure. Under the combined influence of hydrogen bonds in amino and carbamate structures, the absorption peak shifts to a lower wavenumber. Additionally, hydrogen bonding facilitates the formation of intramolecular

six-membered rings, resulting in a more stable structure with improved hydrolytic and chemical resistance [32–34]. Therefore, in Figure 1c, as the SNIPU1 adhesive presents a more marked 1614 cm^{-1} peak, this appears to indicate the formation of more carbamate structures. This is one reason why it shows a better bonding performance than the other SNIPU adhesives.

Figure 1d shows the DSC analysis results investigating the effect of the different polyamines on the thermal properties of SNIPU adhesives. It can be observed that the SNIPU1 adhesive's curing peak temperature ($105\text{ }^{\circ}\text{C}$) was significantly lower than that of the other three adhesives ($116\text{ }^{\circ}\text{C}$, $135\text{ }^{\circ}\text{C}$, $147\text{ }^{\circ}\text{C}$). Moreover, its curing onset temperature ($45\text{ }^{\circ}\text{C}$) and curing end temperature ($155\text{ }^{\circ}\text{C}$) were lower, thus completing the adhesive curing earlier, implying that SNIPU1 cures more rapidly at a lower temperature than the others. This is a key factor in ensuring the rapid formation of strong bonds between wood surfaces. Furthermore, comparing the areas of the DSC curves for the four adhesives, the area for the SNIPU1 adhesive is significantly larger than the others, exhibiting a larger exothermic peak, indicating that its exothermal curing reaction is more intense and that its curing is more complete [35]. Thus, this means that under the same hot-press conditions, the curing and bonding performance of the SNIPU1 adhesive is superior to that of the other adhesives. This confirms the reasonable insight of selecting PEI-10000 as the polyamine in this work. Thus, PEI-10000 can be considered as the preferred polyamine for the preparation of SNIPU adhesives. An endothermic peak appears after $200\text{ }^{\circ}\text{C}$, primarily due to the evaporation of moisture in the adhesive and the decomposition of the adhesive.

2.2. Effect of PEI-10000 Dosage on Adhesives

Figure 2a shows the test of the shear strength of plywood bonded with SNIPU1 adhesives prepared with different proportions of PEI-10000. As the amount of PEI-10000 increases, the shear strength of the adhesive improves, with an optimal shear strength observed at a PEI-10000 content of 45% (*w/w* of sucrose). This increase is due to the greater proportion of urethane bonds formed. This, coupled with hydrogen bonding, leads to more six-membered ring structures, thus enhancing bond stability, hydrolytic resistance, and chemical resistance, hence contributing to the improved shear strength. However, as the PEI-10000 content continues to increase, the shear strength decreases. This may be due to the high viscosity of PEI-10000 itself, which leads to higher viscosity in the synthesized adhesive as its content increases. High viscosity also hinders application and inhibits the adhesive's penetration into the wood surface, this being one of the reasons for the observed decrease in bonding strength, as shown in Figure 2b.

Figure 2c shows that the FTIR spectra were used to identify the structure and functional groups of the SNIPU1 adhesive. The 1614 cm^{-1} peak is the absorption peak from the C=O stretching vibration. It can be observed that SNIPU1-45% presents a more pronounced absorption peak, which indicates that a greater number of carbamate structures has been generated, with a higher proportion of six-membered rings, and an enhanced overall performance. Figure 3 shows the XPS spectra of adhesives with different PEI-10000 additions, as well as the fraction of distinct chemical bonds in the spectra. It shows that SNIPU1-45% presents the highest O=C-N peak area (13.37%), while the other three adhesives show lower areas: 10.79% for SNIPU1-30%, 12.0% for SNIPU1-37.5%, and 12.71% for SNIPU1-52.5%. This further shows that SNIPU1-45% produces a higher proportion of carbamate structures. Thus, the XPS analysis results confirm the FT-IR spectroscopy data to explain why SNIPU1-45% performs so well. The results of XPS spectral analysis and FT-IR spectral analysis further confirm the successful preparation of the NIPU adhesive.

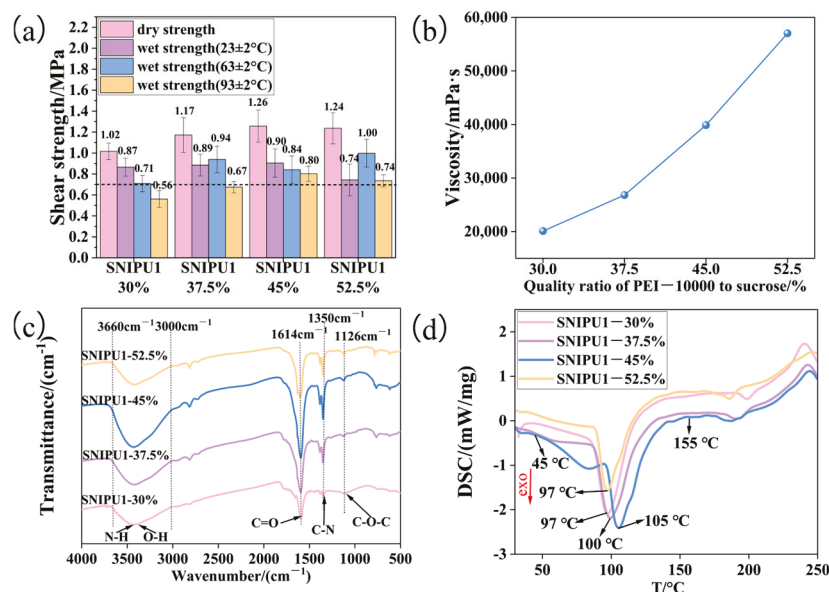


Figure 2. (a) Shear strength of SNIPU1 adhesives. (b) Viscosity of SNIPU1 adhesives. (c) FT-IR curves of SNIPU1 adhesives. (d) DSC curves of SNIPU1 adhesives.

Figure 2d shows the DSC analysis performed to study the effect of the PEI-10000 content on the thermal properties of the adhesive. Compared to other adhesives, the SNIPU1-45% adhesive exhibits a lower curing onset temperature (45 °C), which indicates a faster curing rate and a greater ability to cross-link more rapidly under the same conditions. Additionally, the SNIPU1-45% adhesive shows a larger integrated area of the DSC curve and a bigger exothermic peak, resulting in a more intense curing exotherm and more complete curing. This contributes to its superior performance. An endothermic peak appears after 200 °C, primarily due to the evaporation of moisture in the adhesive and the decomposition of the adhesive.

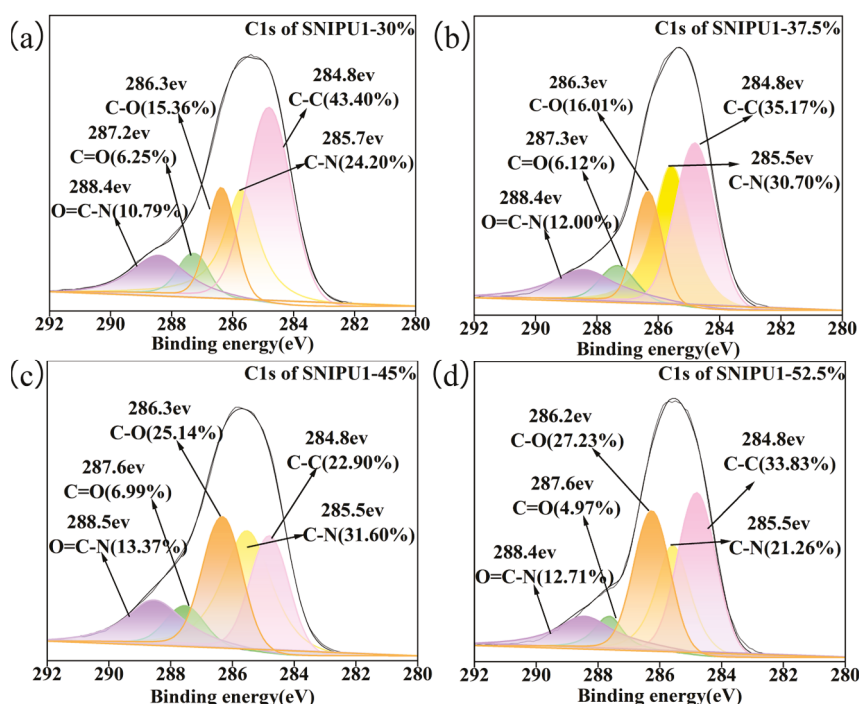
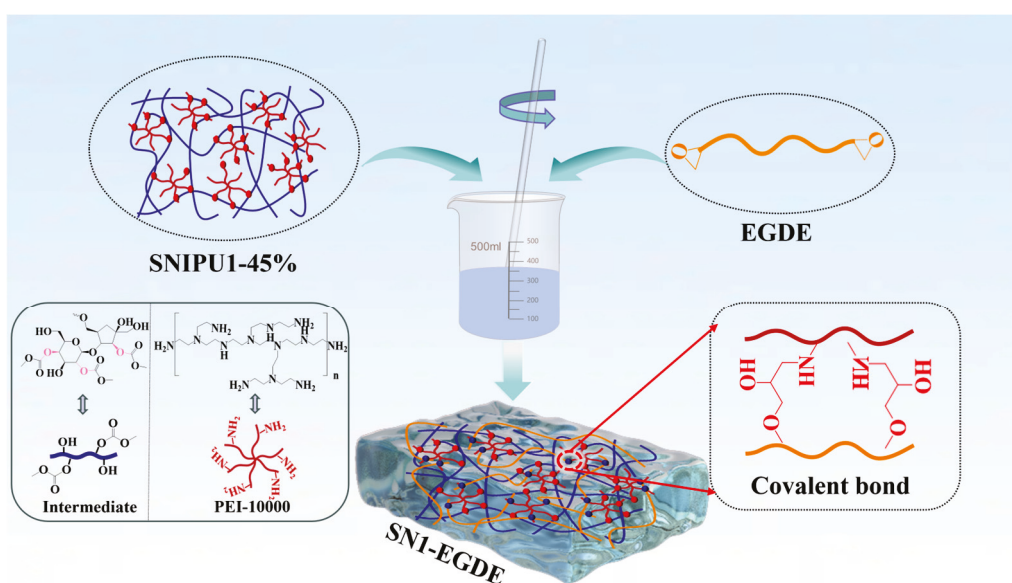


Figure 3. (a) C1s XPS spectra of SNIPU1-30% adhesive. (b) C1s XPS spectra of SNIPU1-37.5% adhesive. (c) C1s XPS spectra of SNIPU1-45% adhesive. (d) C1s XPS spectra of SNIPU1-52.5% adhesive.

2.3. Effect of Modifier Type on SNIPU Adhesive at the Lower Hot-Pressing Temperature of 180 °C

What described above was carried out at a hot-pressing temperature of 200 °C. Too high a hot-pressing temperature may damage the wood, thus it is crucial to reduce the hot-pressing temperature. To minimize the hot-pressing temperature of the SNIPU adhesive, a further cross-linker has been added to optimize the adhesive performance. The performance of the modified adhesive was then tested at 180 °C. The ternary ring structure of epoxy cross-linkers is easily opened, thus making them extremely reactive and suitable for usage as polymer cross-linkers producing stable covalent bonds [36]. Therefore, in this work, four epoxides, namely EGDE (The reaction scheme is shown in Scheme 1), an epoxy resin, a commercial epoxysilane (KH560), and trimethylolpropane triglycidyl ether were used to modify the SNIPU1 adhesive [37]. Figure 4a shows the effect of adding the same proportion of epoxide on the shear strength of SNIPU1. The plywood prepared with the adhesive modified by EGDE exhibited good wet shear strength at a hot-pressing temperature of 180 °C. In contrast, the unmodified SNIPU1 adhesive and the adhesives modified with the other three types of epoxides showed poorer wet shear strengths.



Scheme 1. Schematic representation of the preparation of the SN1-EGDE adhesive and the formation reaction of the SN1-EGDE adhesive structure.

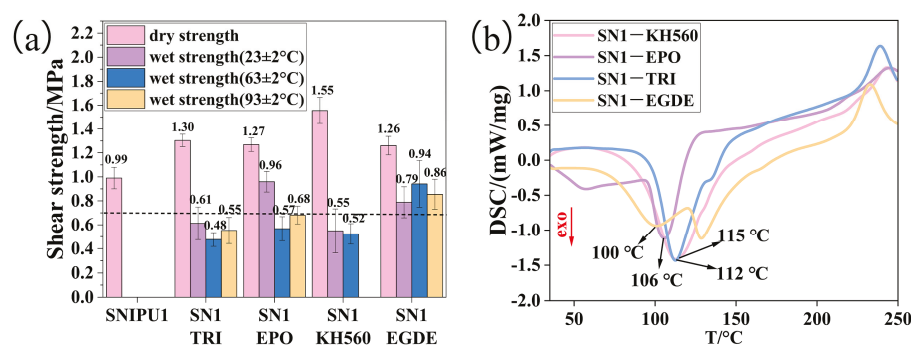


Figure 4. (a) Shear strength of SNIPU1 and SN1E adhesive under hot pressing at 180 °C. (b) DSC curves of SN1 adhesives.

Figure 4b shows the DSC analysis of the thermal properties of the adhesive after the addition of different epoxides. Under the same curing conditions, the curing temperatures of the SN1-EGDE, SN1-EPO, SN1-TRI, and SN1-KH560 adhesives were 100 °C, 106 °C,

112 °C, and 115 °C, respectively. The SN1-EGDE adhesive exhibited the lowest curing temperature, suggesting its superior performance and effective curing. Therefore, the bonding performance of the SN1-EGDE adhesive is better than that of the other adhesives. An endothermic peak appears after 200 °C, primarily due to the evaporation of moisture in the adhesive and the decomposition of the adhesive.

Figure 5 shows the SEM images of the SN1 adhesive. It can be clearly observed that SN1-TRI and SN1-EPO (Figure 5c,d) contain numerous pores, which were formed by the volatilization of substances during the high-temperature curing process. These volatiles disrupt the cross-linked network structure of the adhesive, negatively affecting its performance [38]. The SEM image of the SN1-KH560 adhesive (Figure 5b) shows an uneven surface with significant cracks, which may facilitate water penetration and lead to the breakdown of the cross-linked structure, resulting also in a weaker bonding performance. The SEM image of the SN1-EGDE adhesive (Figure 5a), shows noticeably fewer pores and cracks compared to the other three. Its smoother, denser structure effectively prevents water infiltration in humid environments, contributing to its better bonding performance [39]. All this further supports the rationale for selecting EGDE as the additional cross-linking agent.

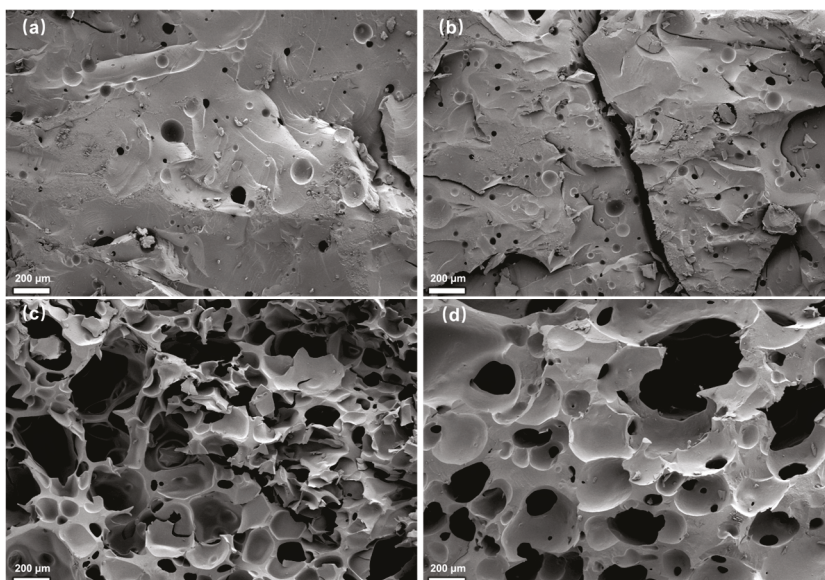


Figure 5. SEM image of SN1-EGDE (a), SN1-KH560 (b), SN1-TRI (c), SN1-EPO (d).

2.4. The Effect of the Amount of the Modifier EGDE on the Adhesive

Figure 6a shows the shear strength of plywood bonded with adhesives containing different amounts of EGDE when hot pressed at 180 °C. When the EGDE content was between 10% and 16% (*w/w* of SNIPU1), the SN1E-13% adhesive exhibited the best shear strength, with a dry strength of 1.40 MPa and a wet shear strength exceeding 0.90 MPa. This is because EGDE is a low-molecular-weight epoxy compound. Compared to existing methods, the increased addition of EGDE introduces more ether bond structures, enhancing the toughness of the system, thereby reducing the brittleness of the cured adhesive and improving its impact resistance. Additionally, the increase in EGDE reduces intermolecular forces within the adhesive, lowering its viscosity and improving its fluidity, making it easier to apply and penetrate the plywood. A higher EGDE content allows the amino groups in the adhesive to react more fully with the epoxy groups of EGDE, forming a denser cross-linked network. This is the key reason for the significant improvement in adhesive performance under a hot-pressing temperature of 180 °C. However, there are also disadvantages. With a further increase in EGDE content, the wet shear strength of

SN1E-16% decreases, possibly due to excessive EGDE reducing the cross-linking density. Moreover, excessive adhesive penetration into the plywood during application results in an overly thin cured adhesive layer, leading to decreased strength. Additionally, an excessive amount of EGDE increases the hydrophilicity of the adhesive, resulting in a higher water absorption rate, which accelerates aging in humid environments [40,41]. Figure 6b shows the physical image of the plywood dry shear strength test, with the wood failure rate indicated in parentheses. The results demonstrate that the sample bonded with SN1E-13% exhibits a higher wood failure rate (50%), indicating superior adhesive performance. Figure 6c shows the XPS spectrum of SN1-EGDE. After the addition of EGDE, the proportion of C-N (18.33%) and O=C-N (4.11%) in SN1-EGDE in the adhesive decreased compared to that before the addition (Figure 3), which may be due to the reaction of the adhesive SNIPU1 with EGDE to introduce more other groups. This confirms the smooth progress of the reaction and validates the reason for the improved strength.

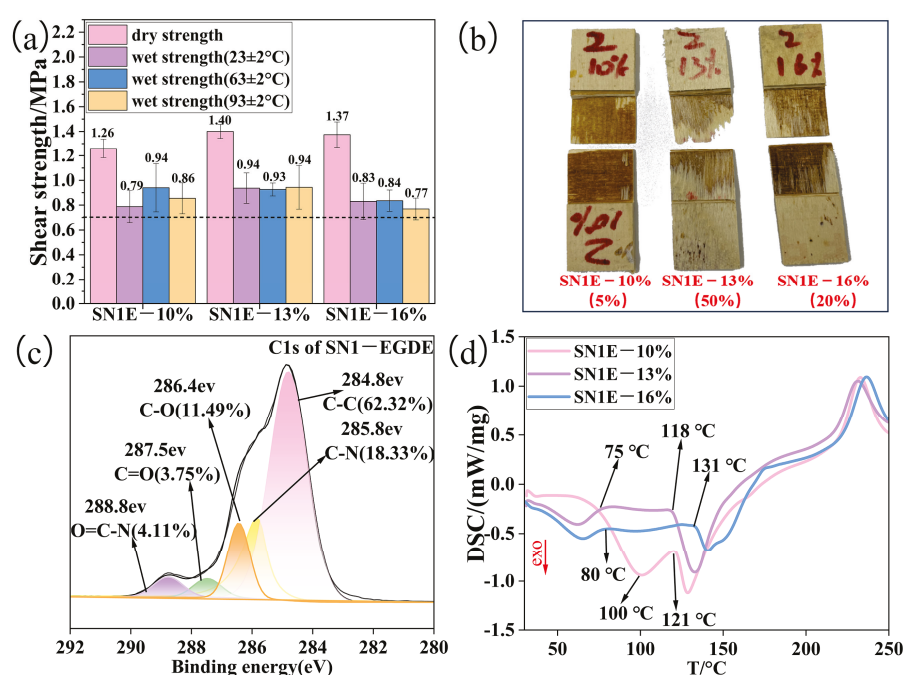


Figure 6. (a) Shear strength of SN1E adhesive under hot pressing at 180 °C. (b) Plywood dry shear strength test diagram. (c) C1s XPS spectra of SN1-EGDE adhesive. (d) DSC curves of SN1E adhesives.

Figure 6d shows the DSC analysis of the thermal properties of adhesives with different amounts of EGDE. SN1E-13% exhibited an exothermic reaction early in the test, which may be due to the reaction between the added EGDE and the adhesive, and the exothermic reaction ended at 75 °C. The larger exothermic peak area observed in the figure indicates a faster and more complete reaction, allowing the adhesive network to form more rapidly and densely. In contrast, the exothermic reaction for SN1E-10% occurred later and did not end until 121 °C, which may be due to the smaller amount of EGDE added, resulting in a smaller reaction contact area. SN1E-13% also began a second exothermic reaction at 118 °C, earlier than SN1E-10% (121 °C) and SN1E-16% (131 °C), indicating that SN1E-13% cures more quickly between the plywood layers. Furthermore, SN1E-13% has a larger exothermic peak, more thorough and complete curing, a stronger exothermic reaction, and a denser bond between the plywood layers, which is one of the reasons for its superior strength. An endothermic peak appears after 200 °C, primarily due to the evaporation of moisture in the adhesive and the decomposition of the adhesive.

3. Materials and Methods

3.1. Materials

The sucrose was procured from Guangdong Guanghua Technology Co., Ltd. Polyethyleneimine (PEI, Mw = 10,000 Da) was purchased from Adamas Beta (Shanghai) Chemical Reagent Co., Ltd. (Shanghai, China). Dimethyl carbonate (AR, $\geq 99\%$), polyethyleneimine (PEI, Mw = 1800 Da), hexanediamine, diethylenetriamine, sodium dodecylbenzene sulfonate, ethylene glycol diglycidyl ether (EGDE), trimethylolpropane triglycidyl ether, and epoxy resin were all purchased from Shanghai Macklin Biochemical Technology Co., Ltd. (Shanghai, China). The silane coupling agent KH560 was purchased from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China).

3.2. Preparation of SNIPU Adhesives

A total of 40 g of sucrose, 30 g of water, 36 g of dimethyl carbonate and 1.2 g of sodium dodecylbenzene sulfonate were added into a flask and heated up to 70 °C to keep warm and react for 2 h, then the polyamine compound was added and heated up to 90 °C, and the temperature was timed to react for 30 min to obtain the sample. In this study, four polyamine compounds, PEI-10000, hexanediamine, diethylenetriamine (DETA), and PEI-1800, were used to prepare the adhesives, and the prepared samples were named SNIPU1, SNIPU2, SNIPU3, and SNIPU4, respectively. Upon adjusting the PEI-10000 addition to 30%, 37.5%, 45%, and 52.5% (*w/w*, based on the weight of dry sucrose), the resulting adhesives were designated SNIPU1-30%, SNIPU1-37.5%, SNIPU1-45%, and SNIPU1-52.5%, respectively. When fixed at SNIPU1-45%, the samples to which the cross-linker ethylene glycol glycidyl ether, alkyl coupling agent KH560, trimethylolpropane triglycidyl ether, and epoxy resin were added were designated SN1-EGDE, SN1-KH560, SN1-TRI, and SN1-EPO, respectively. Meanwhile, when fixed as SN1-EGDE, the amount of addition was varied to 10%, 13%, and 16% (*w/w*, based on the mass of SNIPU1-45%), and the samples obtained were designated SN1E-10%, SN1E-13%, and SN1E-16%, respectively.

3.3. Preparation of Three-Layer Plywood and Bonding Strength Test

The adhesive was uniformly applied to the veneer surface (size 180 mm $\times$ 110 mm $\times$ 2 mm) at 300 g/m² glue application volume, and the three-layer plywood was subjected to a hot-pressing process at 200 °C and 1.2 MPa pressure for a duration of 6 min. The prepared samples were left at room temperature for 24 h, after which they were cut into pieces measuring 100 mm $\times$ 25 mm. They were then tested in accordance with China national standard GB/T 9846-2015 (≥ 0.7 MPa). Each test was repeated 6 times.

3.4. Viscosity Evaluation

Determination of viscosity of SNIPU1 adhesive at room temperature using NDJ digital viscometer (Shanghai Nirun Intelligent Technology Co., Ltd., Shanghai, China). The test was performed using rotor 4, repeated three times at a rotation speed of 6 RPM, and the average value was calculated.

3.5. Water Resistance Test of Cured Adhesives

The SNIPU adhesive samples were subjected to a two-hour baking process at 200 °C in an oven. Subsequently, the samples were immersed in water at temperatures of 93 °C and 63 °C for a period of three hours, after which they were dried in an oven at 80 °C for several hours until a stable weight was achieved. The water resistance of the cured adhesives was calculated using the following formula:

$$\text{water resistance of cured adhesives (\%)} = \frac{m}{M} \times 100 \quad (1)$$

where M and m are, respectively, the mass before and after immersion.

3.6. Fourier Transforms Infrared (FT-IR) Spectroscopy

The adhesive was dried to a constant weight in an oven at 200 ± 2 °C and then ground into a powder for FTIR analysis. The powder was mixed with KBr powder at a mass ratio of 1:100 and pressed into a sheet-like form using a mold, and analyzed using a Varian 1000 spectrometer (Varian Inc., Palo Alto, CA, USA). The scanning range was 4000–500 cm^{-1} , with a resolution of 4 cm^{-1} and 32 scans.

3.7. X-Ray Photo Spectroscopy (XPS) Analysis

The XPS analysis was conducted using the Thermo Scientific K-Alpha+ (Thermo Fisher Scientific, Waltham, MA, USA), with a monochromatic Al $K\alpha$ source having an energy of 1486.6 eV. The pass energy for narrow scan was set at 50 eV, with a step size of 0.100 eV. The binding energy was calibrated using the C1s peak of carbon at 284.8 eV to compensate for charge correction, and the spectra were processed using Thermo Advantage software (5.9921).

3.8. Differential Scanning Calorimetry (DSC) Analysis

DSC analysis was conducted using a NETZSCH DSC 204 F1 (Netzsch Group, Selb, Germany) differential scanning calorimeter, with a heating program from 30 °C to 250 °C at a rate of 10 °C/min, in a sealed alumina crucible, while maintaining a constant nitrogen flow atmosphere (20 mL/min).

3.9. Scanning Electron Microscopy (SEM)

The dried adhesive samples were cut into rectangles with a flat cross-section, measuring 0.5 cm in length, 0.5 cm in width, and less than 1 cm in height. The surface of the samples was then sprayed with gold and examined under a ZEISS Sigma 300 scanning electron microscope in Germany (ZEISS, Jena, Germany), with an accelerating voltage of 5 kV.

4. Conclusions

In the work presented, a novel non-isocyanate biomass sucrose-based adhesive was successfully synthesized by using PEI-10000 (45% sucrose weight) as the primary raw material, with modifications in the type and quantity of polyamine used. The adhesive demonstrated promising performance, with the NIPU1-45% adhesive exhibiting a dry strength of 1.26 MPa, a 24 h cold water (23 °C) wet strength of 0.90 MPa, and 3 h hot water (63 °C and 93 °C) wet strengths of 0.84 MPa and 0.80 MPa, respectively, at a hot-pressing temperature of 200 °C. Furthermore, by incorporating an epoxy-based EGDE-modified adhesive, the hot-pressing temperature was successfully reduced to 180 °C, while still meeting the national standard GB/T 9846-2015 (≥ 0.7 MPa), which demonstrates its potential for industrial applications in the wood industry. This study not only simplifies the adhesive production process but also opens a new research direction for non-isocyanate biomass adhesives, contributing to the development of more sustainable and environmentally friendly materials. The findings also expand the understanding of the molecular design and performance optimization of non-isocyanate polyurethanes, which are crucial for addressing the current limitations in the adhesive technology used in wood bonding.

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References

1. Suryawanshi, Y.; Sanap, P.; Wani, V. Advances in the synthesis of non-isocyanate polyurethanes. *Polym. Bull.* **2019**, *76*, 3233–3246. [CrossRef]
2. Mouren, A.; Avérous, L. Sustainable cycloaliphatic polyurethanes: From synthesis to applications. *Chem. Soc. Rev.* **2023**, *52*, 277–317.
3. Garipov, R.M.; Sysoev, V.A.; Mikheev, V.V.; Zagidullin, A.I.; Deberdeev, R.Y.; Irzhak, V.I.; Berlin, A.A. Reactivity of Cyclocarbonate Groups in Modified Epoxy–Amine Compositions. *Dokl. Phys. Chem.* **2003**, *393*, 289–292.
4. Zhang, P.; Guo, H.; Qin, C.; Yuan, H.; Cao, Y.; Wang, Z.; Jin, C. Solvent-free synthesis of bio-based non-isocyanate polyurethane (NIPU) with robust adhesive property and resistance to low temperature. *Polym. Test* **2021**, *140*, 108616.
5. Huang, Z.; Guo, H.; Yuan, H.; Cao, Y.; Wang, Z.; Jin, C. Bio-based thermosetting non-isocyanate polyurethane as high-performance adhesives for bonding wood. *Eur. Polym. J.* **2025**, *225*, 113733.
6. Zhang, P.; Yuan, H.; Wang, Y.; Cao, Y.; Wang, Z.; Jin, C. Synthesis of CO₂-based biomass derived non-isocyanate polyurethane hybrid adhesives with excellent mechanical properties and water resistance. *Green. Chem.* **2025**, *27*, 2102. [CrossRef]
7. Guan, J.; Song, Y.; Lin, Y.; Yin, X.; Zuo, M.; Zhao, Y.; Tao, X.; Zheng, Q. Progress in Study of Non-Isocyanate Polyurethane. *Ind. Eng. Chem. Res.* **2011**, *50*, 6517–6527.
8. Zhou, Y.; Li, C.; Xu, D.; Wang, Z.; Chen, Z.; Lei, H.; Du, G. A tannin-oxidized glucose wood adhesive with high performance. *Ind. Crops Prod.* **2024**, *222*, 119603. [CrossRef]
9. Bansode, A.; Barde, M.; Asafu-Adjaye, O.; Patil, V.; Hinkle, J.; Via, B.K.; Adhikari, S.; Adamczyk, A.J.; Farag, R.; Elder, T.; et al. Synthesis of Biobased Novolac Phenol–Formaldehyde Wood Adhesives from Biorefinery-Derived Lignocellulosic Biomass. *ACS Sustain. Chem. Eng.* **2021**, *9*, 10990–11002.
10. Chang, Z.; Pang, H.; Huang, A.; Li, J.; Zhang, S. Reinforcement of Bonding Strength and Water Resistance of Soybean Meal-Based Adhesive via Construction of an Interactive Network from Biomass Residues. *Polymers* **2019**, *11*, 967. [CrossRef]
11. Iswanto, A.; Lubis, M.; Sutiawan, J.; Al-Edrus, S.; Lee, S.; Antov, P.; Kristak, L.; Reh, R.; Mardawati, E.; Santoso, A. Latest Advancements in the Development of High-Performance Lignin- and Tannin-Based Non-Isocyanate Polyurethane Adhesive for Wood Composites. *Polymers* **2023**, *15*, 3864. [CrossRef] [PubMed]
12. Anttila, A.-K.; Pirttilä, A.M.; Häggman, H.; Harju, A.; Venäläinen, M.; Haapala, A.; Holmbom, B.; Julkunen-Tiitto, R. Condensed conifer tannins as antifungal agents in liquid culture. *Holz* **2013**, *67*, 825–832. [CrossRef]
13. Li, D.; Xue, B.; Zhao, Q.; Wang, W.; Li, X.; Wen, J.; Wang, Z.; Zhao, W. Green synthesis of lignin-based non-isocyanate polyurethanes as reusable, self-healable and removable adhesives. *Eur. Polym. J.* **2024**, *221*, 113553. [CrossRef]
14. Liu, T.; Li, C.; Xi, X.; Wei, Z.; Wu, Z.; Yan, Z.; Lei, H.; Du, G. Thermosetting Sucrose-Based Wood Adhesive with Enhanced Water Resistance and Bonding Performance by Constructing a Schiff Base Cross-Linking Network. *ACS Appl. Polym. Mater.* **2024**, *6*, 13927–13936. [CrossRef]
15. Li, W.; Yang, C.; Ren, X.; Li, Z.; Yang, H.; Zhang, X.; Huang, T.; Ran, X.; Gao, W.; Ni, K.; et al. Developing sugar-based wood adhesives using Schiff base chemistry derived from carbohydrates. *Colloids Surf. A Physicochem. Eng. Asp.* **2024**, *687*, 133485. [CrossRef]
16. Liu, L.; Jia, Y.; Zheng, L.; Luo, R.; Essawy, H.; Huang, H.; Wang, Y.; Deng, S.; Zhang, J. Development and Characterization of Bio-Based Formaldehyde Free Sucrose-Based Adhesive for Fabrication of Plywood. *Polymers* **2024**, *16*, 640. [CrossRef]
17. Kusumah, S.S.; Umemura, K.; Guswenrivo, I.; Yoshimura, T.; Kanayama, K. Utilization of sweet sorghum bagasse and citric acid for manufacturing of particleboard II: Influences of pressing temperature and time on particleboard properties. *J. Wood Sci.* **2017**, *63*, 161–172. [CrossRef]
18. Sheldon, R.A. Green and sustainable manufacture of chemicals from biomass: State of the art. *Green Chem.* **2014**, *16*, 950–963. [CrossRef]
19. Kobayashi, H.; Fukuoka, A. Synthesis and utilisation of sugar compounds derived from lignocellulosic biomass. *Green. Chem.* **2013**, *15*, 1740–1763. [CrossRef]

20. Xu, Y.-Q.; Hu, X.-F.; Zou, C.; Shi, J.; Du, Q.-Z.; Teng, B.-T.; Yin, J.-F. Effect of saccharides on sediment formation in green tea concentrate. *LWT* **2017**, *78*, 352–360. [CrossRef]
21. Benítez, E.I.; Genovese, D.B.; Lozano, J.E. Effect of typical sugars on the viscosity and colloidal stability of apple juice. *Food Hydrocoll.* **2009**, *23*, 519–525. [CrossRef]
22. Zhao, Z.; Miao, Y.; Yang, Z.; Wang, H.; Sang, R.; Fu, Y.; Huang, C.; Wu, Z.; Zhang, M.; Sun, S.; et al. Effects of Sulfuric Acid on the Curing Behavior and Bonding Performance of Tannin–Sucrose Adhesive. *Polymers* **2018**, *10*, 651. [CrossRef] [PubMed]
23. Zhang, Q.; Yan, R.; Xiong, Y.; Lei, H.; Du, G.; Pizzi, A.; Puangsin, B.; Xi, X. Preparation and characterization of polymeric cellulose wood adhesive with excellent bonding properties and water resistance. *Carbohydr. Polym.* **2025**, *347*, 122705.
24. Keil, T.W.M.; Deiringer, N.; Friess, W.; Merkel, O.M. Evaluation of adsorption of DNA/PEI polyplexes to tubing materials. *Eur. J. Pharm. Biopharm.* **2022**, *179*, 58–64. [CrossRef]
25. Teng, N.; Dai, J.; Wang, S.; Hu, J.; Liu, X. Hyperbranched flame retardant for epoxy resin modification: Simultaneously improved flame retardancy, toughness and strength as well as glass transition temperature. *Chem. Eng. J.* **2022**, *428*, 131226.
26. Li, K.; Geng, X.; Simonsen, J.; Karchesy, J. Novel wood adhesives from condensed tannins and polyethylenimine. *Int. J. Adhes. Adhes.* **2004**, *24*, 327–333.
27. Zhou, Y.; Dong, F.; Chen, X.; Huang, X.; Guo, L.; Liu, H.; Xu, X. A novel rosin-based non-isocyanate polyurethane with high-strength, self-healing, and recyclable properties for wood adhesives. *Ind. Crops Prod* **2024**, *211*, 118203.
28. GB/T9846-2015; Plywood for General Use. Standardization Administration of China: Beijing, China, 2015.
29. Islam, M.N.; Rahman, F.; Das, A.K.; Hiziroglu, S. An overview of different types and potential of bio-based adhesives used for wood products. *Int. J. Adhes. Adhes.* **2022**, *112*, 102992.
30. Seligra, P.G.; Jaramillo, C.M.; Famá, L.; Goyanes, S. Biodegradable and non-retrogradable eco-films based on starch–glycerol with citric acid as crosslinking agent. *Carbohydr. Polym.* **2016**, *138*, 66–74.
31. Ma, Q.; Mohawk, D.; Jahani, B.; Wang, X.; Chen, Y.; Mahoney, A.; Zhu, J.Y.; Jiang, L. UV-curable cellulose nanofiber-reinforced soy protein resins for 3D printing and conventional molding. *ACS Appl. Polym. Mater.* **2020**, *2*, 4666–4676. [CrossRef]
32. Rokicki, G.; Parzuchowski, P.G.; Mazurek, M. Non-isocyanate polyurethanes: Synthesis, properties, and applications. *Polym. Adv. Technol.* **2015**, *26*, 675–905. [CrossRef]
33. Kathalewar, M.S.; Joshi, P.B.; Sabnis, A.S.; Malshe, V.C. Non-isocyanate polyurethanes: From chemistry to applications. *RSC Adv.* **2013**, *3*, 4110–4129. [CrossRef]
34. Camara, F.; Benyahya, S.; Besse, V.; Boutevin, G.; Auvergne, R.; Boutevin, B.; Caillol, S. Reactivity of secondary amines for the synthesis of non-isocyanate polyurethanes. *Eur. Polym. J.* **2014**, *55*, 17–26. [CrossRef]
35. Xiong, Y.; Wu, Z.; Xi, X.; Lei, H.; Li, C.; Chen, Z.; Shi, J.; Du, G. A bio-based soy wood adhesive modified by dual-crosslinking strategy with excellent mechanical strength and water-resistance. *Ind. Crops Prod.* **2024**, *222*, 119417. [CrossRef]
36. Zeng, Y.; Xu, P.; Yang, W.; Chu, H.; Wang, W.; Dong, W.; Chen, M.; Bai, H.; Ma, P. Soy protein-based adhesive with superior bonding strength and water resistance by designing densely crosslinking networks. *Eur. Polym. J.* **2021**, *142*, 110128. [CrossRef]
37. Luo, H.; Yin, Y.; Wang, Y.; Li, Q.; Tang, A.; Liu, Y. Enhanced properties of a soybean adhesive by modification with a cycloaliphatic epoxy resin. *Int. J. Adhes. Adhes.* **2022**, *114*, 103026. [CrossRef]
38. Zhao, Z.; Sakai, S.; Wu, D.; Chen, Z.; Zhu, N.; Gui, C.; Zhang, M.; Umemura, K.; Yong, Q. Investigation of Synthesis Mechanism, Optimal Hot-Pressing Conditions, and Curing Behavior of Sucrose and Ammonium Dihydrogen Phosphate Adhesive. *Polymers* **2020**, *12*, 216. [CrossRef]
39. Ye, R.; Wang, C.; Shi, X.; Zhang, D.; Lai, C.; Chen, X.; Wang, C.; Chu, F. A facile strategy to fabricate low viscosity and high cold tack soy protein-based adhesive for particleboard production. *Ind. Crops Prod.* **2024**, *214*, 118612. [CrossRef]
40. Yao, X.; Liu, H.; Li, C.J.H. Development of Eco-Friendly Soy Meal Adhesives Enhanced by Ethylene Glycol Diglycidyl Ether. *Adv. Polym. Technol.* **2019**, *2019*, 8697047. [CrossRef]
41. Li, J.; Luo, J.; Li, X.; Yi, Z.; Gao, Q.; Li, J. Soybean meal-based wood adhesive enhanced by ethylene glycol diglycidyl ether and diethylenetriamine. *Ind. Crops Prod.* **2015**, *74*, 613–618. [CrossRef]

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Article

Hydrochar from Agricultural Waste as a Biobased Support Matrix Enhances the Bacterial Degradation of Diethyl Phthalate

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Abstract: The hydrothermal carbonization (HTC) of biomass presents a sustainable approach for waste management and production of value-added materials such as hydrochar, which holds promise as an adsorbent and support matrix for bacterial immobilization applied, e.g., for bioremediation processes of sites contaminated with phthalate ester plasticizers such as diethyl phthalate (DEP). In the present study, hydrochar was synthesized from vine shoots (VSs) biomass employing the following parameters during the HTC process: 260 °C for 30 min with a 1:10 (*w/v*) biomass-to-water ratio. The resulting vine shoots hydrochar (VSs-HC) was characterized for porosity, elemental composition, and structural properties using Scanning Electron Microscopy (SEM), Energy-Dispersive X-ray Spectroscopy (EDX), and Raman spectroscopy. Elemental analysis confirmed the presence of key elements in the VSs structure, elements essential for char formation during the HTC process. The VSs-HC exhibited a macroporous structure (>0.5 µm), facilitating diethyl phthalate (DEP) adsorption, bacterial adhesion, and biofilm formation. Adsorption studies showed that the VSs-HC achieved a 90% removal rate for 4 mM DEP within the first hour of contact. Furthermore, VS-HC was tested as a support matrix for a bacterial consortium (*Pseudomonas* spp. and *Microbacterium* sp.) known to degrade DEP. The immobilized bacterial consortium on VSs-HC demonstrated enhanced tolerance to DEP toxicity, degrading 76% of 8 mM DEP within 24 h, compared with 14% by planktonic cultures. This study highlights VSs-HC's potential as a sustainable and cost-effective material for environmental bioremediation, offering enhanced bacterial cell viability, improved biofilm formation, and efficient plasticizer removal. These findings provide a pathway for mitigating environmental pollution through scalable and low-cost solutions.

Keywords: agricultural biomass valorization; bio-based adsorbent; support matrix; immobilized bacterial cells; plasticizers; microbial biodegradation; sustainable ecotechnologies

1. Introduction

The global annual generation (>1 billion tons) of large quantities of agricultural waste presents both a challenge and an opportunity for sustainable resource management [1,2]. Hydrothermal carbonization (HTC) has emerged as a promising solution to transform lignocellulosic biomass into hydrochar under moderate temperature and pressure conditions [3]. The resulting material, namely hydrochar, is a carbon-rich material characterized

by high porosity and substantial surface area, making it suitable for a variety of environmental applications [4,5]. Among the numerous types of agricultural waste, vine shoots (VSs)—an abundant by-product of vineyard annual pruning—represent an ideal resource for hydrochar production [6]. Re-purposing such biomass aligns with the principles of a circular economy by reducing waste while creating high-value materials for industrial and environmental purposes (Figure 1).

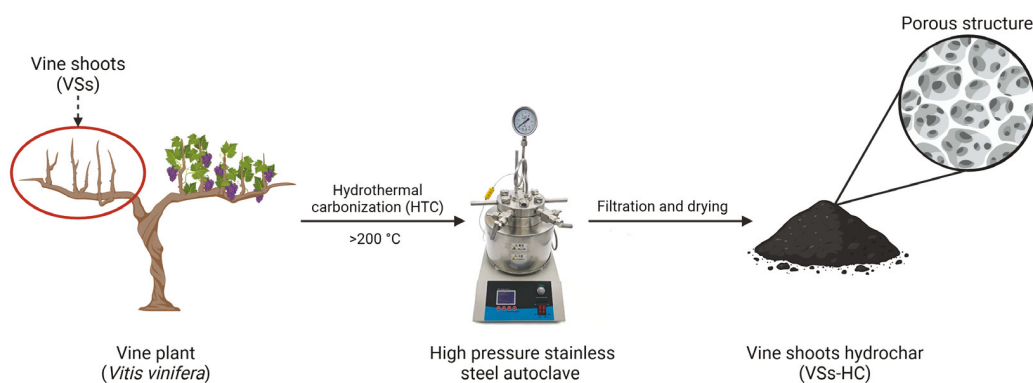


Figure 1. Vine shoot valorization through hydrothermal carbonization and the resulting porous hydrochar.

The porous structure and carbon-rich composition of hydrochar offer significant potential in biotechnological applications, particularly as a support matrix for microbial immobilization [7]. In the context of environmental remediation, hydrochar can facilitate the immobilization of bacterial cells, enhancing their stability and efficiency in degrading persistent pollutants, such as phthalate esters [8,9]. Its high surface area and abundant honeycomb structures promote bacterial adhesion and biofilm formation, while its adsorption properties enable it to retain pollutants for microbial degradation [9,10]. Due to its numerous functional groups, hydrochar can easily interact with aromatic benzene-ring-structure pollutants and break them down to less toxic compounds. Such examples of interactions include hydrophobic groups which can enhance adsorption through π – π interactions (stacking) with the benzene ring of DEP; hydroxyl (–OH) and carboxyl (–COOH) groups, which can contribute to hydrogen bonding with the ester (–COO–) groups; and carbonyl (–C=O) groups, which can facilitate dipole–dipole interactions with aromatic molecules [11,12]. These features make hydrochar particularly suited for addressing the contamination of a wide range of pollutants.

In this study, vine shoot hydrochar (VSs-HC) was employed for the removal of diethyl phthalate (DEP), a representative compound of the dialkyl phthalate ester group. Dialkyl phthalate esters, or just phthalate esters, are widely used plasticizers essential for enhancing the flexibility, durability, and processability of materials [13]. Globally, it is estimated that 5 to 8 million tons of phthalate esters are produced each year, of which approximately 1 million tons are potentially used in agricultural plastics, such as mulch films, irrigation tubing, drip lines, seedling trays, packaging, etc. [14]. A portion of these phthalate esters accumulates in waste dumps, contributing to the contamination of agricultural soils. In these soils, the total amount of phthalate esters is estimated to range from 100 to 1000 tons worldwide, corresponding to concentrations of 0.1 to 10 mg/kg [15,16]. Besides agricultural plastics, phthalate esters are commonly found in food packaging, medical devices, flooring, and cosmetics, among other products [17,18]. The increasing demand for these materials has led to the projected growth of the plasticizer market being expected to exceed 14.32 million metric tons by 2030 [19]. However, these benefits come with significant environmental and health concerns. Phthalate esters are non-covalently bound to polymer

matrices and gradually leach into the environment. This makes them one of the most frequently detected persistent organic pollutants [20]. Their chemical toxicity disrupts biological membranes, and as endocrine disruptors, they pose serious risks to human health [21]. Phthalate esters are typical amphipathic molecules with a high hydrophobicity as well as a relatively good water solubility. These molecules show a tendency to dissolve relatively well in the phospholipid bilayer of biological membranes [22,23]. The accumulation of these amphipathic organic compounds leads to a non-specific increase in membrane fluidity causing permeabilization of the cell membranes that causes damage to cell metabolism [24,25]. Consequently, phthalate esters, as typical aromatic structures with hydrophilic substituents, are known to be among the most toxic substances, making them very hazardous to the environment but also toxic to bacteria capable of degrading them [22,26].

Given these challenges, addressing phthalate ester contamination has become an environmental priority. Traditional remediation methods, such as chemical treatments and physical removal, often fall short due to high costs, incomplete degradation, and the risk of secondary pollution [27]. Bioremediation has emerged as a promising alternative, leveraging microorganisms' metabolic pathways to degrade pollutants into less harmful by-products or mineralize them [28]. Several bacterial strains of the genera *Pseudomonas*, *Bacillus*, *Microbacterium*, and *Rhodococcus* have been reported to effectively biodegrade phthalate esters through enzymatic hydrolysis and oxidative degradation (monooxygenases and dioxygenases) [29–32]. Both mechanisms are complementary, and they often work together in microbial degradation pathways. Despite its potential, the inherent toxicity of DEP often reduces the efficiency of free-living bacterial cells in such processes, creating a need for innovative strategies to enhance bioremediation. One such strategy involves immobilizing bacteria on suitable support materials to improve cell stability, biofilm formation, and tolerance to toxic pollutants [33].

In this study, VSs-HC was employed as a support material for immobilizing a bacterial consortium to enhance the biodegradation of DEP. DEP was selected as a model compound due to its environmental persistence and toxicity. HTC was used to produce hydrochar from VSs biomass, and its properties were characterized using techniques such as Scanning Electron Microscopy (SEM), Raman spectroscopy, Energy-Dispersive X-ray Spectroscopy (EDX), and Ultra-Performance Liquid Chromatography (UPLC). These analyses confirmed the hydrochar's porous structure, composition, and ability to support efficient bacterial immobilization. It is commonly known that immobilized bacterial cells exhibit improved tolerance and stability, effectively degrading high concentrations of toxic organic compounds such as DEP [23,34,35]. By promoting hydrochar as a bio-based solution for addressing phthalate contamination, this study demonstrates a sustainable approach that integrates waste valorization with effective environmental remediation.

2. Results and Discussions

2.1. Hydrochar Preparation and Characterization

2.1.1. VSs Conversion Through HTC

During the HTC process, the raw material structure underwent significant physical transformations and a new structure was shaped. A honeycomb-like structure was revealed under SEM, suggesting that the HTC process effectively decomposed the lignocellulosic structures and improved the pore formation (Figure 2). Compared to the VSs (Figure 2a) biomass, which exhibited rough and heterogeneous fiber structures, the resulting hydrochar (Figure 2b) exhibited well-defined porous structures with diameters varying from 0.5 up to 20 μm , as observed under SEM. According to the IUPAC classification for porous materials [36], the obtained hydrochar contains large and observable macropores (>50 nm).

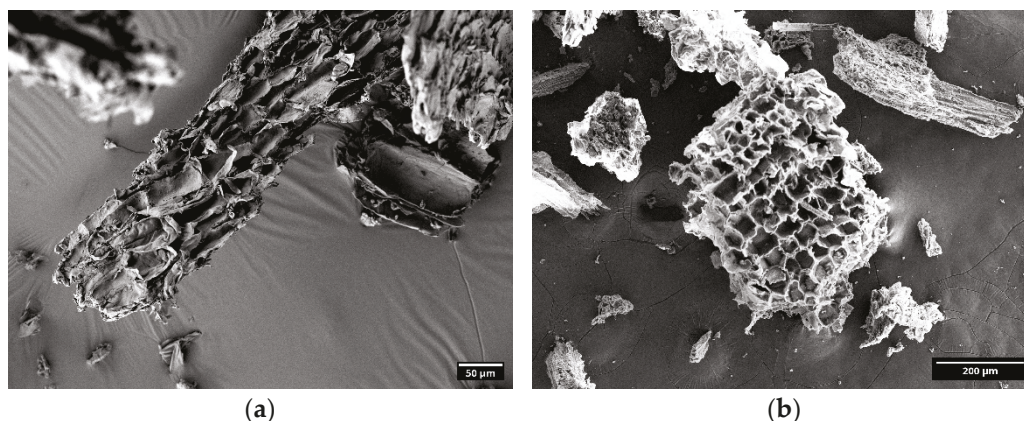


Figure 2. SEM images of (a) VSs biomass; and (b) VSs-HC obtained at 260 °C, 30 min, and 1:10 *w/v* ratio.

2.1.2. SEM-EDX Analysis

Elemental analysis (SEM-EDX) revealed that besides the main constituent carbon (C), hydrochar exhibited comparatively high levels of other elements such as calcium (Ca), oxygen (O), aluminum (Al), silicon (Si), phosphorous (P), and sulphur (S). The presence of calcium (Ca) and silicon (Si) is particularly important, as these elements contribute to the structural stability of hydrochar and influence its thermal degradation behavior and adsorption properties (Figure 3) [37]. From the perspective of microbial immobilization, the carbon-rich surface of hydrochar provides hydrophobic interactions that enhance bacterial attachment. Oxygen facilitates hydrogen bonding and electrostatic interactions, improving bacterial adhesion on the pore structures [38]. Elements such as Ca, P, S, and Si promote biofilm formation by stabilizing extracellular polymeric substances (EPSs) secreted by bacteria, thereby increasing bacterial adhesion and biofilm stability and enhancing pollutant resistance [39]. More than that, due to its divalent nature, Ca acts as a bridging agent between negatively charged bacterial cells and hydrochar surfaces, facilitating bacterial adhesion [40]. Meanwhile, the high levels of chromium (Cr) observed in the SEM-EDX analysis result from the sputter-coated layer (30 nm) of the hydrochar sample and are not a constituent or inherent part of the VSs biomass.

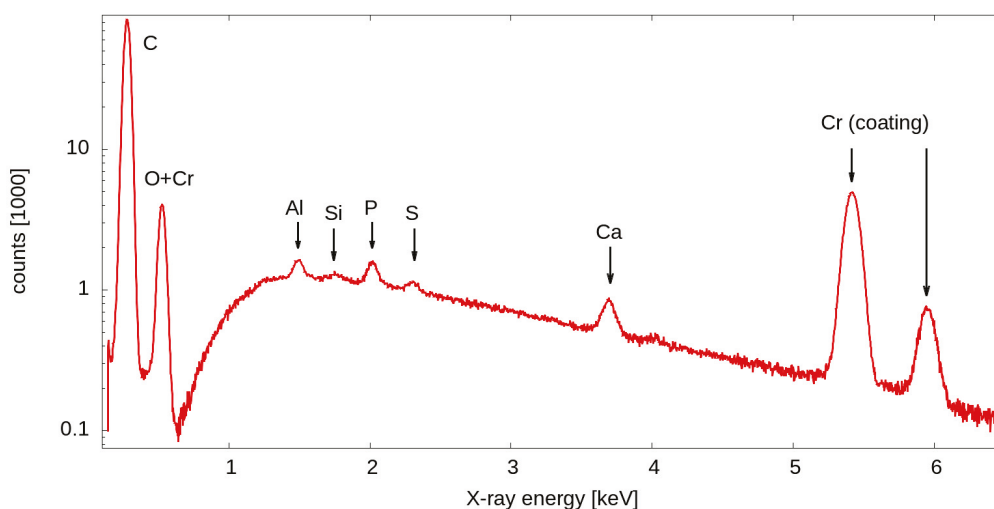


Figure 3. SEM-EDX coupled analysis of the hydrochar surface.

2.1.3. Raman Spectroscopy of VSs-HC

Understanding the structural composition of biomass is crucial for optimizing materials for specific applications, such as microbial immobilization. Raman spectroscopy was employed to analyze the structural features of VSs-HC using a WITec Raman microscope (Figure 4). The spectra revealed distinct peaks at 1370 cm^{-1} and 1583 cm^{-1} , corresponding to the D- and G-bands of graphite-like structures, which are indicative of carbonization.

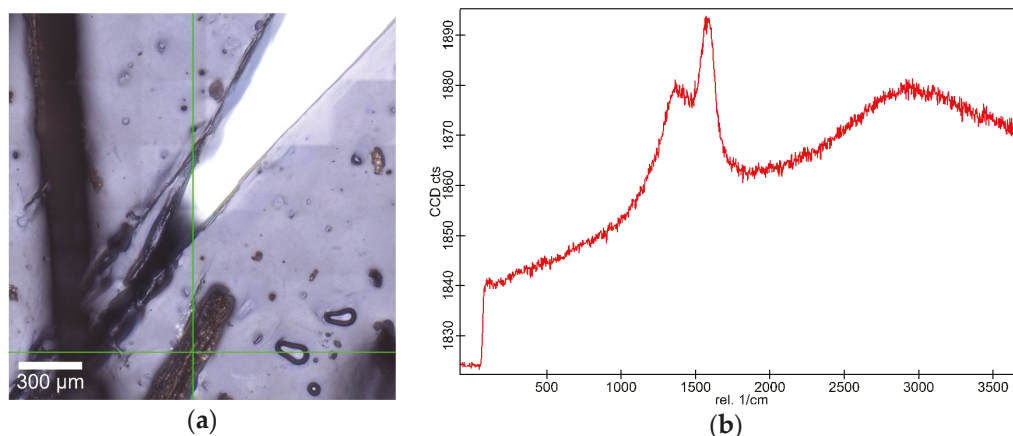


Figure 4. Hydrochar's Raman analysis on (a) targeted location and (b) the identified spectral band of graphite-like structures.

The aforementioned Raman shifts suggest the formation of disordered and graphitic carbon, which has significant implications for microbial immobilization. The presence of graphitic domains enhances electrical conductivity, a critical parameter for electron transfer in bioelectrochemical applications [41]. Additionally, carbonaceous structures, such as hydrochar, provide a favorable surface for microbial adhesion due to their π -electron interactions and the potential presence of oxygen-containing functional groups [42].

Although cellulose and hemicellulose play vital roles in hydrothermal carbonization (HTC) by influencing pore formation and surface chemistry, their identification cannot be confirmed based on the Raman spectra obtained. This means that both biopolymers were broken down during the HTC process into smaller organic compounds, such as 5-hydroxymethylfurfural (HMF), furfural, levulinic acid, and various phenolic derivatives, compounds which do not retain the crystalline structure required for characteristic Raman signals [43]. Additionally, the HTC process promotes the dehydration, decarboxylation, and aromatization of the VSs-HC with the formation of hydrochar, which consists of disordered carbon, oxygen-containing functional groups, and aromatic domains [44]. Therefore, the decomposition of cellulose and hemicellulose lead to new reorganized chemical structures dominated by aromatic and disordered carbon species. As a result, their characteristic Raman spectra disappear, replaced by broad D- and G-bands typical of hydrochar. Nevertheless, the new graphene-like structures are more likely to improve surface properties for microbial attachment and electron transfer processes.

2.2. Adsorption of DEP by Hydrochar

The adsorption efficiency of hydrochar was evident from the rapid reduction in DEP concentration. Within the first hour of contact, 3.59 mM DEP (90%) was adsorbed from 4 mM DEP by using 0.5% (50 mg) VSs-HC, as shown in Figure 5. The DEP adsorption increased gradually over time, and after 24 h, approximately 3.81 mM (95%) of DEP was removed in experiments using only 0.5% VSs-HC.

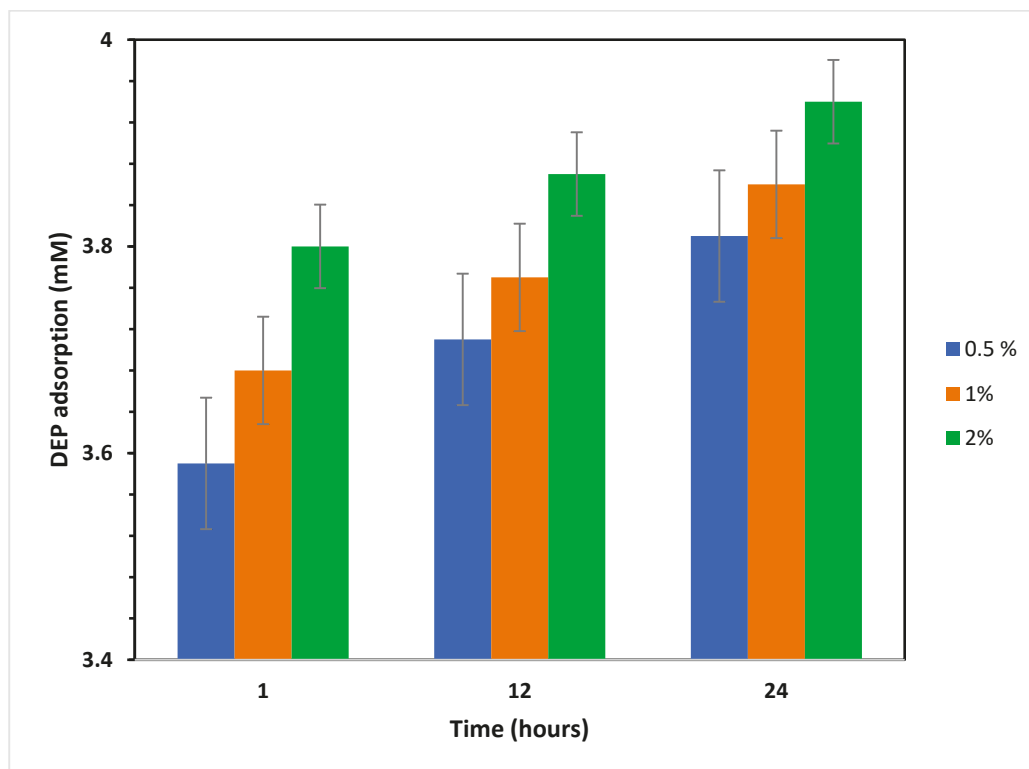


Figure 5. UPLC measurements of DEP adsorption by different amendments of hydrochar after 1 h (T1), 12 h (T12), and 24 h (T24). The initial concentration was 4 mM DEP. Hydrochar concentrations (%) reported for the dry weights (mg) of 0.5 = 50 (blue); 1 = 100 (orange); 2 = 200 (green).

Physicochemical interactions and diffusion processes can explain the mechanisms behind the rapid adsorption (initial phase) and slow adsorption (later phase) of DEP by hydrochar. The porous structure of hydrochar facilitates DEP diffusing into the available pores and binding to active sites (mainly functional groups and surface area). However, as these sites fill up, the process slows down and the available DEP must diffuse deeper into smaller pores or through the hydrochar matrix, which takes longer. Additionally, hydrochar contains various oxygen-containing functional groups (e.g., hydroxyl (-OH), carboxyl (-COOH), and carbonyl (-C=O)), which interact with DEP via hydrogen bonds, electrostatic attraction, or π - π interactions [45]. Over time, as these pores become occupied, the adsorption rate decreases as observed at 12 and 24 h.

Although 2% of VSs-HC (~98% after 24 h) adsorbed more DEP than 0.5% of VSs-HC (~95% after 24 h), a major difference was not observed and further tests were continued with the lowest concentration of hydrochar. Therefore, a hydrochar concentration of 0.5% was identified as optimal for future experimental applications, ensuring both high adsorption capacity and material efficiency.

2.3. Microbial DEP Removal in the Presence and Absence of Hydrochar

The removal of DEP by a microbial consortium, mainly consisting of *Pseudomonas* spp. and *Microbacterium* sp., was tested in the presence and absence of VSs-HC. Figure 6 shows the growth rates of the bacterial consortium in the absence of hydrochar on different concentrations of DEP. The growth rates of 4, 8, 16, and 32 mM were compared to the that of 2 mM, with the latter serving as a control. Although the amount of carbon and energy increased with the addition of 4 mM DEP, the growth rate decreased by about 6%. The toxic effects of DEP became much more pronounced when adding 8 mM, resulting in growth rates of 67% compared to the control. When adding 16 mM DEP, strong inhibitory effects

were observed; the bacterial consortium barely survived with a 2% growth compared with 2 mM DEP (control).

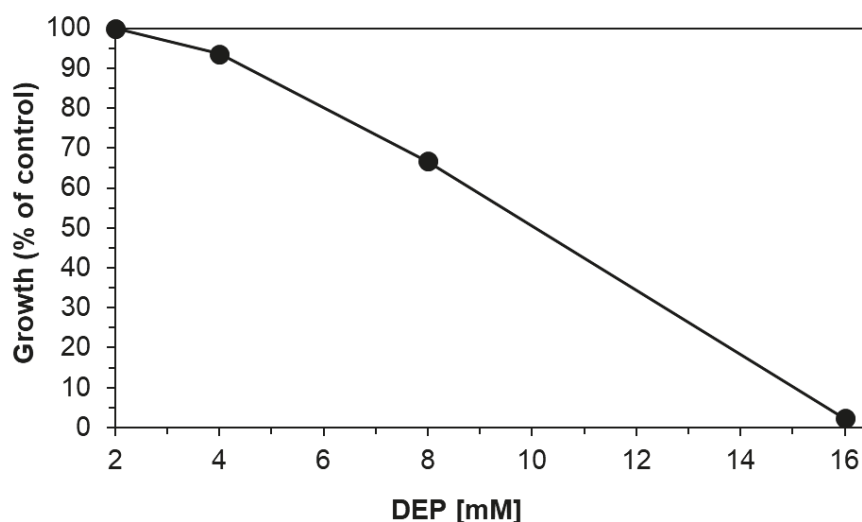


Figure 6. Growth rates (black circles) of the bacterial consortium when incubated with different concentrations of DEP in the absence of hydrochar. The growth rate of the bacterial consortium on 2 mM DEP (=control) was set to 100% and compared to the growth rates on 4, 8, and 16 mM DEP. Growth on 32 mM DEP is 0% of the control.

On the electron micrograph samples (Figure 7), large biofilms can be observed at 8 mM and 16 mM DEP. The observed bacteria present rod shapes and a single flagellum (0.5 to 1 μm in length). These findings confirm that VSs-HC serves as a suitable support matrix for the bacterial cell adhesion and biofilm formation, although at 32 mM DEP, the samples presented lysed cells and scattered fragments of cell membrane.

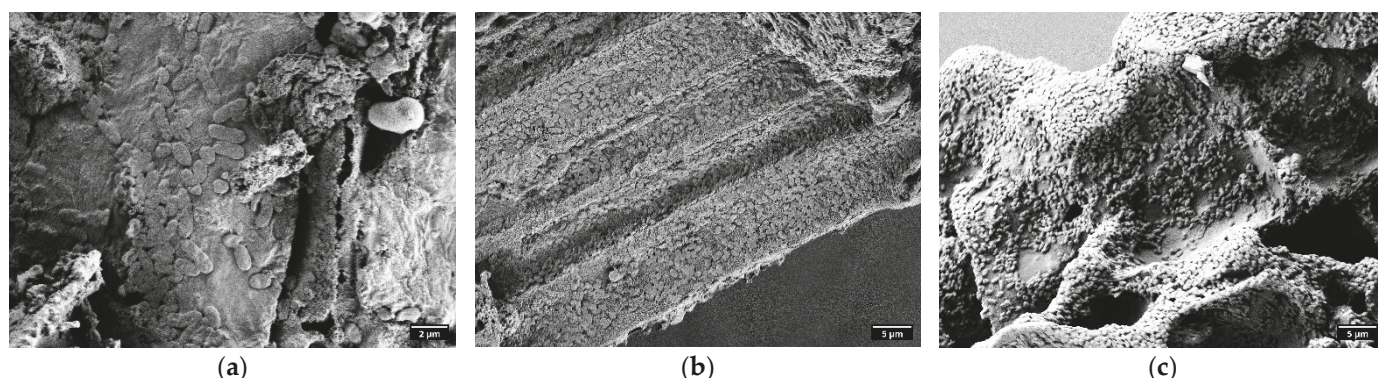


Figure 7. SEM images of VSs-HC after incubation with the bacterial consortium at 8 mM DEP after 1 (a), 12 (b), and 24 h (c).

Figure 8 shows that DEP degradation was lower in the planktonic cell culture (Figure 8a) compared to the immobilized cells on the VSs-HC (Figure 8b). The planktonic cells were partially inhibited at 8 and 16 mM DEP, exhibiting a removal of 11% and 5% in the first 12 h of contact, while at 32 mM DEP the bacterial cells are nearly completely inhibited and no activity is observed. The residual 2% DEP degradation measured in this culture (Figure 8a) was found to remain constant during the experiment, which can be attributed to the sorption by necrotic bacterial biomass into cellular structures (like membranes and proteins) [46].

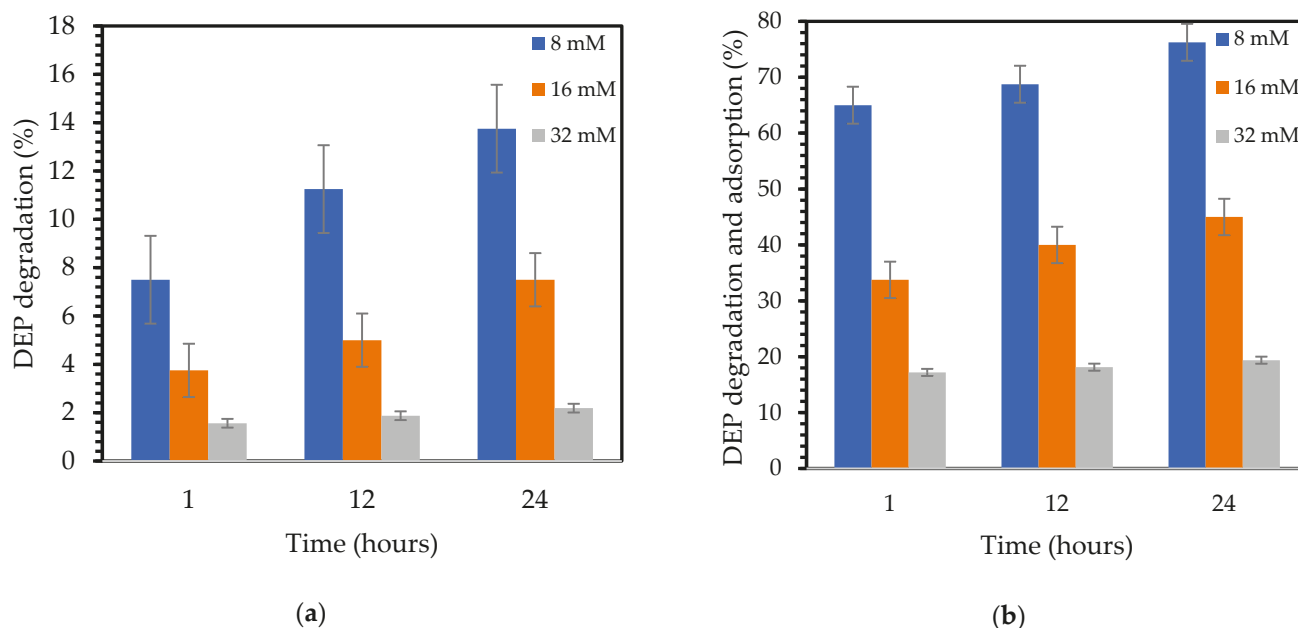


Figure 8. DEP degradation (a) by the microbial consortium in the absence of hydrochar and (b) by the microbial consortium and hydrochar (0.5% *w/v*). Adsorption capacity is reported in mM to reflect the molar concentration of DEP removed from the aqueous solution, consistent with microbiological conventions for substrate measurement. This unit emphasizes the integrated adsorption–biodegradation process rather than hydrochar-specific capacity, which could alternatively be expressed in mg/g or mM/g if normalized to adsorbent mass.

A synergic mechanism of adsorption, by the VSs-HC, and degradation, by the bacterial cells, can be observed. A similar example of combined adsorption and biodegradation processes was found for the removal of the pesticide chlorpyrifos from water by using rice husk biochar and an immobilized bacterial strain of *Aeromonas veronii*. The immobilized bacteria could degrade up to 96% of the chlorpyrifos (30 mg/L) within 24 h, compared with 67% in the planktonic cells [47].

During the first hour of contact, around 65% of the 8 mM DEP was adsorbed and degraded by the VSs-HC and immobilized cells. For 16 mM and 32 mM DEP, the adsorption and degradation of DEP increased by up to 45% and 19% within 24 h of contact. Thus, the adsorption capacity for 8, 16, and 32 mM with 0.5% (*v/w*) hydrochar is high enough to ease the toxic effect caused by these high DEP concentrations on the bacterial consortium and facilitate their growth and biofilm formation. However, 32 mM showed a negligible growth, and within 24 h the bacterial cells were hardly inhibited and the possible synergy can be attributed to the adsorption and sorption (by the necrotic bacterial biomass) processes.

The solubility of DEP (1.08 g/L, equivalent to approximately 5.15 mM) indicates that the concentrations used in this study exceeded the compound’s natural solubility, potentially leading to localized aggregation such as in Figure 7. Notably, the entrapped DEP within the hydrochar’s porous structure could serve as a sustained-release mechanism, enabling prolonged antimicrobial activity while minimizing free DEP concentration in the medium. This entrapment likely mitigates the environmental toxicity of DEP while enhancing its bioavailability and efficacy against microbial communities.

3. Materials and Methods

3.1. Feedstock

One type of raw material was collected from the annual vine pruning of vineyards, namely VSs, and subjected to analysis. The aforementioned raw material was sourced from

northeastern Romania and collected during the autumn season. Firstly, it was air-dried in a ventilated room to minimize photodegradation and alteration. Secondly, the material was ground using a Micro Powder Grinding Mill (Retsch GmbH GM 200, Haan, Germany) at a rotation speed of 320 rpm for 20 min to achieve an approximately uniform particle size. Milled material was subsequently classified by particle size using a Retsch GmbH AS 200 sieve shaker (Haan, Germany), separating it into three distinct fractions: large (1–2 cm), medium (0.5–1 cm), and fine (<0.1 cm). The resulting fractions were collected in a box and stored in a desiccator to prevent moisture absorption until further analysis.

3.2. Hydrochar Preparation

The HTC of VSs was realized by using a micro pressured stainless-steel autoclave, containing a glass vessel with a volume of 15 mL and a maximum pressure of 10 MPa. For each run, 1 g of VSs was introduced to 10 mL sterile distilled water in order to reach the 1:10 (*w/v*) ratio and heated at 260 °C for 30 min. Prior to HTC, the mixture was left to “sink” for 30 min in order to assure a better homogeneity. During the HTC process, the autoclave’s pressure reached approximately 4.5 MPa. After the HTC process was completed, the stainless-steel autoclave was cooled down at room temperature; when it reached 100 °C, it was partially introduced into an ice bath. This procedure sped up the cooling process and provided sufficient time for the autoclaves to depressurize.

The wet hydrochar was filtered through Whatman filter paper (11 µm pore size) in order to separate the dry material from the liquid phase. The obtained solid phase was washed with approximately 50 mL deionized water until the yellowish coloration of the material was no longer present. The liquid phase was collected in 50 mL Duran laboratory bottles and stored in the fridge for further analysis.

The filtered hydrochar was dried at 105 ± 2 °C for 12 h, and then it was weighted until values were constant and stored at room temperature in a closed glass jar for further characterization. For significant data accuracy and reproducibility, all experiments were performed in triplicate.

3.3. Preparation of VSs-HC Samples Incubated with the Bacterial Consortium

Approximately 50 mg of VSs-HC was introduced to a 50 mL Erlenmeyer flask and sterilized for 15 min at 121 °C in order to eliminate any native microbial activity. Experiments were performed in triplicates, and therefore 9 Erlenmeyer flasks containing sterilized VSs-HC were prepared.

The sterilized VSs-HC (50 mg), mineral media (10 mL), bacterial consortium inoculum (500 µL), and DEP (8, 16, and 32 mM) were mixed all together in 50 mL Erlenmeyer flasks at 30 °C and 150 rpm for 24 h. During the incubation time, regular sampling (1 h, 12 h, and 24 h) was performed for UPLC analysis. Approximately 0.5 mL aliquots were taken using Whatman filter syringes (Whatman, Darmstadt, Germany; 0.45 µm pore size). The remaining VSs-HC samples were placed on sterile Petri dishes and carefully dried under a horizontal laminar flow hood for 12 h. After drying, samples were transferred to Eppendorf tubes and labeled for further SEM analysis.

Prior to the bacterial immobilization on VSs-HC, an overnight culture was prepared as a stock culture for the further inoculations. The bacterial consortium was cultivated in a 150 mL Erlenmeyer flask containing 50 mL mineral medium and 200 mg/L yeast extract. The overnight culture was kept for a minimum of 8 h at 30 °C and 150 rpm in order to ensure optimal microbial growth before the immobilization process.

3.4. Scanning Electron Microscopy (SEM) Analysis, EDX Coupled

Field emission electron microscopy (FE-SEM, Zeiss Merlin VP Compact, Carl Zeiss Microscopy, Oberkochen, Germany) was employed to obtain microscopic information on

the morphology and structure of the sample surface. In preparation for SEM, the samples had to be fixed, dehydrated, and dried in order to make them compatible with the high-vacuum conditions in the analysis chamber of the FE-SEM. For that, the samples were chemically fixed in a 2.5% glutaraldehyde solution for 12 h in the fridge, dehydrated in a graded ethanol/water series (30%, 50%, 70%, 80%, 90%, 95%, 100%), subjected to the chemical drying agent hexamethyldisilazane (HMDS, 1:1 HMDS/ethanol, and pure HMDS for 10 min each) and finally air-dried under a fume hood. The fixed and dried samples were then mounted onto standard SEM sample holders (stubs) and were sputter-coated with a 30 nm thick layer of chromium by a Leica SCD500 (Leica Microsystems, Wetzlar, Germany). Imaging was conducted at an electron acceleration voltage of 2 kV with a beam current of approximately 250 pA to 300 pA in secondary electron detection mode. The elemental composition of the sample was obtained by an Energy-Dispersive X-ray (EDX) Spectrometer (Bruker Quantax XFlash 5060F, Bruker Nano GmbH, Berlin, Germany) which is coupled to the FE-SEM. In order to achieve the efficient ionization of chemical elements (heavier than carbon) in the sample, an electron energy of 10 keV was used. Spectra were obtained and evaluated with the software package Bruker Esprit Version 1.9 (Bruker Nano GmbH, Berlin, Germany).

3.5. Raman Spectroscopy

For the analysis of the degree of carbonization of VSs-HC, a WITec Alpha 300RA confocal Raman microscope (WITec—Wissenschaftliche Instrumente und Technologie GmbH, Ulm, Germany) was used. Raman spectra were acquired on chunks (<1 mm) of the VSs-HC using a solid-state laser for excitation with a wavelength of 532 nm. Raman spectral acquisition focused on the D- and G-bands of graphite centered at 1370 cm^{-1} and 1583 cm^{-1} , respectively [48].

3.6. Chemicals

The diethyl phthalate (DEP) of 99% purity used in this study was acquired from Thermo Fisher Scientific (Waltham, MA, USA). Other chemicals and solvents utilized throughout the experiments were of analytical grade, ensuring high purity suitable for accurate and reproducible results. All compounds were utilized as received without any additional purification, maintaining consistency in experimental conditions. This approach minimizes variability and aligns with standard practices for chemical analysis and environmental remediation studies.

3.7. Isolation and Cultivation of Bacterial Consortium

A bacterial consortium mainly consisting of *Pseudomonas* spp. and *Microbacterium* sp. capable of efficiently mineralizing different phthalate esters was previously isolated from a biofilm scratch taken from a polyurethane tubing (obtained from the lab of Hermann J. Heipieper). The microbial consortium was cultivated in a mineral medium as described by Hartmans [49] with 200 mg/L yeast extract at 30 °C and 180 rpm in 50 mL scale. As the main carbon and energy source, DEP was added in different concentrations (2, 4, 8, 16, and 32 mM). Growth inhibition (Formula (2)) observed at concentrations higher than 2 mM DEP was calculated by comparing the percentage difference in the growth rates μ (h^{-1}) (Formula (1)) between cultures containing more than 4 mM DEP and higher (μ_{xmM}) with that of the culture grown with 2 mM DEP ($\mu_{2\text{mM}}$).

Formula (1): Calculation of bacterial growth rates μ (h^{-1})

$$\mu\ (\text{h}^{-1}) = [\ln(\text{OD}_{t_1}) - \ln(\text{OD}_{t_0})]/(t_1 - t_0) \quad (1)$$

Formula (2): Relative growth (%)

$$\text{Relative growth (\%)} = [\mu_{xmM} \times 100] / \mu_{2mM} \quad (2)$$

Note: OD represents optical density, a measure of bacterial concentration typically determined by absorbance at a specific wavelength (e.g., 560 nm). OD_{t1} and OD_{t0} denote optical density at times t_1 and t_0 , respectively.

In addition to the above-described conditions, 10 mL cultures were incubated with 0.5, 1, and 2% (*v/w*) VSs-HC in a separate experimental batch at 4 mM DEP. For 0.5% (*v/w*) hydrochar, this was also performed at 8, 16, and 32 mM. VSs-HC samples containing DEP were filtered using Whatman filter syringes (0.45 μm pore size) and the resulting aliquots (0.5 mL) were collected in 1.5 mL Eppendorf tubes and stored at -20°C for further analysis. During the whole sampling and filtering process, Erlenmeyer flasks were kept at 30°C and 150 rpm, while being collected at the corresponding times (1, 12, and 24 h). The experiments were performed in triplicate.

3.8. Analysis of DEP by Ultra-Performance Liquid Chromatography (UPLC)

From the cultures containing the bacterial consortium, different concentrations of DEP samples (0.5 mL) were taken with Whatman filter syringes (0.45 μm pore size), placed in 1.5 mL Eppendorf tubes, and stored at -20°C until further use. Before analysis, samples were thawed at room temperature, supplemented with 0.5 mL methanol, and shaken at 150 rpm for 5 min (Vortex-Genie 2, Scientific Industries, New York, NY, USA). Aliquots (2.0 μL) of the resulting mixture were directly subjected to measurements. UPLC analysis was performed using an AcquityTM UPLC system (Waters, Eschborn, Germany) equipped with an AcquityTM UPLC BEH C18 column (1.7 μm particle size, 2.1×50 mm; Waters, Eschborn, Germany) as described before by Hofmann and Schlosser [50]. Also, the AcquityTM UPLC system was coupled to a PAD detector (absorbance at 278 nm; Waters, Eschborn, Germany) and Empower 3 software. The measurements were performed in isocratic elution at a flow rate of $0.5 \text{ mL} \times \text{min}^{-1}$. Elution was performed with a 50:50 (*v/v*) mixture of methanol/water.

4. Conclusions

VSs-HC produced through HTC at 260°C for 30 min at a 1:10 *w/v* ratio exhibited remarkable potential, firstly as an excellent adsorptive material and secondly as a natural support matrix for the immobilization of a bacterial consortium. SEM analysis confirmed the presence of macropores ($>0.5 \mu\text{m}$ in diameter) on the VSs-HC, which provided a suitable adhesion surface for bacterial colonization and biofilm formation. Rich in elements such as C, Ca, O, Si, Al, etc., it can be easily converted through HTC to obtain a porous structure.

Raman spectroscopy confirmed the presence of D- and G-bands of graphite-like structures, indicative of carbonization. The HTC process breaks cellulose and hemicellulose into smaller organic compounds and reshapes the chemical structure of hydrochar, creating new carbon structures. Nevertheless, this structural modification leads to improvements in functional groups, microbial adhesion, and electron transfer processes.

VSs-HC's adsorption properties enable rapid DEP adsorption, with 90% adsorption in just 1 h of contact. Furthermore, in the experiments with planktonic and immobilized bacterial cells, major differences were observed. At 8 mM and 16 mM DEP, hydrochar provides a stable and protective environment for biofilm formation with rates of 65% and 34% DEP degradation, while planktonic cells show 8% and 4% DEP degradation in 1 h. In the immobilized bacterial cells, a maximum of 76% DEP removal was measured with 8 mM after 24 h compared with 14% in the planktonic cells. The DEP-degrading bacterial

consortium remains viable within the VSs-HC matrix. A synergic mechanism of adsorption and degradation can be noticed between VSs-HC and the bacterial consortium.

These findings highlight the potential of VSs-HC as a sustainable biosorbent and support matrix for bacterial cells. Therefore, VSs biomass valorization through HTC can be a proper solution for mitigating the environmental impact of organic pollutants such as DEP and valorizing agricultural waste.

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References

1. Avitabile, V.; Baldoni, E.; Baruth, B.; Bausano, G.; Boysen-Urban, K.; Caldeira, C.; Camia, A.; Cazzaniga, N.; Ceccherini, G.; De Laurentiis, V.; et al. *Biomass Supply and Uses in the EU*; Publications Office of the European Union: Luxembourg, 2023; Available online: <https://data.europa.eu/doi/10.2760/368529> (accessed on 10 December 2024).
2. Kumar, R.K.S.; Sasikumar, R.; Dhilipkumar, T. Exploiting agro-waste for cleaner production: A review focusing on biofuel generation, bio-composite production, and environmental considerations. *J. Clean. Prod.* **2024**, *435*, 140536. [CrossRef]
3. Lewandowski, W.M.; Rym, M.; Kosakowski, W. Thermal Biomass Conversion: A Review. *Processes* **2020**, *8*, 516. [CrossRef]
4. Gupta, N.; Mahur, B.K.; Izrayeel, A.M.D. Biomass conversion of agricultural waste residues for different applications: A comprehensive review. *Environ. Sci. Pollut. Res.* **2022**, *29*, 73622–73647. [CrossRef]
5. Armanu, E.G.; Secula, M.S.; Tofanica, B.M.; Volf, I. The Impact of Biomass Composition Variability on the Char Features and Yields Resulted through Thermochemical Processes. *Polymers* **2024**, *16*, 2334. [CrossRef]
6. Nabais, J.M.V.; Laginhas, C.; Carrott, P.J.M.; Carrott, M.M.L.R. Thermal conversion of a novel biomass agricultural residue (vine shoots) into activated carbon using activation with CO₂. *J. Anal. Appl. Pyrolysis* **2010**, *87*, 8–13. [CrossRef]
7. Armanu, G.E.; Secula, M.S.; Cimpoesu, C.; Heipieper, H.J.; Volf, I. A biobased nano/micro-structured material for microorganisms' immobilization. In Proceedings of the 24th International Multidisciplinary Scientific GeoConference SGEM, Albena, Bulgaria, 29 June–8 July 2024; p. 24.
8. Gallifuoco, A.; Papa, A.A.; Passucci, M.; Spera, A.; Taglieri, L.; Di Carlo, A. An Exploratory Study of Hydrochar as a Matrix for Biotechnological Applications. *Ind. Eng. Chem. Res.* **2023**, *62*, 11805–11813. [CrossRef]
9. Bargmann, I.; Martens, R.; Rillig, M.C.; Kruse, A.; Kücke, M. Hydrochar amendment promotes microbial immobilization of mineral nitrogen. *Z. Pflanzenernähr. Bodenkd.* **2014**, *177*, 59–67. [CrossRef]
10. Garver, M.P.; Liu, S. Chapter 27—Development of Thermochemical and Biochemical Technologies for Biorefineries. In *Bioenergy Research: Advances and Applications*; Elsevier: Amsterdam, The Netherlands, 2014; pp. 457–488. [CrossRef]
11. Zhang, X.; Zhang, J.; She, Y.; Li, Y.; Cheng, H.; Ji, R.; Bian, Y.; Han, J.; Jiang, X.; Song, Y.; et al. Comparison of the performance of hydrochar, raw biomass, and pyrochar as precursors to prepare porous biochar for the efficient sorption of phthalate esters. *Sci. Total Environ.* **2022**, *10*, 157511. [CrossRef] [PubMed]

12. Cheng, H.; Zhou, Y.; Beiyuan, J.; Li, X.; Min, J.; Su, L.; Zhang, L.; Ji, R.; Xue, J. Insights into the effect of hydrochar-derived dissolved organic matter on the sorption of diethyl phthalate onto soil: A pilot mechanism study. *Sci. Total Environ.* **2024**, *20*, 169101. [CrossRef] [PubMed]
13. Huang, L.; Zhu, X.; Zhou, S.; Cheng, Z.; Shi, K.; Zhang, C.; Shao, H. Phthalic Acid Esters: Natural Sources and Biological Activities. *Toxins* **2021**, *13*, 495. [CrossRef]
14. Lü, H.; Mo, C.H.; Zhao, H.M.; Xiang, L.; Katsoyiannis, A.; Li, Y.W.; Cai, Q.Y.; Wong, M.H. Soil contamination and sources of phthalates and its health risk in China: A review. *Environ. Res.* **2018**, *164*, 417–429. [CrossRef]
15. PlasticsEurope. Plastics—The Facts 2020: An Analysis of European Plastics Production, Demand and Waste Data. 2020. Available online: <https://plasticseurope.org/knowledge-hub/plastics-the-facts-2020/> (accessed on 14 December 2024).
16. Tran, H.T.; Nguyen, M.K.; Hoang, H.G.; Justin, M.; Hutchison, M.J.; Vu, C.T. Phthalate esters in the environment: A critical review of their occurrence and ecological risk. *Chemosphere* **2022**, *307*, 135989. [CrossRef]
17. Kabulovna, D.S.; Turapovna, H.E.; Parpievna, G.N. Use of Plasticizers in Polymers. *Int. J. Innov. Eng. Res. Technol.* **2023**, *10*, 224–230.
18. Baloyi, N.D.; Tekere, M.; Maphangwa, K.W.; Masindi, V. Insights Into the Prevalence and Impacts of Phthalate Esters in Aquatic Ecosystems. *Front. Environ. Sci.* **2021**, *9*, 684190. [CrossRef]
19. Fortune Business Insights, Plasticizers Market Size, Share & COVID-19 Analysis, By Type (Phthalates and Non-Phthalates), By Application (Wire & Cable, Flooring & Wall Covering, Film & Sheet, Consumer Goods, Coated Fabrics, and Others), and Regional Forecast, 2023–2030. Available online: <https://www.fortunebusinessinsights.com/plasticizers-market-104572> (accessed on 22 December 2024).
20. Staples, A.C.; Peterson, R.D.; Parkerton, F.T.; Adams, J.W. The environmental fate of phthalate esters: A literature review. *Chemosphere* **1997**, *35*, 667–749. [CrossRef]
21. Meeker, J.D.; Sathyanarayana, S.; Swan, S.H. Phthalates and other additives in plastics: Human exposure and associated health outcomes. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **2009**, *364*, 2097–2113. [CrossRef]
22. Sikkema, J.; de Bont, J.A.M.; Poolman, B. Mechanisms of membrane toxicity of hydrocarbons. *Microbiol. Rev.* **1995**, *59*, 201–222. [CrossRef]
23. Neumann, G.; Kabelitz, N.; Zehnsdorf, A.; Miltner, A.; Lippold, H.; Meyer, D.; Schmid, A.; Heipieper, H.J. Prediction of the adaptability of *Pseudomonas putida* DOT-T1E to a second phase of a solvent for economically sound two-phase biotransformations. *Appl. Environ. Microbiol.* **2005**, *71*, 6606–6612. [CrossRef]
24. Weber, F.J.; de Bont, J.A.M. Adaptation mechanisms of microorganisms to the toxic effects of organic solvents on membranes. *Biochim. Biophys. Acta* **1996**, *1286*, 225–245. [CrossRef]
25. Heipieper, H.J.; Keweloh, H.; Rehm, H.J. Influence of phenols on growth and membrane permeability of free and immobilized *Escherichia coli*. *Appl. Environ. Microbiol.* **1991**, *57*, 1213–1217. [CrossRef]
26. Heipieper, H.J.; Neumann, G.; Cornelissen, S.; Meinhardt, F. Solvent-tolerant bacteria for biotransformations in two-phase fermentation systems. *Appl. Microbiol. Biotechnol.* **2007**, *74*, 961–973. [CrossRef] [PubMed]
27. Muter, O. Current Trends in Bioaugmentation Tools for Bioremediation: A Critical Review of Advances and Knowledge Gaps. *Microorganisms* **2023**, *11*, 710. [CrossRef]
28. Bala, S.; Garg, D.; Thirumalesh, B.V.; Sharma, M.; Sridhar, K.; Inbaraj, B.S.; Tripathi, M. Recent Strategies for Bioremediation of Emerging Pollutants: A Review for a Green and Sustainable Environment. *Toxics* **2022**, *10*, 484. [CrossRef]
29. Gao, D.W.; Wen, Z.D. Phthalate esters in the environment: A critical review of their occurrence, biodegradation, and removal during wastewater treatment processes. *Sci. Total Environ.* **2016**, *541*, 986–1001. [CrossRef] [PubMed]
30. Liang, D.W.; Zhang, T.; Fang, H.H.P.; He, J. Phthalates biodegradation in the environment. *Appl. Microbiol. Biotechnol.* **2008**, *80*, 183–198. [CrossRef] [PubMed]
31. Yang, T.; Ren, L.; Jia, Y.; Fan, S.; Wang, J.; Wang, J.; Nahurira, R.; Wang, H.; Yan, Y. Biodegradation of Di-(2-ethylhexyl) Phthalate by *Rhodococcus ruber* YC-YT1 in Contaminated Water and Soil. *Int. J. Environ. Res. Public Health* **2018**, *15*, 964. [CrossRef]
32. Jin, D.C.; Liang, R.X.; Dai, Q.Y.; Zhang, Y.R.; Wu, L.X.; Chao, L.W. Biodegradation of Di-n-Butyl Phthalate by *Rhodococcus* sp. JDC-11 and Molecular Detection of 3,4-Phthalate Dioxygenase Gene. *J. Microbiol. Biotechnol.* **2010**, *20*, 1440–1445. [CrossRef]
33. Lokesh, P.; Shobika, R.; Omer, N.S.; Reddy, M.; Saravanan, P.; Rajeshkannan, R.; Saravanan, V.; Venkatkumar, S. Bioremediation of plastics by the help of microbial tool: A way for control of plastic pollution. *Sustain. Chem. Environ.* **2023**, *3*, 100027. [CrossRef]
34. Armanu, E.G.; Bertoldi, S.; Chrzanowski, L.; Volf, I.; Heipieper, H.J.; Eberlein, C. Benefits of Immobilized Bacteria in Bioremediation of Sites Contaminated with Toxic Organic Compounds. *Microorganisms* **2025**, *13*, 155. [CrossRef]
35. Keweloh, H.; Heipieper, H.J.; Rehm, H.J. Protection of bacteria against toxicity of phenol by immobilization in calcium alginate. *Appl. Microbiol. Biotechnol.* **1989**, *31*, 383–389. [CrossRef]
36. White, J.R.; Budarin, V.; Luque, R.; Clark, H.J.; Macquarrie, J.D. Tuneable porous carbonaceous materials from renewable resources. *Chem. Soc. Rev.* **2009**, *38*, 3401–3418. [CrossRef]

37. Wijitkosum, S.; Jiwnok, P. Elemental Composition of Biochar Obtained from Agricultural Waste for Soil Amendment and Carbon Sequestration. *Appl. Sci.* **2019**, *9*, 3980. [CrossRef]
38. Fang, J.; Gaom, B.; Chen, J.; Zimmerman, R.A. Hydrochars derived from plant biomass under various conditions: Characterization and potential applications and impacts. *Chem. Eng. J.* **2015**, *267*, 253–259. [CrossRef]
39. Sharma, S.; Mohler, J.; Mahajan, S.D.; Schwartz, S.A.; Bruggemann, L.; Aalinkeel, R. Microbial Biofilm: A Review on Formation, Infection, Antibiotic Resistance, Control Measures, and Innovative Treatment. *Microorganisms* **2023**, *11*, 1614. [CrossRef] [PubMed]
40. Bos, R.; van der Mei, H.C.; Busscher, H.J. Physico-chemistry of initial microbial adhesive interactions—Its mechanisms and methods for study. *FEMS Microbiol. Rev.* **1999**, *23*, 179–230. [CrossRef]
41. Lekshmi, G.S.; Bazaka, K.; Ramakrishna, S.; Kumaravel, V. Microbial electrosynthesis: Carbonaceous electrode materials for CO₂ conversion. *Mater. Horiz.* **2023**, *10*, 292–312. [CrossRef]
42. Li, S.; Cheng, C.; Thomas, A. Carbon-Based Microbial-Fuel-Cell Electrodes: From Conductive Supports to Active Catalysts. *Adv. Mater.* **2017**, *29*, 1602547. [CrossRef]
43. Sevilla, M.; Fuertes, A.B. The production of carbon materials by hydrothermal carbonization of cellulose. *Carbon* **2009**, *47*, 2281–2289. [CrossRef]
44. Titirici, M.M.; Antonietti, M.; Baccile, N. Hydrothermal carbon from biomass: A comparison of the local structure from poly- to monosaccharides and pentoses/hexoses. *Chem. Mater.* **2008**, *20*, 5926–5932. [CrossRef]
45. Sharma, K.; Nayariseri, A.; Singh, S.K. Biodegradation of plasticizers by novel strains of bacteria isolated from plastic waste near Juhu Beach, Mumbai, India. *Sci. Rep.* **2024**, *14*, 30824. [CrossRef]
46. Schuermans, S.; Kestens, C.; Marques, P.E. Systemic mechanisms of necrotic cell debris clearance. *Cell Death Dis.* **2024**, *15*, 557. [CrossRef] [PubMed]
47. Manikandan, K.S.; Mariyam, A.; Gowda, N.K.; Singh, A.; Nair, V. Mechanistic understanding of biochar-bacteria system for enhanced chlorpyrifos bioremediation in water and soil medium. *Chem. Eng. J.* **2024**, *483*, 149119. [CrossRef]
48. Reich, S.; Thomsen, C. Raman spectroscopy of graphite. *Philos. Trans. R. Soc. A* **2004**, *362*, 2271–2288. [CrossRef]
49. Hartmans, S.; Smits, J.P.; van der Werf, M.J.; Volkerling, F.; de Bont, J.A.M. Metabolism of Styrene Oxide and 2-Phenylethanol in the Styrene-Degrading Xanthobacter Strain 124x. *Appl. Environ. Microbiol.* **1989**, *55*, 2850–2855. [CrossRef] [PubMed]
50. Hofmann, U.; Schlosser, D. Biochemical and physicochemical processes contributing to the removal of endocrine-disrupting chemicals and pharmaceuticals by the aquatic ascomycete *Phoma* sp. UHH 5-1-03. *Appl. Microbiol. Biotechnol.* **2016**, *100*, 2381–2399. [CrossRef]

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Article

Thermoplastic Phenomena and Morphological Changes upon Fast Pyrolysis of Biomass and Model Compounds

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Abstract: The work reports preliminary results on the morphological changes that biomass particles experience at high heating rates in a heated strip reactor (HSR) at $T = 1000\text{--}1600\text{ }^{\circ}\text{C}$ under an inert atmosphere. Samples included a natural lignocellulosic biomass (pinewood) as well as biomass components: cellulose, hemicellulose (xylan) and lignin. On top of that, reference compounds have been investigated, namely naphthalene pitch, a paraffinic wax and glucose. During the heat-up phase, the investigated biomass mainly retains the original morphology and size, while the single components exhibit different behaviors. Hemicellulose undergoes a fluid stage and eventually forms millimetric spherical char particles. Cellulose does not become fully fluid but softens and forms millimetric char aggregates of different shapes. Lignin particles hardly soften and stick together in a curved slab. Comparison with model compounds allows us to infer that the degree of melting and the viscosity of the melt are responsible for the final particle shape. In fact, naphthalene pitch and glucose appear to be more viscous during pyrolysis and lead to the formation of three-dimensional columns a few millimeters high. Wax undergoes extensive melting, but the relatively low viscosity and the absence of crosslinking reactions eventually lead only to the formation of droplets.

Keywords: biomass; pyrolysis; softening; morphology; thermoplastic; spherodization

1. Introduction

During the heating and pyrolysis process in a boiler, carbon-based fuels such as coals, biomass and wastes undergo important structural reorganization, in parallel with volatile matter release. The biomass char particles produced at the end of pyrolysis may be spherical or irregular in shape, shrunken or swollen, and more or less porous. The shape and porosity of char particles have important implications on transport phenomena and fluid dynamics in boilers. These features contribute to the burning mode, ultimately impacting on conversion rate and efficiency. The relevance of these features is indeed acknowledged in coal combustion CFD (computational fluid dynamics) codes, which require particle size, swelling factor and porosity as input parameters. Several groups investigated the shape changes in coal [1] and biomass [2–8] particles in high-heating-rate ($dT/dt > 10^3\text{ }^{\circ}\text{C/s}$) devices. For brevity, just a few are cited here. Gil et al. [1] documented using SEM analysis that during fast pyrolysis in oxy-fuel atmospheres, coal particles soften and generate

nearly spherical chars. The tendency towards spherodization at very high heating rates, which are typical of PF (pulverized fuel-fired) boilers, has also been reported for several biomasses; however, this is not a generalized phenomenon, and very different behaviors can also be found. Cetin et al. [2] investigated pine, eucalyptus and sugar cane bagasse in a drop tube and reported that bagasse was less prone to softening than the two wood species. Dell’Ora et al. [3] reported a more pronounced tendency towards spherodization for softwood compared to hardwood. McNamee et al. [5] investigated the influence of torrefaction on spherodization, reporting an increased tendency towards spherodization for torrefied eucalyptus and willow compared to untreated samples [5]. Panahi et al. [6] used char sampling (optical microscopy and SEM) and high-resolution videography to monitor the progressive shape changes of single biomass particles throughout pyrolysis in a drop tube. They observed a high tendency toward spherodization for miscanthus and a less pronounced tendency for beech. Panahi et al., in a further work [7], reported spherodization for five torrefied biomasses (corn straw, willow, pine, eucalyptus and miscanthus), even though only two spherodized as raw fuels as well (eucalyptus and miscanthus) [7], while Holmgren et al. [8] found that wheat biomass retained its original flake shape.

The physical and chemical processes that determine the shape and morphology of char particles have been addressed to some extent for coals in the past [9], but they are not completely understood. The reason for the observed differences among different biomasses is even more intriguing.

It is common sense that the swelling of solid fuels upon heat-up and pyrolysis would be related to the plasticity of the pyrolysis products, especially during the stage of incipient char formation, when the solidification of the carbon structure and the release of volatiles occur in parallel and in competition with each other. According to network pyrolysis models, like the chemical percolation devolatilization (CPD) model [9], after the labile bridges are broken, the fragments form the so-called “metaplast”. Part of the metaplast is released as volatile matter, while part of it reattaches to the solid matrix via crosslinking reactions. The fluidity of the metaplast and the rate of crosslinking could likely play a role in the formation of spherical vs. irregular char particles as well as on the formation of more graphitic vs. amorphous carbon. It could be expected that for biomass, the presence of different components (cellulose, hemicellulose, lignin, etc.) may contribute to the complexity of observed behavior.

The existence of an intermediate liquid phase during biomass pyrolysis has been debated in the literature for several decades [10–22]. In 1974, Nordin et al. [12] noticed that cellulose and biomass pass through a condensed phase during flash pyrolysis. It has been explained that during the pyrolysis of biomass, the lignocellulosic components depolymerize and the heavy oligomeric compounds with boiling points higher than the temperatures of the depolymerization reactions form a liquid intermediate phase [10–21,23]. Real-time visualization studies have shown that these heavy components in the liquid phase can be ejected in the form of aerosols through bubble explosions [13–20]. This phenomenon explains the presence of oligomeric products in bio-oil, which are typically not well characterized [22]. Dauenhauer et al. [13] recorded with a high-speed camera the evolution of cellulose during fast pyrolysis at 700 °C. Bubbling and evaporation of the melted phase promoted aerosol ejection and the shrinking of the liquid phase, without a trace of char formation. In 2011, Teixeira et al. [14] used a high-speed camera to visualize the mechanism of aerosol formation by bubbles bursting in the intermediate liquid phase during the pyrolysis of cellulose and sucrose; a new mechanism called reactive boiling ejection was proposed. Zhou et al. [20], using a high-speed camera, showed that, during lignin pyrolysis, lignin first melts. Then, bubbles are formed within the liquid phase, leading to particle swelling. Finally, evaporation and bubble collapse lead to particle shrinking.

Montoya et al. [21] examined the intermediate liquid phase formed during fast pyrolysis of biomass and of biomass components (cellulose, organo-solv lignin, milled wood lignin, xylan, de-ashed xylan, sugarcane bagasse and de-ashed sugarcane bagasse) by high-speed visualization techniques. The purpose was to identify the fraction responsible for the conservation of particle morphology. They confirmed that cellulose, lignin and de-ashed xylan were completely transformed into an intermediate liquid phase. Xylan, as received, did not fully melt and maintained its general shape during pyrolysis. These results suggest that the presence of mineral matter has a critical importance in allowing lignocellulosic materials to retain their original morphology.

Interesting clues about the thermoplastic behavior of biomass pyrolysis can be found in Dufour et al. [23], who examined the behavior of the main lignocellulosic components, namely cellulose, hemicellulose (xylan) and lignin. However, their study is limited to a relatively low temperature (about 450 °C) and heating rate (5 °C/min). The thermoplastic behavior of biomass and its constituents at very high heating rates, which are typical of fast pyrolysis systems (e.g., fluidized beds and pulverized firing boilers), requires further investigation, given the significance of such phenomena in defining particle morphology and shape, and ultimately, combustion performance in industrial burners.

The present work aims to contribute to understanding how the intrinsic properties of biomass and model compounds influence their structural transformations during pyrolysis. This knowledge is crucial for optimizing biomass processing to develop high-performance, sustainable pyrolysis and combustion processes. These issues may also be of interest for developing or enhancing lignocellulosic fibers and advanced materials, as they provide insights into biomass processing under extreme thermal conditions.

In this framework, the present work reports the results of an experimental campaign aimed at characterizing the melting phenomena and morphological changes in carbonaceous materials upon fast pyrolysis in a heated strip reactor (HSR), with heating rates up to 10^4 °C/s and temperatures of 1000–1600 °C. The investigated samples include natural lignocellulosic biomasses, as well as isolated components, namely cellulose, hemicellulose (xylan) and lignin. The study is complemented by the investigation of selected reference compounds with different chemical compositions: naphthalene pitch, glucose and wax. A comparison with model compounds suggests that the degree of melting and the viscosity of the melt are responsible for the final particle shape.

2. Results

2.1. Biomass

Sequences of images taken by a thermocamera, observing particle samples on top of the heated strip, are presented and commented on in the following. Figure 1 reports the temperature profile depending on the time of two selected spots (p1 and p2) of particles with diameters of 0.01 cm during the pinewood pyrolysis at target strip temperature $\approx$ 1500 °C. The thermocamera is capable of registering frames from 630 °C (as detected with the pyrometer), even though it is calibrated from 860 °C. In all the experiments, particles are laid on the strip and care is taken to spread the sample uniformly, trying to avoid the formation of thick bulks of samples.

Figure 1 shows that reactor strip and particles are heated up simultaneously after $t = 2.5$ s. The strip temperature first rises rapidly in 0.5 s to 1360 °C, and then to 1480 °C after additional an 1.5 s. In the figure, 860 °C corresponds to the lowest value of the calibration temperature of the thermocamera. After the power is turned off, the strip and the particles cool down quickly in 2 s. Initially, the particles distributed on the strip can be assumed to be at the same temperature as the strip itself, while the development of

agglomerates causes a temperature gradient between the strip and the top layers of the samples, which can surpass 100 °C.

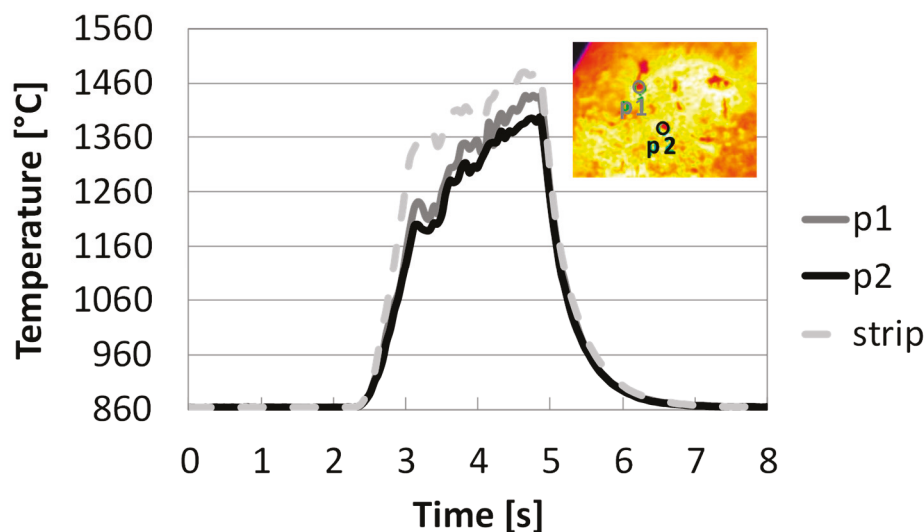


Figure 1. Temperature profile depending on the time of the reactor strip and two selected spots during pyrolysis in HSR at a target strip temperature ≈ 1500 °C (top view) (P1: dark grey solid line; P2: black solid line; Strip: dotted grey line).

Figure 2A reports the images obtained during experiments in the HSR of pinewood pyrolysis at a target strip temperature ≈ 1500 °C. The sample at the beginning of the experiments is seen as a layer of powder. Volatile flux is visible as a gray smoke in the figures, starting at $t = 0.39$ s and ending at $t = 0.60$ s. Time t_0 is chosen as strip temperature reaches 630 °C. At the end of the experiment, a few dispersed particles are recovered from the strip. Figure 2B reports lateral views of pyrolysis in the HSR of pinewood at a target strip temperature ≈ 1300 °C. Although pyrolysis increases in this case, the underlying phenomena remain similar. At the end of the experiment, the sample takes the form of fine char powder.

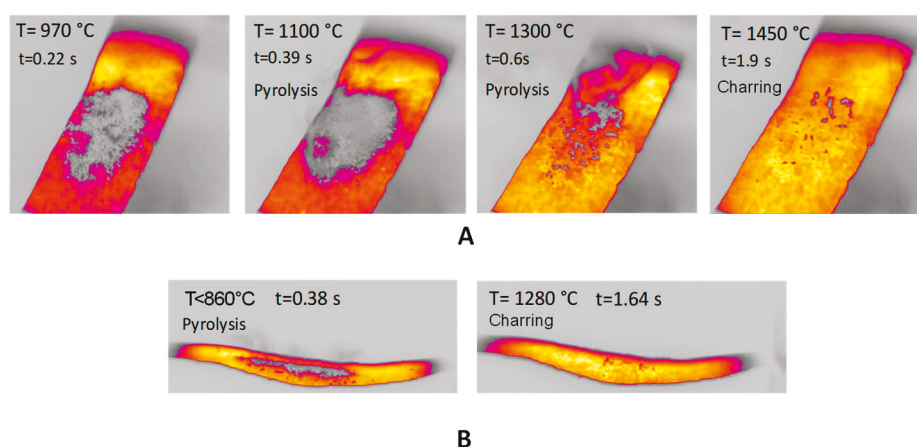


Figure 2. (A) HSR pinewood pyrolysis at a target strip temperature ≈ 1500 °C (top view); (B) HSR pinewood pyrolysis at a target strip temperature ≈ 1300 °C (lateral view).

2.2. Biomass Components

Figures 3–8 report images captured during pyrolysis in the HSR of the three lignocellulosic components investigated at a target strip temperature in the range of 1000–1300 °C: hemicellulose, cellulose and lignin.

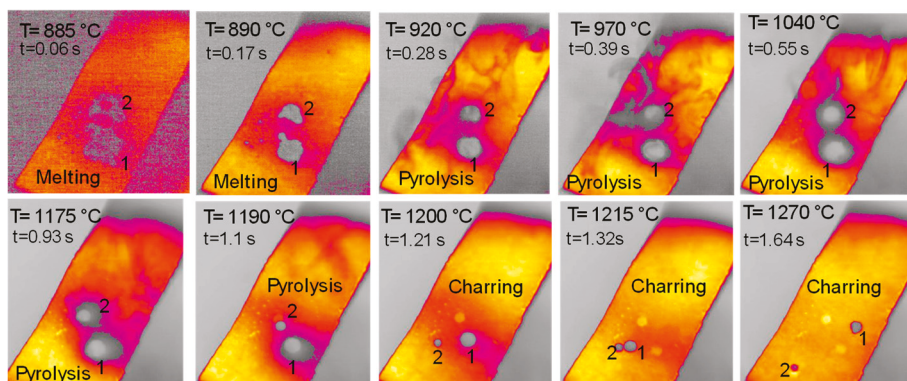


Figure 3. HSR experiment of hemicellulose at a target strip temperature ≈ 1300 °C (top view) [24].

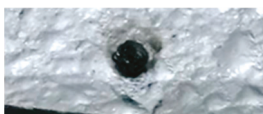


Figure 4. Char particle of hemicellulose collected in the HSR at a target strip temperature ≈ 1300 °C [24].

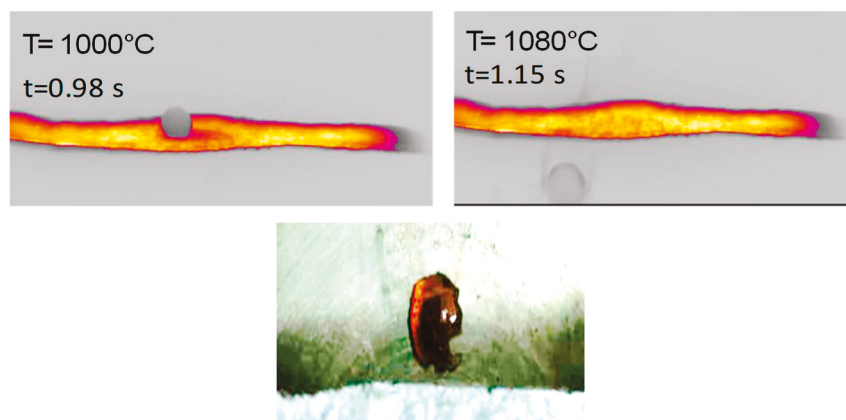


Figure 5. (Top) HSR experiment of hemicellulose at a target strip temperature of ≈ 1000 °C (lateral view). (Bottom) Brownish particle recovered at the end of the experiment [24].

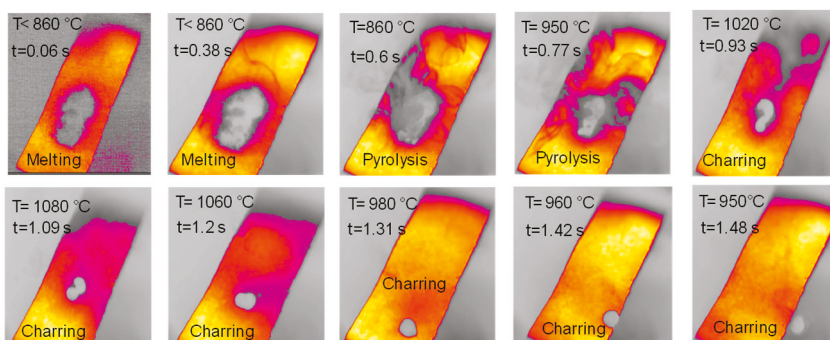


Figure 6. HSR experiment of cellulose at a target strip temperature ≈ 1000 °C (top view).



Figure 7. Particle recovered at the end of the cellulose pyrolysis experiment at a target strip temperature of ≈ 1000 °C.

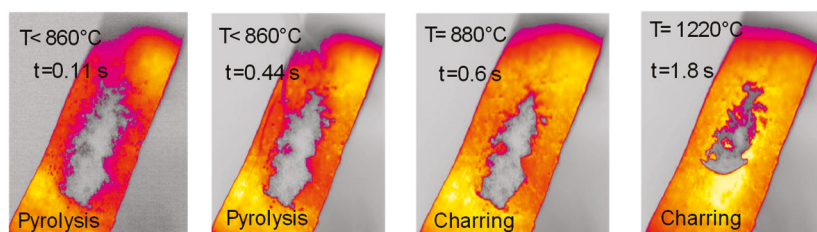


Figure 8. HSR experiment of lignin at a target strip temperature ≈ 1300 °C (top view).

The behavior of hemicellulose in the HSR is documented in the top view, shown in Figure 3, and the lateral view, shown in Figure 5, of the thermocamera during two similar pyrolysis experiments. Note that experiments on hemicellulose have also been reported in [24]. The sample of hemicellulose has an original cubical particle shape. In the experiment shown in Figure 3, two balks of particles of 1×0.8 cm and 0.8×0.6 cm are initially deposited on the strip. Between 0.06 s and 0.17 s, they generate two almost spherical agglomerates with a diameter of 0.6 and 0.5 cm and smoothed edges. Pyrolysis occurs when the strip's temperature range is 900–1200 °C, and an intense release of volatiles forms a cloud of hot gas around the two clusters. As previously discussed, with larger agglomerates, the temperature gradient between the strip and the upper layers of the agglomerates is about 100 °C, but rolling phenomena can possibly lower these values. Shrinkage phenomena are observed at the end of the pyrolysis stage. Between 1.21 and 1.64 s, the charring stage takes place with a progressive shrinkage of the particle. Figure 4 reports the char particle (first spot) collected at the end of the experiment. Time, temperature and observed particle sizes of the two clusters during the experiments at a target strip temperature ≈ 1300 °C are reported in Table 1.

Table 1. Analysis of HSR experiment on hemicellulose at a target strip temperature of 1300 °C [24].

Stage	Time [s]	Temperature [°C]	Particles Dimensions [cm]	
			1st Spot	2nd Spot
	0 ³	630 ³	1×1 ¹	0.8×0.8 ¹
Melting	0.06	885	0.8×0.8 ¹	0.8×0.6 ¹
Melting	0.17	890	0.6 ²	0.5 ²
Pyrolysis	0.28	920	0.5 ²	0.4 ²
Pyrolysis	0.39	970	0.5 ²	0.4 ²
Pyrolysis	0.55	1040	0.5 ²	0.4 ²
Pyrolysis	0.93	1175	0.45 ²	0.35 ²
Pyrolysis	1.1	1190	0.4 ²	0.3 ²
Charring	1.21	1200	0.4 ²	0.2 ²
Charring	1.32	1215	0.3 ²	0.2 ²
Charring	1.64	1270	0.2 ²	0.1 ²

¹ Length (L) $\times$ width (W); ² diameter; ³ time $t = 0$ is taken as the time when the strip reaches 630 °C, which is the lower temperature limit of the pyrometer.

Lateral views of hemicellulose at strip temperatures of around 1000 °C are reported in Figure 5. These clearly show the formation of a spherical intermediate stage resembling molten droplets. At $t = 1.15$ s, the sphere moves along the strip and eventually falls off. A brownish particle is found on the reactor floor.

The sample of cellulose has an original spherical particle shape. The results for cellulose, documented in Figure 6, show that during heat-up a more complex and interesting behavior is found; a marked release of volatiles occurs between $t = 0.38$ s and $t = 0.77$ s, when the temperature reached 940 °C, and is accompanied by a progressive agglomeration of the particles lying on the strip. At approximately $t = 1.2$ s, a spherical cluster with a

diameter of 0.5 cm forms, which migrates across the strip and eventually falls off. At the end of the experiment, a solid particle is recovered on the floor of the reactor, as shown in Figure 7. The recovered solid particle does not look like a char particle but more like an agglomeration of smaller sticky particles. Table 2 reports the time, temperature and observed particle sizes during the experiments in the HSR at a target strip temperature ≈ 1000 °C.

Table 2. Analysis of HSR experiment of cellulose at a target strip temperature of ≈ 1000 °C.

Stage	Time [s]	Temperature [°C]	Particles Dimensions [cm]
			1st Spot
	0 ³	630 ³	1×2 ¹
Melting	0.06	<860	1×2 ¹
Melting	0.38	<860	1×2 ¹
Pyrolysis	0.6	860	0.7×2 ¹
Pyrolysis	0.77	940	0.5×1 ¹
Charring	1.09	1080	0.4×0.8 ¹
Charring	1.2	1060	0.4×0.6 ¹
Charring	1.31	980	0.5 ²
Charring	1.42	860	0.5 ²
Charring	1.48	860	0.5 ²

¹ Length (L) $\times$ width (W); ² diameter; ³ time $t = 0$ is taken as the time when the strip reaches 630 °C, which is the lower temperature limit of the pyrometer.

The sample of lignin has an original elongated particle shape. For lignin pyrolysis (Figure 8), volatile efflux is observed from $t = 0.44$ s to $t = 0.60$ s. At approximately $t = 0.60$ s, an agglomeration of sticky char particles is formed, which detaches itself from the strip, and eventually curves upwards. At the end of the experiment, the char sample appears as a single slightly curved slab (boat-shaped).

2.3. Model Compounds

Figure 9 reports the top views of the pyrolysis experiments in the HSR on naphthalene pitch at a target temperature $T \simeq 1600$ °C. Notably, the behavior of naphthalene pitch is very interesting. In Figure 9, at $t = 0.33$ s, the powder dispersed on the strip undergoes pyrolysis, and the volatile efflux is quite strong, as also shown in the later photograms. At $t = 0.50$ s, the sample forms three distinct clusters on the strip. These clusters progressively grow in size in the vertical direction, forming three columns. At the end of the test, three porous black char columns of 7–10 mm in height are recovered on the strip (Figure 10). On the other hand, Figure 11 reports the lateral views of naphthalene pitch pyrolysis at a target temperature 1300 °C, with the evolution of volatiles and the formation of much shorter columns.

Figure 12 reports a sequence of images taken during an experiment with glucose in the HSR. In this case, the behavior is slightly similar to that observed for hemicellulose, with particles deposited unevenly on the strip forming multiple denser spots. Upon heat treatment, between $t = 0.17$ s and $t = 0.39$ s, an intense release of volatiles occurs. The multiple clusters of particles then merge on the strip to form a distinct sphere, which later progressively shrinks, ultimately forming one char column. Figure 13 reports a sequence of images taken during an experiment on wax in the HSR. Also in this case, the behavior is similar to that observed for hemicellulose, with an agglomeration of all particles into a spherical droplet, which moves on the strip and eventually falls off. The sample recovered on the floor of the reactor looks like a solidified wax drop.

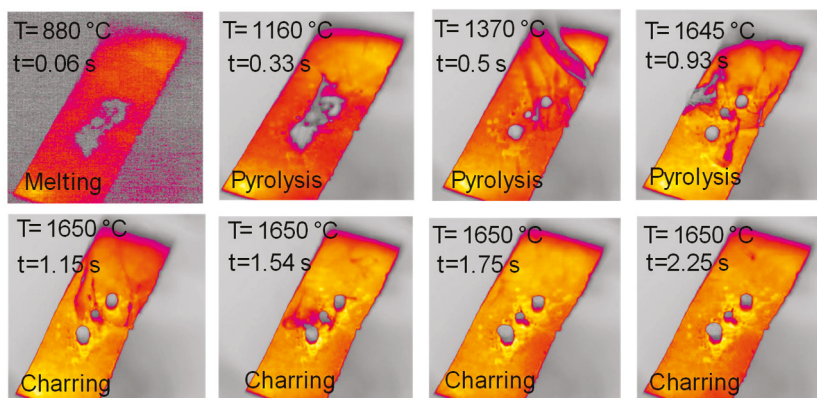


Figure 9. HSR experiment of naphthalene pitch pyrolysis at a target strip temperature ≈ 1600 °C (top view).



Figure 10. Particle recovered at the end of the naphthalene pitch pyrolysis experiment at a target strip temperature ≈ 1600 °C.

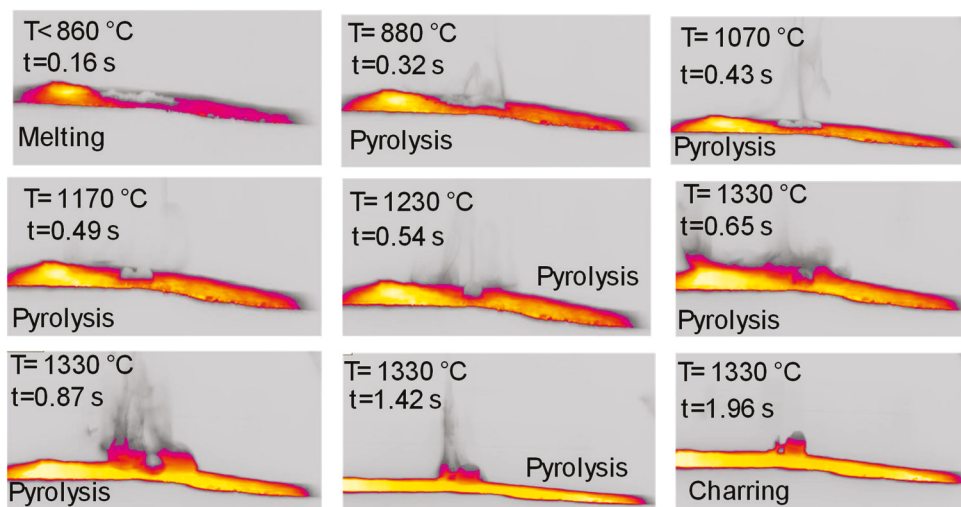


Figure 11. HSR experiment of naphthalene pitch pyrolysis at a target strip temperature ≈ 1300 °C (lateral view).

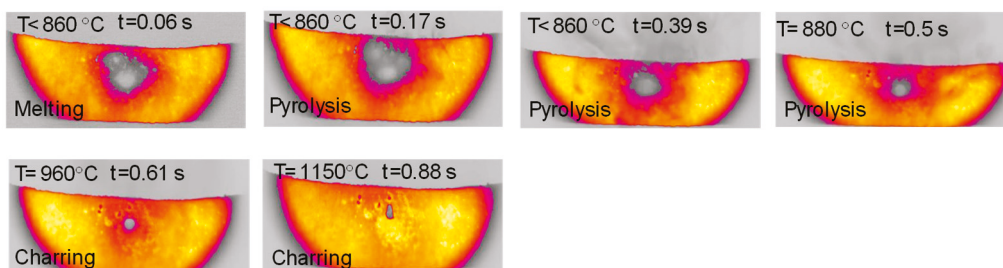


Figure 12. HSR experiment of glucose pyrolysis at a target strip temperature ≈ 1200 °C (top view).

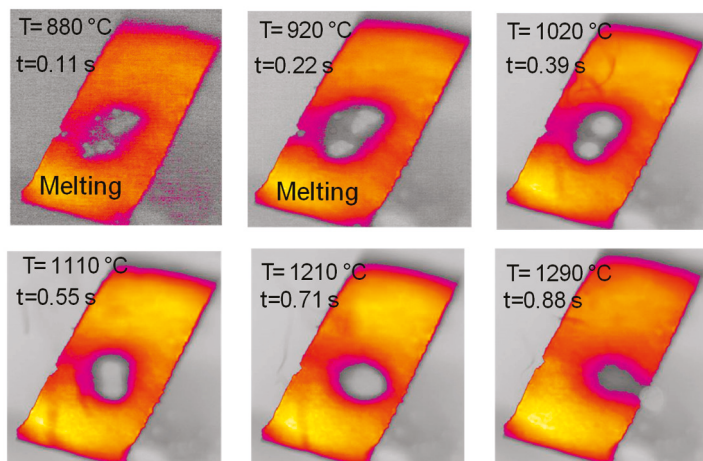


Figure 13. HSR experiment of wax pyrolysis at a target strip temperature ≈ 1300 °C (top view).

3. Discussion

Wax, glucose and naphthalene pitch were chosen as representatives of aliphatic tars and “thermoplastic” organic compounds of biogenic (rich of oxygen) and fossil origin (mainly aromatics, without oxygen), respectively.

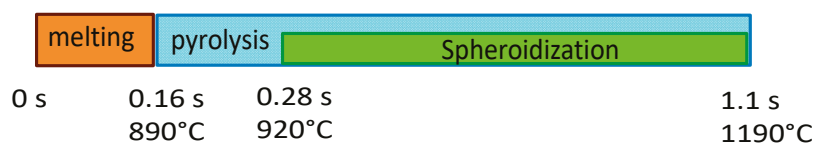
The most remarkable behavior upon pyrolysis in the HSR was found for naphthalene pitch, followed by glucose, with the formation of molten agglomerates which progressively grow in height. Wax, on the other hand, forms liquid droplets. This behavior could be attributed to the decreasing viscosity of the melted phase moving from naphthalene pitch over glucose to wax.

The particles of the investigated natural biomass, upon treatment on the heated strip, remained separate and did not show remarkable phenomena of melting and agglomeration. However, several examples of spherodization from lignocellulosic materials have been reported in the literature [2–8]. The investigated individual lignocellulosic components, however, showed melting and thermoplastic effects in the HSR.

Figure 14 reports the timeline sequence of events registered during the experiments of cellulose and hemicellulose pyrolysis in the HSR. Hemicellulose reaches a clear fluid state, and in this form, it forms drops, similarly to wax. As pyrolysis proceeds, if the drop remains on the strip for enough time, it eventually forms a single spherical char particle. If the drop falls from the strip, it freezes into a glassy solid, with a shape similar to that produced by cooled wax. Cellulose has an intermediate behavior, forming a single large char particle with an imperfect spherical shape at the end of the devolatilization step. Lignin particles (not reported in Figure 14) experience more moderate melting, which leads to the particles sticking together in a slab.

Interesting clues about the thermoplastic behavior of biomass at a low heating rate (5 °C/min) and temperatures up to 400 °C have been provided by Dufour et al. [23], who performed an in situ study on the rheology and mobility of miscanthus, cellulose, lignin and hemicellulose throughout pyrolysis. At first analysis, the present HSR’s results and Dufour’s low temperature results are opposite; in the HSR, the biomass component with the highest propensity to pass through a fluid viscous state is hemicellulose, followed by cellulose and lignin, while in Dufour’s experiments, the opposite was found. However, these results align when the argumentation presented by Dufour et al. [23] is more carefully considered.

Hemicellulose pyrolysis



Cellulose pyrolysis

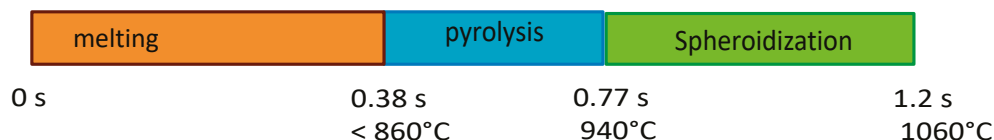


Figure 14. Timeline results from HSR experiments of hemicellulose at a target strip temperature ≈ 1300 °C and cellulose pyrolysis at a target strip temperature ≈ 1000 °C.

At the low heating rate and temperatures of [23], hemicellulose melted and softened from 200 °C. Dufour attributed the fluidity of this material to the mobility of primary pyrolysis products, since hemicellulose pyrolysis started at 200 °C. In the HSR experiments, because of the severe heating conditions, the pyrolysis rate is higher; thus, the fluid state of this material is reasonably favored in comparison to the mild heating conditions examined in [23].

As for lignin, in Dufour et al.'s work [23], it is reported that this component underwent glass transition before the start of pyrolysis (it was completely fluid at 200–225 °C). However, they also noted that lignin solidified as soon as pyrolysis and crosslinking reactions took off at around 350 °C. In the HSR, where these reactions are fast, it is therefore reasonable that the tendency of lignin to form fluid is hindered and counterbalanced by the tendency to form a crosslinked char.

Finally, for cellulose, Dufour et al. [23] reported that some increase in fluidity was observed at 250 °C, attributing it to the tendency of disordered carbohydrates to become liquid even before pyrolysis determines an appreciable mass loss. The formation of a more viscous melt compared to that of hemicellulose is also confirmed by the present experiments in the HSR, even though the progress of the pyrolysis reactions is, of course, more rapid under the heating conditions of the HSR.

The work of Dufour et al. [23] also highlighted potential interactions among the biomass constituents in natural biomass. For example, cellulose could impose its thermoplastic behavior on the overall structure and reduce the overall tendency toward melting. This could justify the inertia to melting and modest thermoplastic phenomena observed in the present work for the natural biomass investigated (pinewood). However, evidence of spheroidization during the fast pyrolysis of other biomasses has been reported in the literature; therefore, a more extensive dataset of lignocellulosic biomass should be investigated in future works in order to elucidate whether and what interactions exist among lignocellulosic components in natural biomass of different origins and whether other constituents, such as extractives and minerals, could further interfere.

4. Materials and Methods

A selection of seven different samples is used for the experimental campaign:

1. pinewood—a typical woody biomass supplied by Luleå University of Technology (Luleå, Sweden) [25];
2. hemicellulose—xylan (from corncob) supplied by Carl Roth (CAS No. 9004-63-5, Karlsruhe, Germany) [26];

- cellulose—vivapur MCC sphere supplied by JRS (CAS No. 9004-34-6, Rosenberg, Germany) [26];
- lignin—dealkaline lignin supplied by Fisher Scientific (CAS No. 9005-53-2, Göteborg, Sweden) [26].
- naphthalene pitch—a 100% mesophase pitch synthesized by polymerization of naphthalene, kindly provided by Mitsubishi Gas Chemical Company (CAS No. 25135-16-4, Tokyo, Japan) [27];
- paraffinic wax;
- D-(+)-glucose supplied by Sigma Aldrich (CAS No. 14431-43-7, Milano, Italy).

The samples have been sieved to obtain particle sizes of 100–200 μm .

Figure 15 reports a photo of the equipment, the HSR and the equipment used in the experimental campaign. In the HSR, a pyrolytic graphite foil is used as the sample holder. The HSR is heated by Joule effects up to 2000 $^{\circ}\text{C}$, with a heating rate of 10^4 $^{\circ}\text{C}/\text{s}$. The stainless steel reactor is equipped with two optical openings of quartz for a pyrometer or a thermal camera.

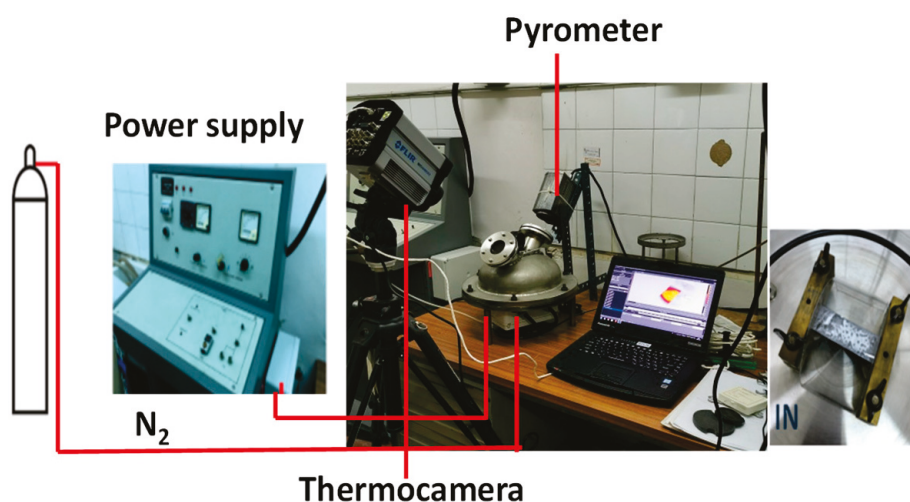


Figure 15. Heated strip reactor.

The HSR has been extensively used to investigate the fast pyrolysis of coal and biomass in nitrogen and carbon dioxide, with contextual analysis of the primary tars, by positioning a quartz bridge on top of the strip to collect the condensable products [26,28].

A fast FLIR thermal camera (FLIR SC6811), capable of capturing 38,000 frames, was used. The thermal camera can capture frames starting from 630 $^{\circ}\text{C}$. Calibration was performed with the quartz window between the camera and the heated strip. Furthermore, a LAND R pyrometer, sensitive in the range of 630–2000 $^{\circ}\text{C}$, was employed. Blank tests were conducted with various voltage and current settings to calibrate the thermal camera's temperature readings against those of the pyrometer. In all the experiments, particles were laid on the strip and care was taken to spread the sample uniformly, trying to avoid the formation of thick bulks of samples. Our previous work [29] showed that the particles closely in contact with the strip can be considered to be the same temperature as the strip. Temperature differences between the bottom and upper surface of the particles were limited to 50 $^{\circ}\text{C}$ for particle sizes in the order of 100 μm , reaching 200 $^{\circ}\text{C}$ for larger particles. Once the sample was loaded, the reactor vessel was sealed and purged with nitrogen to eliminate any oxygen traces inside. The thermal camera captured the images and temperatures of the particles as they heated up. The heat treatments were varied between 1000 and 1600 $^{\circ}\text{C}$, with a residence time set to 4–5 s.

After the power was turned off, the strip underwent rapid thermal cooling, the vessel was opened, and the residual solid sample (char) was recovered. The images captured by the thermal camera were processed to create time-temperature frames of the samples during heating, allowing for the tracking of particle morphology changes over time. Notably, in most of the experiments, the thermocamera acquired images from the top; however, some tests were repeated with a different positioning of the thermocamera in order to acquire lateral views.

5. Conclusions

The present work reports preliminary results on the morphological changes that solid particles of different types and origins experience upon very fast heating in an inert atmosphere. Samples included naphthalene pitch, paraffinic wax and natural lignocellulosic biomass (pinewood), as well as biomass components, namely cellulose, hemicellulose and lignin. The results, as summarized in Table 3, where the photos collected at the end of the pyrolysis experiments are reported, reveal distinct behaviors for the different materials:

- pinewood sample pyrolysis takes place without any relevant change in size or shape or evidence of melting;
- the lignin sample produces a curved slab of sticky powder char (boa-shaped) suggesting the formation of a highly viscous stage;
- cellulose forms an irregular spherical char, which moves on the strip and eventually falls down;
- hemicellulose forms a molten drop, which moves on the strip and eventually falls down. In cases where the melted drop remains on the strips, it turns into a spherical char particle.

Reference/model compounds have also been investigated:

- wax forms a molten drop, which moves on the strip and eventually falls down;
- naphthalene pitch forms three black porous char columns;
- glucose forms a char column.

These effects have been discussed and attributed to the melting and thermoplasticity of the materials upon high temperature pyrolysis, which certainly require in-depth analysis and theoretical interpretation in the follow-up of the research activity. The present investigation highlights evidence of an intermediate liquid phase in lignocellulosic components. The existence of the liquid phase within the particles has critical implications for defining mass and energy transfer during pyrolysis. Indeed, many physiochemical phenomena occur simultaneously within the liquid phase as solid/liquid reactions, mass and heat transfer in the liquid-solid-gas phases, and bubbling (nucleation, coalescence, growth). The HSR was chosen for its particular feature of reaching elevated temperatures with fast heating rates, heating up the sample in contact with the strip; however, it did not provide information on the change of viscosity with temperature.

Table 3. Char morphologies.

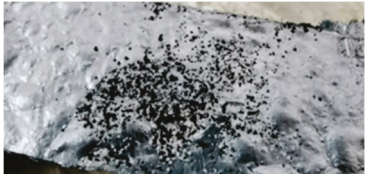
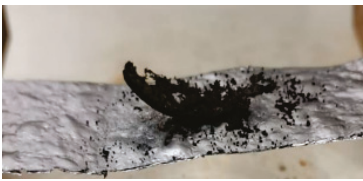
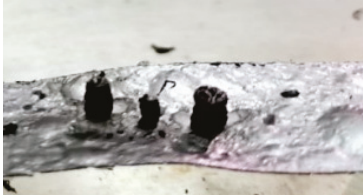
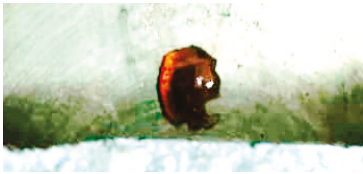
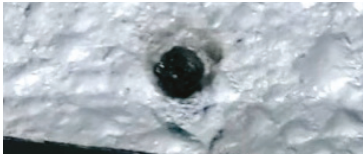
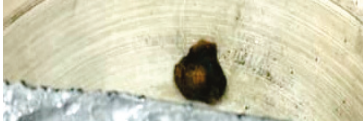
Char powder: Biomass (pinewood)	
------------------------------------	--

Table 3. Cont.

Char curved slab: Lignin	
Porous char columns: Naphthalene pitch, glucose	
Molten drop: Hemicellulose, wax	
Spherical char: Hemicellulose	
Irregular spherical char: Cellulose	

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References

1. Gil, C.M.V.; Riaza, J.; Álvarez, L.; Pevida, C.; Pis, J.J.; Rubiera, F. Oxy-fuel combustion kinetics and morphology of coal chars obtained in N₂ and CO₂ atmospheres in an entrained flow reactor. *Appl. Energy* **2012**, *91*, 67–74. [CrossRef]
2. Cetin, E.; Moghtaderi, B.; Gupta, R.; Wall, T.F. Influence of pyrolysis conditions on the structure and gasification reactivity of biomass chars. *Fuel* **2004**, *83*, 2139–2150. [CrossRef]
3. Dall’Ora, M.; Jensen, P.A.; Jensen, A.D. Suspension combustion of wood: Influence of pyrolysis conditions on char yield, morphology, and reactivity. *Energy Fuels* **2008**, *22*, 2955–2962. [CrossRef]

4. Reed, A.R.; Williams, P.T. Thermal processing of biomass natural fibre wastes by pyrolysis. *Int. J. Energy Res.* **2004**, *28*, 131–145. [CrossRef]
5. McNamee, P.; Darvell, L.I.; Jones, J.M.; Williams, A. The combustion characteristics of high-heating-rate chars from untreated and torrefied biomass fuels. *Biomass Bioenergy* **2015**, *82*, 63–72. [CrossRef]
6. Panahi, A.; Levendis, Y.A.; Vorobiev, N.; Schiemann, M. Direct observations on the combustion characteristics of Miscanthus and Beechwood biomass including fusion and spherodization. *Fuel Process. Technol.* **2017**, *166*, 41–49. [CrossRef]
7. Panahi, A.; Vorobiev, N.; Delichatsios, M.A.; Schiemann, M.; Levendis, Y.A. Combustion Details of Raw and Torrefied Biomass Fuel Particles with Individually-Observed Size, Shape and Mass. *Combust. Flame* **2019**, *207*, 327–341. [CrossRef]
8. Holmgren, P.; Wagner, D.R.; Strandberg, A.; Molinder, R.; Wiinikka, H.; Umeki, K.; Broström, M. Size, Shape, and Density Changes of Biomass Particles During Rapid Devolatilization. *Fuel* **2017**, *206*, 342–351. [CrossRef]
9. Fletcher, T.H. Review of 30 Years of Research Using the Chemical Percolation Devolatilization Model. *Energy Fuels* **2019**, *33*, 12123–12153. [CrossRef]
10. Lédé, J. Cellulose pyrolysis kinetics: An historical review on the existence and role of intermediate active cellulose. *J. Anal. Appl. Pyrolysis* **2012**, *94*, 17–32. [CrossRef]
11. Di Blasi, C. Heat transfer mechanisms and multi-step kinetics in the ablative pyrolysis of cellulose. *Chem. Eng. Sci.* **1996**, *51*, 2211–2220. [CrossRef]
12. Nordin, S.; Nyren, J.; Back, E. An indication of molten cellulose produced in a laser beam. *Text. Res. J.* **1974**, *44*, 915–917. [CrossRef]
13. Dauenhauer, P.J.; Colby, J.L.; Balonek, C.M.; Suszynski, W.J.; Schmidt, L.D. Reactive boiling of cellulose for integrated catalysis through an intermediate liquid. *Green Chem.* **2009**, *11*, 1555–1561. [CrossRef]
14. Teixeira, P.; Mooney, A.R.; Kruger, K.G.; Williams, J.S.; Suszynski, C.L.; Schmidt, W.J.; Schmidt, L.D.; Dauenhauer, D.P. Aerosol generation by reactive boiling ejection of molten cellulose. *Energy Environ. Sci.* **2011**, *4*, 4306. [CrossRef]
15. Goring, D.A.I. Thermal softening of lignin hemicellulose and cellulose. *Pulp Pap. Mag. Can.* **1963**, *64*, T517–T527.
16. Deng, C.; Wu, H. Characteristics of chars formed during rapid pyrolysis of biomass model components at high temperature. *Proc. Combust. Inst.* **2023**, *39*, 3353–3360. [CrossRef]
17. Saka, S.; Asmadi, M.; Kawamoto, H. Gas- and solid/liquid-phase reactions during pyrolysis of softwood and hardwood lignins. *J. Anal. Appl. Pyrolysis* **2011**, *92*, 417–425.
18. Zhu, G.; Zhu, X.; Xiao, Z.; Yi, F. Study of cellulose pyrolysis using an in situ visualization technique and thermogravimetric analyzer. *J. Anal. Appl. Pyrolysis* **2012**, *94*, 126–130. [CrossRef]
19. Yi, F.; Zhu, G.; Zhu, X.; Zhou, R.; Xiao, Z. Pyrolysis characteristics of bean dregs and in situ visualization of pyrolysis transformation. *Waste Manag.* **2012**, *32*, 2287–2293.
20. Zhou, S.; Pecha, B.; van Kuppevelt, M.; McDonald, A.G.; Garcia-Perez, M. Slow and fast pyrolysis of Douglas-fir lignin: Importance of liquid-intermediate formation on the distribution of products. *Biomass Bioenergy* **2014**, *66*, 398–409. [CrossRef]
21. Montoya, J.; Pecha, B.; Janna, F.C.; Garcia-Perez, M. Identification of the fractions responsible for morphology conservation in lignocellulosic pyrolysis: Visualization studies of sugarcane bagasse and its pseudo-components. *J. Anal. Appl. Pyrolysis* **2017**, *123*, 307–318. [CrossRef]
22. Russo, C.; Cerciello, F.; Senneca, O.; Apicella, B. Challenges and progresses in the chemical investigation of high molecular weight species in condensed pyrolysis products of coal and biomass. *J. Anal. Appl. Pyrolysis* **2024**, *177*, 106280. [CrossRef]
23. Dufour, A.; Castro-Díaz, M.; Marchal, P.; Brosse, N.; Olcese, R.; Bouroukba, M.; Snape, C. In Situ Analysis of Biomass Pyrolysis by High Temperature Rheology in Relations with ¹H NMR. *Energy Fuels* **2012**, *26*, 6432–6441. [CrossRef]
24. Cerciello, F.; Freisewinkel, E.; Coppola, A.; Ontyd, C.; Tarlinski, D.; Schimann, M.; Senneca, O.; Salatino, P.; Allouis, C.; Scherer, V.; et al. An experimental and modelling study of particle changes and volatiles release. *Fuel* **2024**, *357*, 29983. [CrossRef]
25. Trubetskaya, A.; Jensen, P.R.; Jensen, A.D.; Garcia Llamas, A.D.; Umeki, K.; Glarborg, P. Effect of fast pyrolysis conditions on biomass solid residues at high temperatures. *Fuel Proc. Technol.* **2016**, *143*, 118–129. [CrossRef]
26. Senneca, O.; Cerciello, F.; Russo, C.; Wutscher, A.; Muhler, M.; Apicella, B. Thermal treatment of lignin, cellulose and hemicellulose in nitrogen and carbon dioxide. *Fuel* **2020**, *271*, 117656. [CrossRef]
27. Mochida, I.; Korai, Y.; Ku, C.H.; Watanabe, F.; Sakai, Y. Chemistry of synthesis, structure, preparation and application of aromatic-derived mesophase pitch. *Carbon* **2000**, *38*, 305–328. [CrossRef]
28. Cerciello, F.; Apicella, B.; Russo, C.; Cortese, L.; Senneca, O. Effects of pressure on lignocellulosic biomass fast pyrolysis in nitrogen and carbon dioxide. *Fuel* **2021**, *287*, 119604. [CrossRef]
29. Senneca, O.; Allouis, C.; Chirone, R.; Russo, S. Set up of an experimental apparatus for the study of fragmentation of solid fuels upon severe heating. *Exp. Therm. Fluid Sci.* **2010**, *34*, 366–372. [CrossRef]

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Article

Facile Preparation Route of Cellulose-Based Flame Retardant by Ball-Milling Mechanochemistry

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Abstract: In this study, a sustainable cellulose-based flame-retardant additive was developed, characterized, and incorporated into polypropylene (PP). Microcrystalline cellulose (Cel) was chemically modified with P₂O₅ using the solvent-free ball-milling mechanochemistry approach at room temperature. This modification enabled phosphorus grafting onto cellulose, significantly enhancing the cellulose charring ability and improving the thermal stability of the char as revealed by thermogravimetric analysis (TGA). The resulting product, Cel-P, containing 4.15 wt.% phosphorus, was incorporated and uniformly dispersed as a flame-retardant (FR) additive at 30 wt.% in PP through melt processing. The PP+30-Cel-P composite demonstrated improved char formation and FR properties, including reduction of both peak heat release rate (pHRR) and total heat release (THR) in mass loss cone calorimetry (MLC). Moreover, lower light absorptivity was obtained by smoke opacity tests as compared to PP filled with unmodified cellulose.

Keywords: cellulose; flame retardancy; mechanochemistry; phosphorylation; bio-based

1. Introduction

Today plastic materials are offering numerous advantages such as lightweight, easy processing, and reasonable cost, making them indispensable in various sectors such as transport (automotive, aeronautics, railways), sports, textiles, and construction [1]. However, these materials also present significant environmental challenges due to the origin of the raw materials required for their production and related end-of-life aspects. Most plastics are derived from fossil resources and are inevitably contributing to greenhouse gas emissions increase. Minimizing the ecological impact of plastics requires improved materials end-of-life management, primarily through efficient recycling [2] and the development of new materials from renewable resources, especially if integrated into circular economies [3].

Additionally, the durability of performance-enhancing additives used in plastics is crucial for their sustainable application. This is especially true for flame-retardant (FR) additives, which are incorporated to improve fire resistance and ensure that fire safety standards and technical requirements are met for each specific application [4].

Flame-retardant additives are designed to prevent polymer ignition, reduce heat release during combustion, and limit flame propagation by interfering with the physical or chemical processes involved in polymer combustion. Certain natural molecules and biopolymers can be effective in this application. For example, cellulose has an intrinsic ability to generate carbon during thermal degradation [5,6], although it requires enhancement to act effectively as a FR additive in polymers [7]. One of the most widely studied strategies

is the formation of a char layer on the surface of the burning material. This barrier helps slow heat diffusion into the material and reduces the release of volatiles into the gas phase, thereby lowering the fuel supply to the flame.

Developing such FRs from renewable resources is a promising approach to supporting low-carbon footprint materials. Cellulose, the most abundant organic raw material in nature, shows significant potential as a bio-sourced FR additive due to its high carbon content and hydroxyl groups, which promote natural charring and enable chemical modification.

However, using cellulose alone does not yield sufficient FR properties, primarily because of its low resistance to thermo-oxidative degradation. Enhancing its thermal stability often requires combination with other FR additives (additive approach) [8,9], or, more effectively, chemically grafting of reactive molecules—typically phosphorus-containing—onto the cellulose (reactive approach) [8,10–12].

The reactive route also remains the most interesting one from an environmental point of view, as it usually enables a higher level of bio-based content to be achieved in the final material. However, this is only true if the chemical modification process used to treat cellulose is eco-friendly and has a low environmental impact; otherwise, the environmental advantage of using cellulose will be lost.

Traditional methods used for modifying cellulose often rely on corrosive, concentrated phosphoric acid or derivatives; require purification steps that consume large amounts of organic solvents; and involve energy-intensive drying techniques.

The development of milder and more environmentally friendly cellulose phosphorylation processes has become essential. Lee et al. demonstrated cellulose phosphorylation via an enzyme-based process, utilizing hexokinase and adenosine-5'-triphosphate (ATP) in the presence of magnesium ions on cellulose nanofibers [13].

Over the past decade, mechanochemistry has emerged as a valuable tool, offering the possibility of achieving chemical transformations with minimal waste and often without any solvent. This method involves initiating chemical reactions through the grinding of reagents [14,15]. Ball milling is one of particularly efficient mechanochemical processes, as it is cost-effective, is straightforward to implement, and enables the processing of large volumes. The rapid rotation of the balls generates shear forces and increases the temperature of the reaction mixture [16,17]. However, solid-state mechanochemistry also presents certain limitations, particularly when it comes to modifying thermoplastic polymers. These materials tend to soften and melt under heat, causing solid-state milling to primarily result in the absorption and dissipation of mechanical energy. As a consequence, the process fails to generate the specific local temperature and pressure effects required for efficient modification [18].

Given the limited examples of large-scale processes available in the literature, synthetic mechanochemistry has often been overlooked due to a lack of evidence at the manufacturing scale. However, equipment enabling rapid scale-up is already available. For instance, we can cite the eccentric mills and horizontal high-energy ball mills by Simoloyer® (<https://www.siebertechnik-tema.com>) and Eccentric Mill (<https://www.continuumtech.ca>, accessed on 10 December 2024), which can process up to 200 L and 1522 L, respectively. Nonetheless, it must be acknowledged that scaling up is far from a linear extrapolation of small-scale research; it requires more than simply employing larger equipment [19].

Recently, mechanochemical phosphorylation has been used to modify cellulose nanofibers, aimed at producing paper with enhanced fire resistance [20,21]. In both studies, phosphorylation was successfully achieved using phosphorus pentoxide (P_2O_5) in a ball mill. P_2O_5 was preferred because it did not produce any by-product, unlike chlorinated phosphorus agents for example, which release HCl during their grafting. However, the presence of P_2O_5 in contact with cellulose can also impact it and cause some structural modifications, which should be carefully avoided. In fact, P_2O_5 can be mix-milled with cellulose for breaking its crystalline structure and increasing glucose yield during the chemical hydrolysis process [22].

In our study, we developed a phosphorylated cellulose (Cel-P) from microcrystalline cellulose by ball-milling mechanochemistry. The Cel-P product was purified by several washing/centrifugation steps in water and characterized by thermogravimetric analysis (TGA), Fourier transform infrared spectroscopy (FTIR), inductively coupled plasma (ICP) analysis, and scanning electron microscopy–energy dispersive X-ray analysis (SEM-EDX). Unmodified cellulose (Cel) and phosphorylated cellulose (Cel-P) were finally blended into PP at 30 wt.% by melt processing. The effect of their incorporation on the thermal and flame retardant properties of PP composites was studied using TGA, cone calorimetry, and smoke opacity tests.

2. Results and Discussion

2.1. Cellulose Modification

2.1.1. Structural Characterization

The treatment of cellulose with P_2O_5 in the ball mill was designed to graft phosphoric acid onto the surface of microcrystalline cellulose microparticles (see experimental). ICP analysis of Cel-P indicated a phosphorus content of 4.15 wt.%, indicating that approximately 33% of the phosphorus initially introduced into the blend was successfully grafted. The phosphorus grafting rate achieved through mechanochemistry appeared lower compared to the relative content obtained via a more conventional solvent-based process. For instance, using phosphonic acid and urea, the P content reached approximately 16.5 wt.% [8], while employing phosphoric acid and aqueous ammonia resulted in a P content of 22.51 wt.% as determined by X-ray photoelectron spectroscopy [12]. This difference was most likely due to the specificity of the phosphorus-grafting mechanisms. In the ball-mill process, grafting occurs mainly on the surface of the cellulose microparticles, whereas in the protocol using urea and phosphoric acid, cellulose is solubilized, allowing functionalization to occur directly on unfolded cellulosic molecular chains. However, it is important to highlight that the phosphorus content achieved in our study was higher than what has been reported for other solvent-based modification reactions. For instance, in a study by Yuan et al. on the modification of microcrystalline cellulose using phytic acid, a maximum phosphorus content of only 0.630 wt.% was achieved [23].

Quantifying the P content by ICP enables us to evaluate the average number of substituent groups attached per monomer unit, known as the degree of substitution (DS). DS of cellulose by grafted phosphorous groups can be estimated using the empirical formula [24].

$$DS = \frac{M_{\text{cellulose}} \times P\%}{100 \times M(P) - \Delta M \times P\%} \quad (1)$$

$M_{\text{cellulose}}$ is the molecular weight of cellulose D-glucose monomer unit (162 g/mol), P% is the weight % of phosphorus in modified cellulose (4.15 wt.%), $M(P)$ is the molar mass of phosphorus (31 g/mol), and ΔM is the molar mass of substituent—molar mass of leaving group ($M(H_3PO_4) - M(H) = 97$ g/mol).

According to this calculation, a degree of substitution of 0.25 was obtained. In comparison, phosphorylation cellulose with phosphonic acid and urea as catalysts at 150 °C for 2 h yielded a DS of 1.3 [8].

The analysis of phosphorus content in Cel-P was complemented by an assessment of phosphorus localization on its surface. To this end, SEM-EDX analyses were carried out for mapping the atomic density of P. Figure 1 clearly evidences the distribution of P along the entire surface of modified cellulose (Cel-P), demonstrating the effectiveness of the process used to functionalize the cellulose surface.

Despite confirmation of phosphoric acid grafting on the cellulose surface by both SEM-EDX and ICP, FTIR spectra (Figure 2) showed no fundamental difference between Cel and Cel-P. A shoulder at around 850 cm^{-1} could be assigned to the new characteristic C-O-P bonds. This slight difference was mainly due to the low grafting rate of phosphoric acid, with a DS of only 0.25.

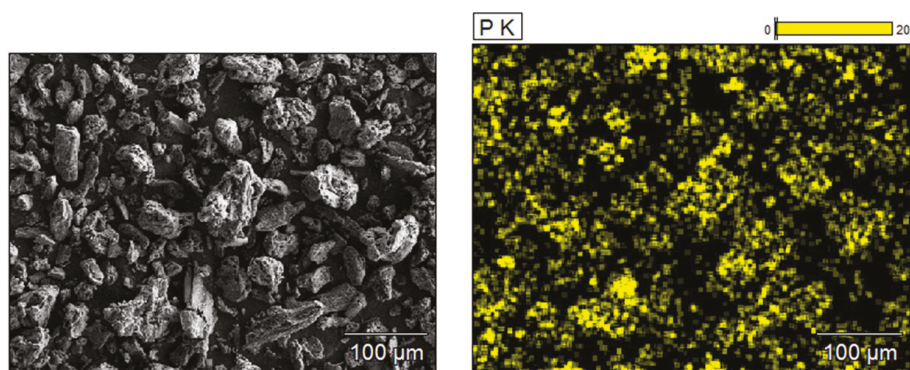


Figure 1. SEM-EDX micrographs showing the map of distribution of P on the surface of Cel-P. SEM image of Cel-P particles (left) and map of distribution of P on the surface (right).

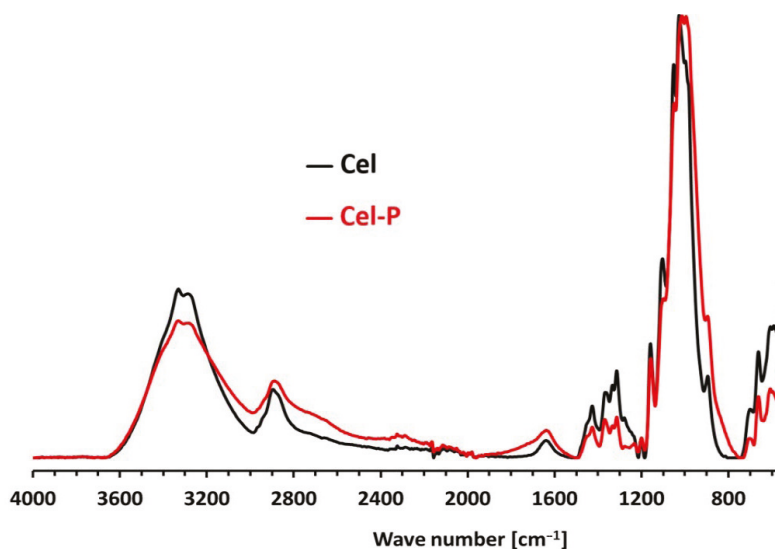


Figure 2. FTIR spectra of Cel and Cel-P.

2.1.2. Thermogravimetric Degradation

In order to assess the effect of phosphorylation on cellulose thermal stability, TGA was performed under air and N_2 on both Cel and Cel-P. Weight loss curves vs. temperature and data are represented in Figure 3 and Table 1, respectively. The degradation profiles of the two celluloses are highly dependent on the nature of the atmosphere used. Under N_2 , both samples underwent thermal degradation in a single step, whereas in air, the DTG curves displayed two distinct decomposition peaks. We can also note a slight mass loss below 100 °C, corresponding to a minor weight reduction (<5 wt.%), which is attributed to the evaporation of water adsorbed on the surface of both cellulose samples in both atmospheres.

Modification of cellulose with P_2O_5 in the ball mill induces a change in its thermogravimetric degradation pathway. As can be seen from the thermograms, the Cel exhibited a better thermal stability than the modified one in both atmospheric conditions. In fact, the temperature at which unmodified cellulose displayed 20 wt.% weight loss was around 305 °C under air and 328 °C under N_2 , whereas it was only 215 °C for phosphorylated cellulose, whatever the nature of the gas. Moreover, the degradation of Cel-P began at around 160 °C, which limited the range of polymers in which it can be melt-processed. In our case, the composites were processed at 180 °C, meaning that a slight degradation of Cel-P during mixing cannot be entirely ruled out.

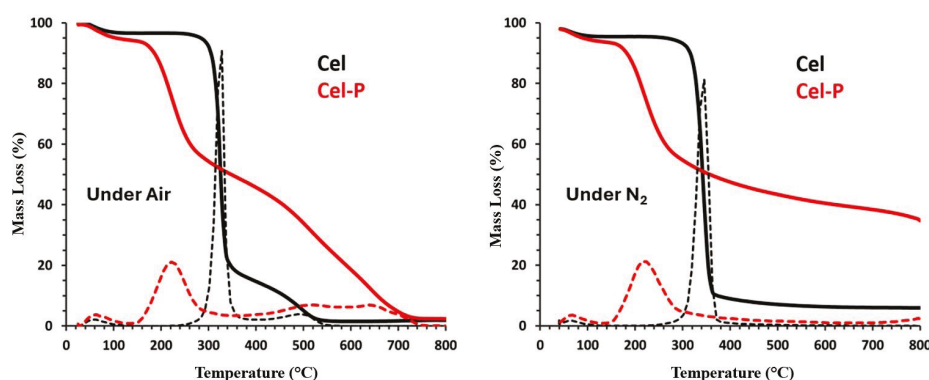


Figure 3. TGA (continuous curves) and DTG (dotted curves) of Cel and Cel-P, under air and under N₂, at 10 °C/min. TGA (continuous curves) and TGA.

Table 1. TGA results for Cel, Cel-P, PP, and related composites, based on thermograms obtained under air and nitrogen, with heating rate of 10 °C.min^{−1}.

Composition	Under Air				Under N ₂			
	T _{-20 wt.%} (°C)	T _{-40 wt.%} (°C)	T _{degrad} (°C)	Residue (%)	T _{-20 wt.%} (°C)	T _{-40 wt.%} (°C)	T _{degrad} (°C)	Residue (%)
Cel	305	320	320; 500	0	328	336	345	6
Cel-P	215	276	222; 515; 645	2	215	267	224	35
PP	440	450	455	0				
PP+30-Cel	344	445	330; 460	5				
PP+30-Cel-P	440	455	218; 463	13				

The low thermal stability of Cel-P is attributed to the presence of phosphoric-acid-grafted groups, which was already reported to catalyze cellulose dehydration, internal rearrangement reaction, and the formation of dehydrated cyclic char structures [25,26]. These effects account for the improved thermal stability of the residue observed above 400 °C. This enhancement is particularly advantageous as it provides the modified cellulose with effective flame-retardant properties. During combustion, a protective char layer forms, acting as a barrier that reduces the release of flammable volatiles into the gas phase and minimizes heat transfer through the material. In fact, even if Cel started to degrade later, it decomposed very rapidly to reach completion at 550 °C under air, while only 6% remained at 400 °C under N₂. In contrast, the amount of residue formed during thermal decomposition of Cel-P was significantly greater. Under air, the second degradation stage, corresponding to the degradation of the residue, occurred more slowly, and the amounts of residue at 340 °C and 450 °C were about 50% and 40%, respectively, for Cel-P, and only 20% and 10%, respectively, in the case of unmodified cellulose. The phenomenon was even more pronounced under air, with Cel degrading almost completely (6%) at 800 °C, while a residue of 35% remained with Cel-P.

Phosphorylation of cellulose induced a fundamental change in its thermogravimetric behavior. On the one hand, the presence of phosphoric acid units induced premature thermal degradation from around 215 °C, and on the other, it led to an improvement in its capacity to form chars in greater quantities and with better thermal resistance.

2.2. PP Composite Properties

2.2.1. Morphology and CEL-P Dispersion

Cel and Cel-P were incorporated into PP at 30 wt.%. Ensuring proper dispersion of the Cel-P particles within the polymer matrix was essential, as poor dispersion could hinder the FR effect of the additive, thereby compromising the composite final performance. SEM micrographs, acquired in chemical contrast mode, highlighting heavier elements such

as oxygen and phosphorus (appearing brighter), are shown in Figure 4. These images clearly demonstrate that (1) ball milling did not impact the particle size, as the particle sizes of Cel and Cel-P in both composites remained comparable, and (2) both Cel and Cel-P particles were well-dispersed throughout the matrix. This finding is further supported by the SEM-EDX micrographs (Figure 5), revealing a good distribution of oxygen and phosphorus elements, characteristic for Cel-P.

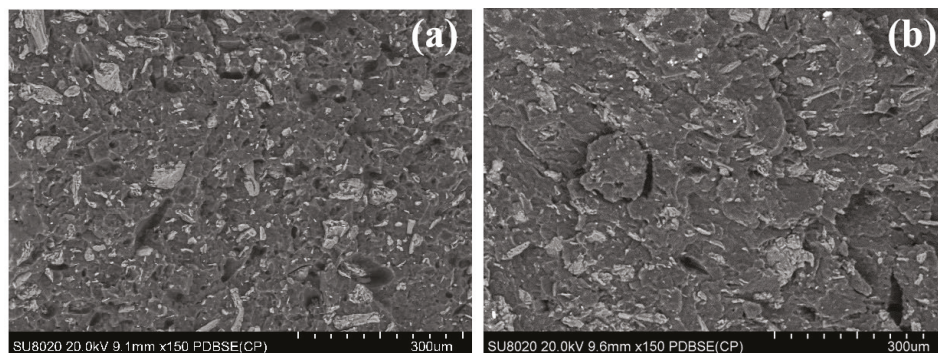


Figure 4. SEM micrographs of cryo-fractured surface of (a) PP+30-Cel and (b) PP+30-Cel-P composite, acquired in chemical contrast mode.

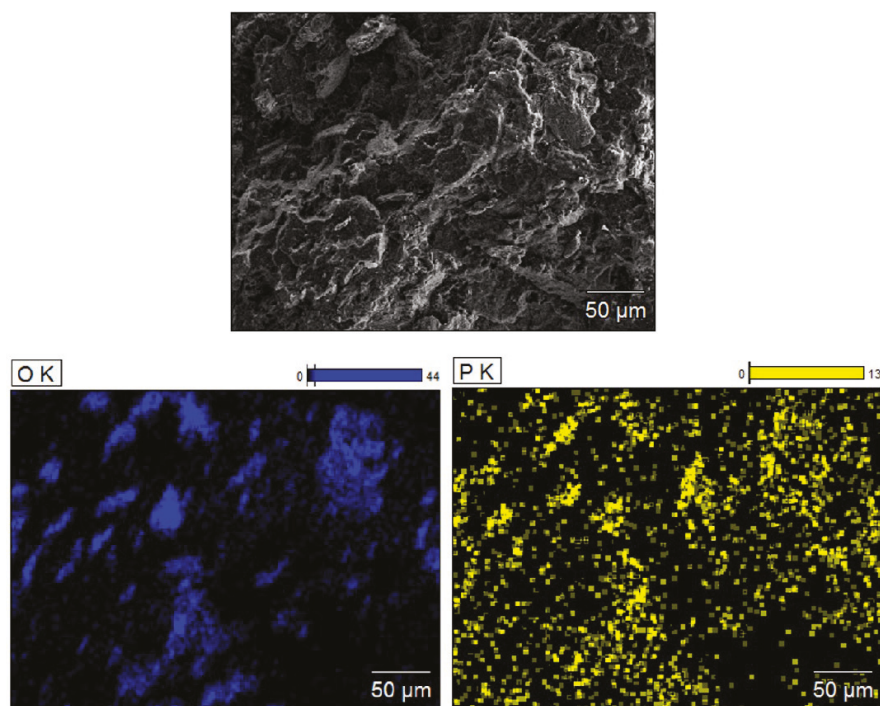


Figure 5. SEM (Top) and SEM-EDX (down) micrographs of the cryo-fractured surface of PP+30-Cel-P composite, showing the dispersion of oxygen (on the left) and phosphorus elements (right).

2.2.2. Thermogravimetric Properties

The incorporation of cellulose, regardless of its chemical functionalization, significantly altered the thermogravimetric behavior of PP (Figure 6). While neat PP degraded completely in a single step, the degradation of PP–cellulose composites occurred in two stages, with the process initiating at lower temperatures. The first stage likely corresponded to the degradation of cellulose and its derivatives, while the second stage can be attributed to the degradation of PP and the residue formation. The temperature at which thermal degradation begins was therefore dictated by the thermal degradation characteristics of the cellulose used. The initial degradation stage occurred at a lower temperature with Cel-P (218 °C) than with unmodified cellulose (330 °C), closely aligning with the previously

described thermogravimetric behavior of the two types of cellulose (Figure 3). However, the degradation rate was slower in the presence of Cel-P, as 20% and 40% mass losses occurred at higher temperatures with Cel-P. Specifically, $T_{-20\%}$ and $T_{-40\%}$ were at 440 °C and 455 °C for PP+30-Cel-P, compared to only 344 °C and 445 °C for PP with unmodified cellulose. This result aligns well with TGA analyses of the two celluloses alone (Cel and Cel-P), highlighting a slowing in degradation rate after the initial stage due to the increased carbonization potential of Cel-P. This same phenomenon also accounted for the enhanced thermal stability of the PP+30-Cel-P composite, which produced a higher amount of residue at the end of the test, reaching 13% at 800 °C, compared to only 5% with unmodified cellulose. Even a small content in phosphorus grafted onto the cellulose significantly improved its thermogravimetric behavior and that of the composite.

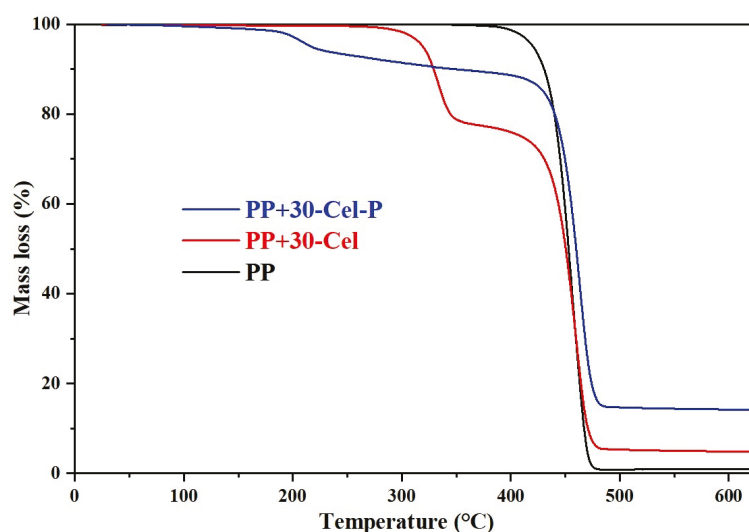


Figure 6. TGA thermograms of neat PP and composites containing 30 wt.% Cel and Cel-P, under air at 10 °C.min^{−1}.

2.2.3. Cone Calorimetry

The mass loss cone calorimeter (MLC) test was used for evaluating the effect of cellulose chemical modification on the fire behavior of PP composites. MLC provides the heat release rate (HRR) curve and allows the measurement of various combustion-related parameters, including time to ignition (TTI), peak of heat release rate (pHRR), total heat release (THR), and mass loss during combustion.

The HRR curves for PP and its composites containing either Cel or Cel-P at 30 wt.% are compared in Figure 7. The key parameters of the fire retardancy of the PP composite values were extracted and are provided in Table 2. The HRR vs. time curve recorded when testing PP alone shows that this polymer burned quickly and completely, with an ignition at 60 s accompanied by a high pHRR of 725 kW.m^{−2} and no residue formation (Figure 8).

Table 2. Results of the mass loss cone calorimeter test at 35 kW.m^{−2}.

Composition	TTI (s)	pHRR (kW.m ^{−2})	THR (MJ.m ^{−2})	pHRR Change (%)
PP	60	725	74	----
PP+30-Cel	48	774	79	+7
PP+30-Cel-P	34	514	62	−30

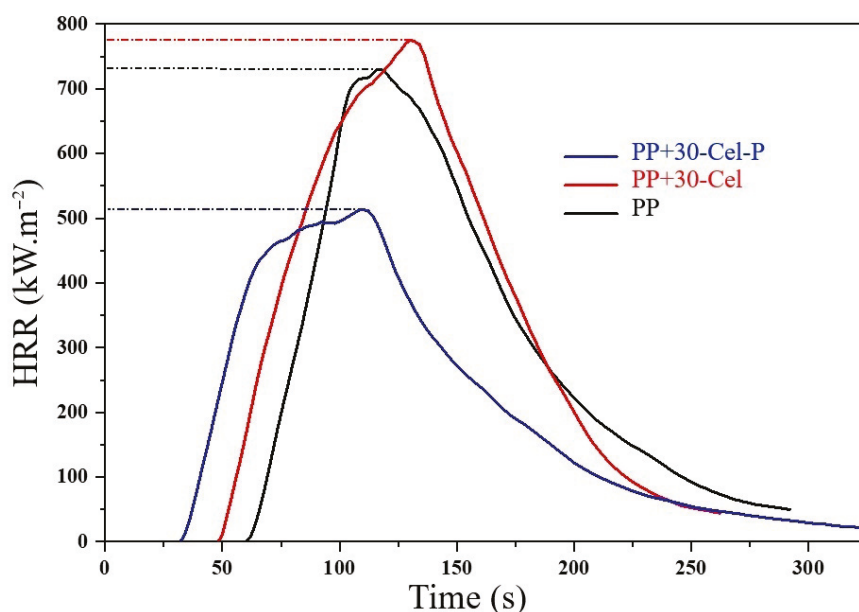


Figure 7. HRR curves of neat PP and PP composites, obtained by the mass loss cone calorimeter test at 35 kW.m^{-2} .

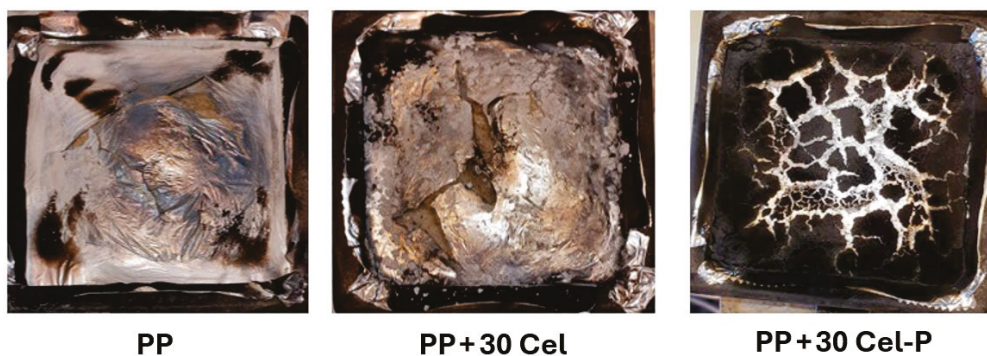


Figure 8. Images of char residues after MLC test of PP and cellulose-based composites.

It is also worth mentioning that the incorporation of Cel resulted in a reduction of composite resistance to ignition down to 50 s and to any enhancement in pHRR that remained high (774 kW.m^{-2}), both due to the combustible nature of cellulose. Cellulose alone, although capable of forming a residue during pyrolytic degradation (Figure 3), did not provide any protection for PP during combustion. The use of phosphorylated cellulose is recommended for this type of application, as it forms a more robust char resistant to thermo-oxidative degradation, as already demonstrated by TGA for both Cel-P (Figure 3) and PP+30-Cel-P composite (Figure 6).

Replacing Cel with Cel-P effectively reduced the pHRR down to 514 kW.m^{-2} , achieving a 30% reduction. This improvement was attributed to the formation of a protective char layer during combustion, which limited the release of combustible volatiles into the gas phase. However, this residue decomposed almost entirely by the end of the test, leaving only small fragments as remnants of the combustion process (Figure 8). The use of phosphorylated cellulose also brought about an additional improvement by reducing the total heat release (THR) (Table 2). When unmodified cellulose was used, THR increased from 74.1 MJ.m^{-2} for PP up to 79 MJ.m^{-2} for PP+30-Cel, but decreased to 62 MJ.m^{-2} for the composite containing Cel-P. Despite these improvements, an even more pronounced reduction in the ignition resistance of the composite was observed with Cel-P, as shown by a decrease in the TTI to 34 s. This behavior is linked to the reduced thermal stability of phosphorylated

cellulose and corresponding PP composite, as indicated by TGA, due to the catalytic effect of the phosphoric acid groups.

The use of Cel-P provided notable improvements in the fire behavior of PP but reduced its ignition resistance. Combinations with other biobased FR, such as phosphorylated lignin [27], could help mitigating this negative effect and even enhancing performance.

2.2.4. Smoke Density

The effect of incorporating Cel and Cel-P on the density of smoke during combustion of PP composites was evaluated in a smoke chamber following ASTM D2843 [28]. In fact, it is very important to consider this parameter that also represents a critical element of fire safety. Curves of light absorptivity vs. time are shown in Figure 9.

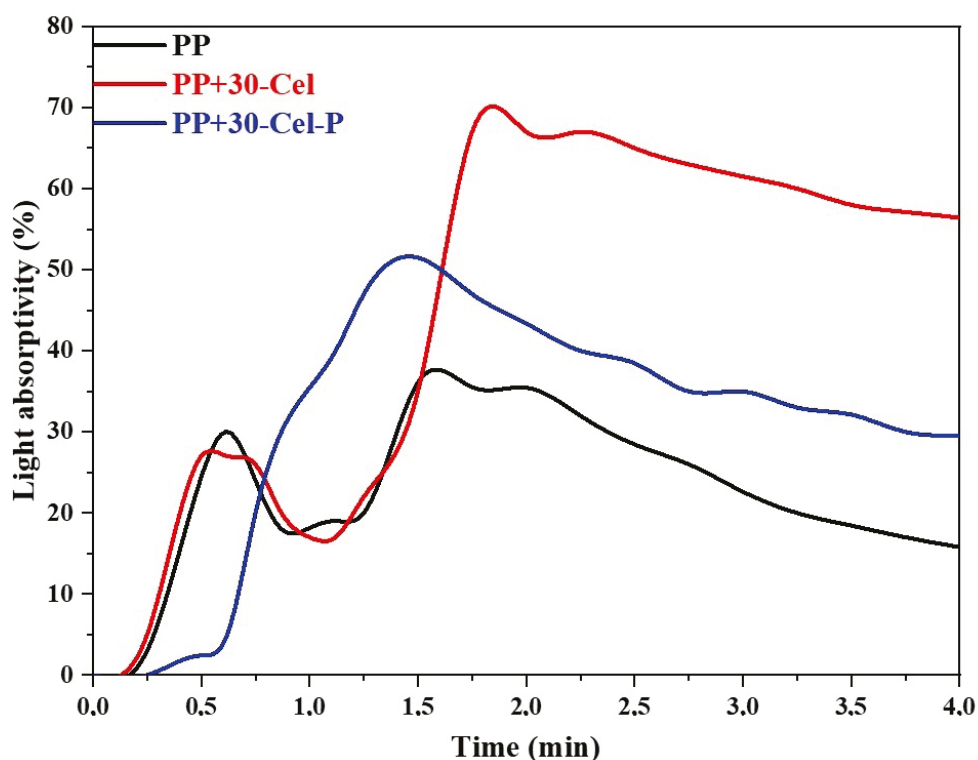


Figure 9. Light absorptivity curves of neat PP, PP+30-Cel, and PP+30-Cel-P composites.

The combustion behavior of the three materials differed significantly. PP alone produced the least smoke, which can be attributed to three main factors: (1) PP combustion is not impeded by the presence of FR additives, allowing it to proceed toward complete combustion; (2) PP ignites quickly, leading to combustion of the fumes in the chamber and thus the reduction of their amount, which accounts for the initial peak observed between 0.5 and 0.75 min; and (3) PP tends to drip, meaning that a substantial portion remained unburned during the test, causing the secondary peak after 1.5 min.

In the case of the PP+30-Cel composite, ignition of the material led to the appearance of the first peak, as in the case of PP, except that the absence of droplets led to further smoke production, more so than in the case of PP. In addition, the contribution of cellulose has to be added, which also contributes to smoke production, reaching very high levels (70%).

On the other hand, composites containing Cel-P showed a totally different behavior. The absence of the first peak resulted from the absence of material ignition. When continuously exposed to flame, the material neither ignited nor dripped. The absence of ignition, combined with the formation of a char, means that the amount of smoke continued to increase until it reached around 50%, then decreased slightly due to the sporadic appearance of a few small flames and the evacuation of a little smoke from the bottom of the apparatus.

In summary, the results of these tests highlight a superior effect of the PP+30-Cel-P composite, despite its tendency to produce denser smoke as compared to neat PP. While the smoke opacity was higher than that of PP, it remained lower than that observed for PP blended with unmodified cellulose. Notably, this increased opacity was mainly due to the absence of droplet formation. In contrast, the lower smoke level observed in neat PP resulted from two adverse effects: gas ignition and droplet formation. Furthermore, the presence of phosphorus-based FR, specifically Cel-P in our case, aims to disrupt the fire triangle and hinder combustion, leading to incomplete combustion that generates more smoke than when the polymer burns alone.

3. Materials and Methods

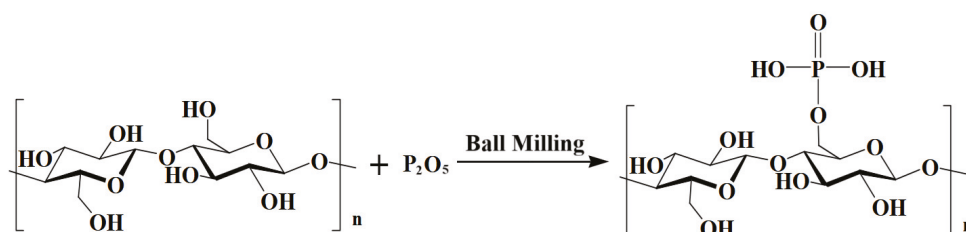
3.1. Materials

Polypropylene (PP) used in this study is a high-impact-modified copolymer (PP 402 CB12) adapted for injection-molding applications, provided by INEOS Olefins & Polymer Europe (Cologne, Germany). This grade is characterized by a melt flow rate (230 °C, 2.16 kg) = 12 g/10 min, tensile strength at yield = 25 MPa, and Izod impact strength = 6 kJ.m⁻² and 10 kJ.m⁻² (at −20 °C and +23 °C, respectively). Microcrystalline cellulose and phosphorus pentoxide (P₂O₅, purity 99%) were purchased from Sigma Aldrich (Burlington, MA, USA).

3.2. Preparation of Phosphorylated Cellulose

Cellulose was chemically modified with P₂O₅ through ball milling in a PM 400 planetary mill (Retsch, Haan, Germany). A mixture of 20 g microcrystalline cellulose and 26 g P₂O₅ was introduced in a 500 mL stainless steel jar along with 60 stainless steel balls and processed at room temperature and 200 rpm. The milling was set for one hour, with a 30 sec pause every 10 min. We set the ball-milling time to 1 h to allow sufficient time for the grafting reaction to occur while minimizing the risk of cellulose degradation during the process. Indeed, Liu et al. [22] demonstrated that the degree of polymerization (DP) of cellulose decreases more rapidly during ball milling in the presence of P₂O₅, with a significant drop after 1 h of treatment at 500 rpm. Cellulose degradation during ball milling leads to the formation of water-soluble oligomers.

After milling, the resulting Cel-P mixture was exposed to air until slightly pasty, then rinsed with cold deionized water to remove any unreacted phosphorus and eventual cellulosic water-soluble oligomers, which dissolve in water. The mixture was then washed/centrifuged 4 times at 4 °C at 10,000 rpm for 10 min using a refrigerated benchtop centrifuge Sigma 6-16KS) from Sigma Laborzentrifugen GmbH (Osterode am Harz, Germany). Finally, the recovered powder, i.e., phosphorylated cellulose, was dried in an oven at 40 °C. Scheme 1 illustrates the possible reactions during ball milling between cellulose and P₂O₅.



Scheme 1. Schematic representation of the reaction between cellulose and P₂O₅.

3.3. Composite Preparation by Melt Compounding

The composite preparation was performed using a Brabender bench-scale kneader (Brabender GmbH & Co. KG, Duisburg, Germany) at 180 °C, with an initial mixing phase of 3 min at 30 rpm, followed by 7 min at 90 rpm. Both PP and additives were pre-dried overnight in an oven at 60 °C. The 100 × 100 × 3 mm³ and 50 × 50 × 3 mm³ plates for

cone calorimetry and smoke density measurements, respectively, were molded using an Agila PE20 hydraulic compression press (Menen, Belgium) at 180 °C. The sample was first placed on the heated section for 3 min, then pressed for 1.5 min at 50 bar, followed by three degassing steps, then pressed again for 3 min at 150 bar, before being transferred to the cold section for pressing over 5 min.

3.4. Characterization and Testing

Inductively coupled plasma (ICP) analysis was used for the determination of phosphorus content in the modified cellulose and was performed with an Optima 7300dV ICP-OES instrument from Perkin Elmer (Waltham, MA, USA). Prior to ICP analysis, the sample was digested using nitric acid. The content of phosphorus was determined using calibration curves obtained from ICP analyses of standard solutions at different concentrations (5 and 10 ppm) in combination with unmodified cellulose.

FTIR spectra were recorded using a Bruker ALPHA II spectrometer (Bruker Optics, Ettlingen, Germany), in the wavenumber range from 4000 cm^{-1} to 400 cm^{-1} , with 2 cm^{-1} resolution and an accumulation of 32 scans.

Thermal stability of Cel, Cel-P, PP, and related composites was studied by TGA using TGA II equipment from Mettler Toledo (Greifensee, Switzerland). Approximately 5 mg of the sample was subjected to a temperature ramp from 30 °C to 800 °C at a heating rate of 10 °C.min⁻¹ under both air and N₂ in the case of cellulose samples and only under air for composites. The decomposition temperatures corresponding to 20% (T_{-20%}) and 40% (T_{-40%}) mass loss, onset thermal degradation, and residual weights at 800 °C were determined.

Scanning electron microscopy–energy dispersive X-ray analysis (SEM-EDX) was used to evaluate the dispersion of phosphorus in Cel-P, as well as through PP+30-Cel-P composite. Analysis was performed using an SU-8020 Hitachi instrument (Hitachi, Tokyo, Japan).

Fire behavior of PP and PP composites was evaluated through a mass loss cone (MLC) from Fire Testing Technology (East Grinstead, West Sussex, UK) following ISO 13927 standard (<https://www.iso.org/standard/82203.html> accessed on 19 November 2024). Samples (100 × 100 × 3 mm³) were exposed to an external heat flow of 35 kW/m². Key metrics, including peak heat release rate (pHRR), total heat release (THR), and time to ignition (TTI), were recorded to analyze fire performance.

Smoke density in the PP samples was measured according to ASTM D2843 (<https://www.astm.org/d2843-22.html> accessed on 19 November 2024). Specimens (50 × 50 × 3 mm³) were placed on a metal screen in a test chamber and exposed to an open flame under 276 kPa pressure, using a propane burner for 4 min. Light transmission loss through the smoke volume generated under these controlled burning conditions was used to quantify smoke density.

4. Conclusions

Ball milling mechanochemistry was used to develop a sustainable, biobased FR through the chemical modification of microcrystalline cellulose with P₂O₅ under mild process conditions. The reaction with P₂O₅ was carried out for 1 h, and the recovered powder was washed/centrifuged with deionized water. ICP analysis estimated the phosphorus content in Cel-P to 4.15 wt.%, corresponding to a degree of substitution of about 0.25%. This derivatization ensures a uniform presence of phosphorus on the surface of cellulosic microparticles, improving the thermal resistance of the char formed during cellulose degradation followed by TGA, even under an oxidizing atmosphere. However, the presence of phosphoric acid groups also induces premature cellulose thermal degradation.

The incorporation of Cel-P into PP improved the composite fire performance, as evidenced by a 30% reduction in the pHRR and a decrease in THR in the MLC test, although it unfortunately reduced the ignition time. In smoke density testing, the PP+30-Cel-P composite showed lower smoke opacity than PP+30-Cel but still higher than neat PP. This was attributed to the presence of phosphorylated cellulose, which acts as a phosphorus-based FR known to disrupt the combustion process, shifting it from complete to incomplete com-

bustion and thereby generating more smoke. Additionally, certain phenomena occurring during the test artificially reduce smoke production in PP and PP+30-Cel, such as droplet formation, which decreases the amount of burned material, and the presence of flames, which inhibits smoke formation.

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References

1. Cronin, J.; Hadley, C.; Skandalis, A. Plastic: A Passenger Market Icon. *Consum. Mark. Cult.* **2022**, *25*, 485–497. [CrossRef]
2. Evode, N.; Qamar, S.A.; Bilal, M.; Barceló, D.; Iqbal, H.M.N. Plastic Waste and Its Management Strategies for Environmental Sustainability. *Case Stud. Chem. Environ. Eng.* **2021**, *4*, 100142. [CrossRef]
3. Atiweh, G.; Mikhael, A.; Parrish, C.C.; Banoub, J.; Le, T.-A.T. Environmental Impact of Bioplastic Use: A Review. *Heliyon* **2021**, *7*, e07918. [CrossRef] [PubMed]
4. Specker, A. *Plastics and Flame Retardants: Balancing Safety and Sustainability*; World Resources Forum: St. Gallen, Switzerland, 2024; Available online: <https://www.wrforum.org/think-piece/plastics-and-flame-retardants-balancing-safety-and-sustainability/> (accessed on 19 November 2024).
5. Cho, J.; Davis, J.M.; Huber, G.W. The Intrinsic Kinetics and Heats of Reactions for Cellulose Pyrolysis and Char Formation. *ChemSusChem* **2010**, *3*, 1162–1165. [CrossRef]
6. Mamleev, V.; Bourbigot, S.; Yvon, J. Kinetic Analysis of the Thermal Decomposition of Cellulose: The Main Step of Mass Loss. *J. Anal. Appl. Pyrolysis* **2007**, *80*, 151–165. [CrossRef]
7. Khakalo, A.; Jaiswal, A.K.; Kumar, V.; Gestranis, M.; Kangas, H.; Tammelin, T. Production of High-Solid-Content Fire-Retardant Phosphorylated Cellulose Microfibrils. *ACS Sustain. Chem. Eng.* **2021**, *9*, 12365–12375. [CrossRef]
8. Costes, L.; Laoutid, F.; Khelifa, F.; Rose, G.; Brohez, S.; Delvosalle, C.; Dubois, P. Cellulose/Phosphorus Combinations for Sustainable Fire Retarded Polylactide. *Eur. Polym. J.* **2016**, *74*, 218–228. [CrossRef]
9. Yin, W.; Chen, L.; Lu, F.; Song, P.; Dai, J.; Meng, L. Mechanically Robust, Flame-Retardant Poly(Lactic Acid) Biocomposites via Combining Cellulose Nanofibers and Ammonium Polyphosphate. *ACS Omega* **2018**, *3*, 5615–5626. [CrossRef]
10. Dahiya, J.; Rana, S. Thermal Degradation and Morphological Studies on Cotton Cellulose Modified with Various Arylphosphorodichloridites. *Polym. Int.* **2004**, *53*, 995–1002. [CrossRef]
11. Aoki, D.; Nishio, Y. Phosphorylated Cellulose Propionate Derivatives as Thermoplastic Flame Resistant/Retardant Materials: Influence of Regioselective Phosphorylation on Their Thermal Degradation Behaviour. *Cellulose* **2010**, *17*, 963–976. [CrossRef]
12. Zhang, S.; Chen, H.; Zhang, Y.; Zhang, Y.; Kan, W.; Pan, M. Flame Retardancy of High-Density Polyethylene Composites with P,N-Doped Cellulose Fibrils. *Polymers* **2020**, *12*, 336. [CrossRef] [PubMed]
13. Božič, M.; Liu, P.; Mathew, A.P.; Kokol, V. Enzymatic Phosphorylation of Cellulose Nanofibers to New Highly-Ions Adsorbing, Flame-Retardant and Hydroxyapatite-Growth Induced Natural Nanoparticles. *Cellulose* **2014**, *21*, 2713–2726. [CrossRef]
14. Liu, X.; Li, Y.; Zeng, L.; Li, X.; Chen, N.; Bai, S.; He, H.; Wang, Q.; Zhang, C. A Review on Mechanochemistry: Approaching Advanced Energy Materials with Greener Force. *Adv. Mater.* **2022**, *34*, 2108327. [CrossRef]
15. Krusenbaum, A.; Grätz, S.; Tamir, T.; Borchardt, L.; Kim, J. The Mechanochemical Synthesis of Polymers. *Chem. Soc. Rev.* **2022**, *51*, 2873–2905. [CrossRef]
16. Burmeister, C.F.; Kwade, A. Process Engineering with Planetary Ball Mills. *Chem. Soc. Rev.* **2013**, *42*, 7660–7667. [CrossRef]
17. Zhang, T.; Kuga, S.; Wu, M.; Huang, Y. Chitin Nanofibril-Based Flame Retardant for Paper Application. *ACS Sustainable Chem. Eng.* **2020**, *8*, 12360–12365. [CrossRef]
18. Kuga, S.; Wu, M. Mechanochemistry of Cellulose. *Cellulose* **2019**, *26*, 215–225. [CrossRef]
19. Reynes, J.F.; Isoni, V.; García, F. Tinkering with Mechanochemical Tools for Scale Up. *Angew. Chem.* **2023**, *135*, e202300819. [CrossRef]

20. Zhang, T.; Wu, M.; Kuga, S.; Ewulonu, C.M.; Huang, Y. Cellulose Nanofibril-Based Flame Retardant and Its Application to Paper. *ACS Sustain. Chem. Eng.* **2020**, *8*, 10222–10229. [CrossRef]
21. Fiss, B.G.; Hatherly, L.; Stein, R.S.; Friščić, T.; Moores, A. Mechanochemical Phosphorylation of Polymers and Synthesis of Flame-Retardant Cellulose Nanocrystals. *ACS Sustain. Chem. Eng.* **2019**, *7*, 7951–7959. [CrossRef]
22. Liu, X.; Yan, P.; Xu, Z.; Zhang, Z.C. The Effect of Mix-Milling with P₂O₅ on Cellulose Physicochemical Properties Responsible for Increased Glucose Yield. *Carbohydr. Polym.* **2021**, *258*, 117652. [CrossRef] [PubMed]
23. Yuan, H.-B.; Tang, R.-C.; Yu, C.-B. Flame Retardant Functionalization of Microcrystalline Cellulose by Phosphorylation Reaction with Phytic Acid. *Int. J. Mol. Sci.* **2021**, *22*, 9631. [CrossRef] [PubMed]
24. Klemm, D.; Philipp, B.; Heinze, T.; Hewinze, U.; Wagenknecht, W. *Comprehensive Cellulose Chemistry, Volume 2: Functionalization of Cellulose*; Wiley: Hoboken, NJ, USA, 1998.
25. Kandola, B.K.; Horrocks, A.R.; Price, D.; Coleman, G.V. Flame-Retardant Treatments of Cellulose and Their Influence on the Mechanism of Cellulose Pyrolysis. *J. Macromol. Sci. Part C* **1996**, *36*, 721–794. [CrossRef]
26. Dobelev, G.; Rossinskaja, G.; Telysheva, G.; Meier, D.; Faix, O. Cellulose Dehydration and Depolymerization Reactions during Pyrolysis in the Presence of Phosphoric Acid. *J. Anal. Appl. Pyrolysis* **1999**, *49*, 307–317. [CrossRef]
27. Costes, L.; Laoutid, F.; Aguedo, M.; Richel, A.; Brohez, S.; Delvosalle, C.; Dubois, P. Phosphorus and Nitrogen Derivatization as Efficient Route for Improvement of Lignin Flame Retardant Action in PLA. *Eur. Polym. J.* **2016**, *84*, 652–667. [CrossRef]
28. ASTM D2843; Standard Test Method for Density of Smoke from the Burning or Decomposition of Plastics. ASTM International: West Conshohocken, PA, USA, 2022.

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Review

Tailoring Polymer Properties Through Lignin Addition: A Recent Perspective on Lignin-Derived Polymer Modifications

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Abstract: Lignin, an abundant and renewable biopolymer, has gained significant attention as a sustainable modifier and building block in polymeric materials. Recent advancements highlight its potential to tailor mechanical, thermal, and barrier properties of polymers while offering a greener alternative to petroleum-based additives. This review provides an updated perspective on the incorporation of lignin into various polymer matrices, focusing on lignin modification techniques, structure–property relationships, and emerging applications. Special emphasis is given to recent innovations in lignin functionalization and its role in developing high-performance, biodegradable, and recyclable materials such as polyurethanes, epoxy resins, phenol-formaldehyde resins, lignin-modified composites, and lignin-based films, coatings, elastomers, and adhesives. These lignin-based materials are gaining attention for potential applications in construction, automated industries, packaging, textiles, wastewater treatment, footwear, supporting goods, automobiles, printing rollers, sealants, and binders.

Keywords: lignin; polyurethane; epoxy resins; phenol-formaldehyde resin; PLA; films; additives

1. Introduction

Lignin is a complex organic biopolymer found in the plant cell walls, providing rigidity and resistance to decay. It is an abundant byproduct of the pulp and paper industry (~50 million tons of lignin), ethanol and textile industry (~30 million tons of lignin), and the agricultural sector [1,2]. However, less than 2% of lignin from these sources is currently utilized, with the remaining predominantly burned as low-cost fuel, raising significant environmental concerns [3–5]. Therefore, converting lignin into value-added products is crucial for addressing future economic and ecological challenges. Lignin is mainly composed of monolignol units [*p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S)], interconnected through aryl ether or carbon–carbon bonds to form their three-dimensional (3D) network (Figure 1) [6]. The various functionalized aromatic groups present in lignin make it a promising candidate to replace petrochemicals. Additionally, lignin is naturally hydrophilic, and its rigid structure imparts unique properties to polymer matrices.

Due to its aromatic hydroxyl functionalities and abundant π – π conjugation, lignin offers biocompatibility, flame retardancy, UV resistance, antibacterial and antioxidant activities, thermal stability, and the ability to modify mechanical properties [7–9]. These characteristics have made lignin the focus of numerous studies and reviews, highlighting its versatility as a polymer additive. It has been successfully incorporated into lignin-derived polyurethanes (PUs), epoxy resins, phenol-formaldehyde resins, polyesters, polylactic acid (PLA), bio-based fillers, additives, films, coatings, stimuli-responsive, and biomedical

materials [10–22]. The incorporation of lignin in these materials offers a more sustainable and often higher-performing alternative to their petrochemical-based counterparts [23,24].

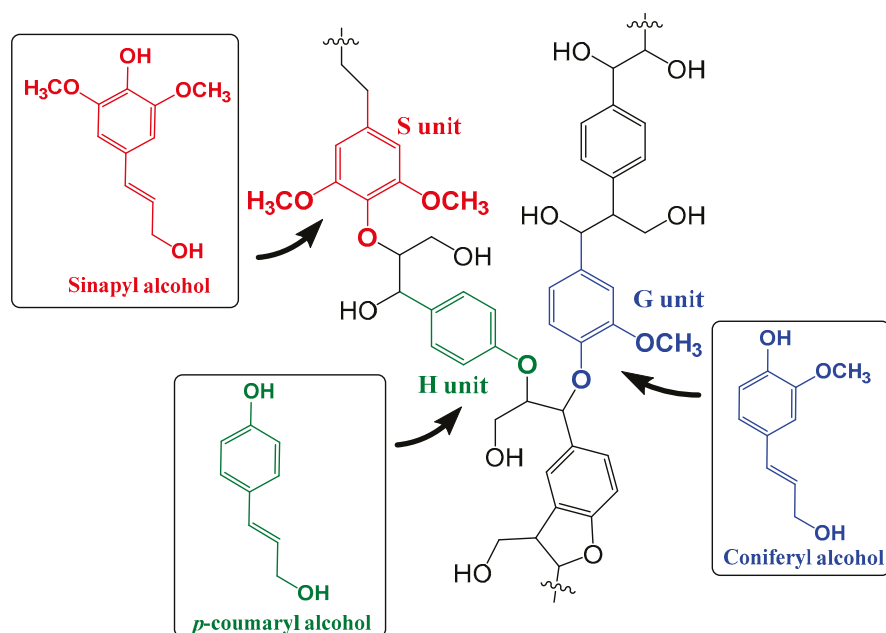


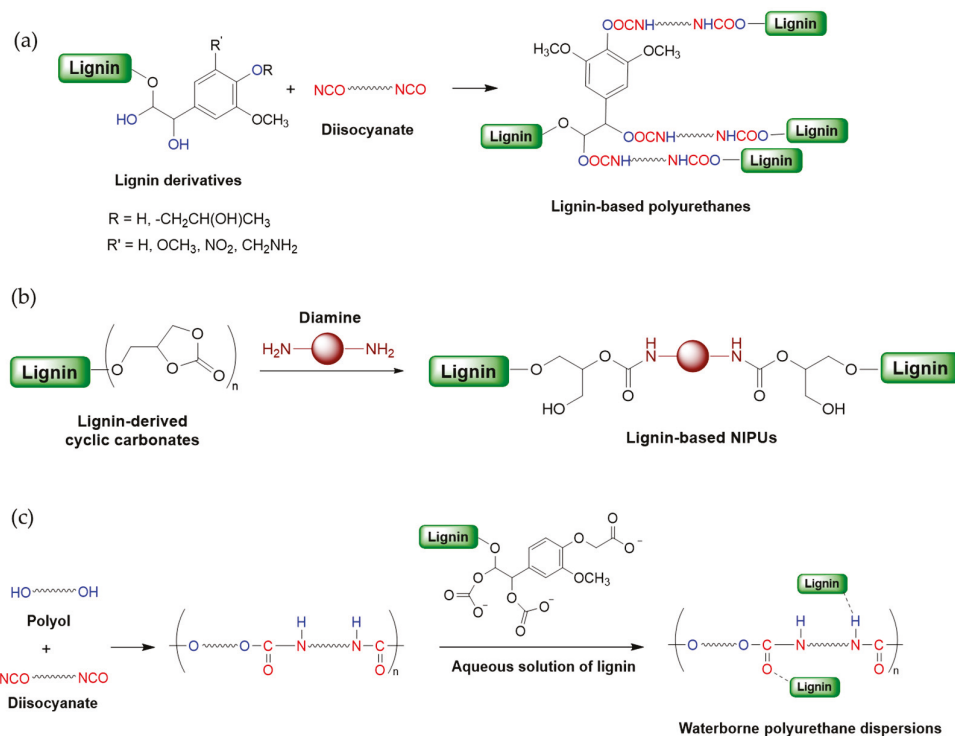
Figure 1. Schematic representation of the structure of lignin, composed of monolignol units.

Despite lignin's abundance, its heterogeneity, complexity, and structural variability challenge its comprehensive study and utilization. To address these issues, lignin can be extracted, depolymerized, or chemically and physically modified to produce smaller derivatives with enhanced functionalities [25–27]. Also, depending upon the extraction method, lignin can be categorized into different types such as kraft lignin, alkali lignin, soda lignin, hydrolyzed lignin, etc., and the lignin composition is different in each lignin type [28–30]. Lignin can be discussed at several levels of structural complexity, which must be carefully distinguished. “Whole lignin” refers to the unmodified, extracted material, typically containing a wide molecular weight distribution and a mixture of structural subunits (e.g., kraft, soda, organosolv lignin). “Lignin fractions” are obtained through physical or chemical separation processes that reduce polydispersity or isolate particular molecular weight windows. “Lignin-derived monomers and oligomers”, on the other hand, are breakdown products obtained by depolymerization techniques such as oxidative cleavage, hydrogenolysis, or solvolysis. These distinct forms vary not only in reactivity and solubility but also in their suitability for incorporation into polymer matrices. As such, clarity in terminology is critical when interpreting or comparing the literature on lignin-based polymers.

This review aims to classify and analyze current strategies for lignin incorporation into polymer matrices across diverse synthetic routes and applications, including polyurethanes (PUs), resins, PLA composites, and lignin-based films, coatings, and adhesives. Emphasis is placed on chemical modifications, structure–property relationships, and sustainability metrics. Moreover, lignin can be utilized in more applications, ranging from hydrogels or aerogels [31–36], capacitors, or lithium–sulfur batteries, [37–41], light-driven shape memory polymers [42,43], pH-sensitive lignin-based polymers, [44]. several nano lignin applications [45–49], and as an application in high-sulfur content materials [50–54], which will not be discussed in this review.

2. Lignin as a Precursor in Polyurethanes (PUs)

Polyurethane (PU) is one of the most versatile polymers, and it is used in different industries, including coating, adhesive, textile, foams, sealants, automotive, and aerospace [55–57]. PUs are synthesized through a step-growth addition polymerization process by reacting polyols (compounds containing multiple hydroxyl groups) with diisocyanates (compounds containing two isocyanate groups). The reaction typically involves the formation of urethane linkages. Traditional PU production relies on petroleum-based polyols, but the hydroxyl groups in lignin provide an opportunity for its utilization as a renewable polyol alternative, providing biodegradability, cost-effectiveness, and reduced environmental impact (Scheme 1a). Despite its potential, lignin's complex, irregular structure and low reactivity due to limited available hydroxyl groups hinder its direct use. To enhance its compatibility and reactivity with isocyanates, lignin is often chemically modified through hydroxy alkylation, phenolation, methylation, esterification, and amination methods to increase the number of accessible hydroxyl groups and improve solubility and dispersion within polymer matrices [58–60]. Such modifications significantly improve the performance and processability of lignin-based PUs (Table 1). This section will explore the most recent approaches to integrating unmodified and modified lignin into PU formulations, including synthesis pathways of lignin-based non-isocyanate and waterborne polyurethane (Scheme 1).



Scheme 1. General reaction scheme for lignin-based polyurethane formation. (a) Traditional isocyanate pathway from unmodified or modified lignin (b) Non-isocyanate pathway from cyclic carbonate functionalized lignin and amines (c) Waterborne polyurethane synthesis pathway from aqueous solution of lignin.

Table 1. Summary of the lignin-based polyurethanes.

Lignin Type	Lignin Treatment/ Modification Method	NCO Source	Application	Optimal Lignin wt. %	Reported Key Properties	Ref.
Pine alkali lignin	Purification	HDI	Coating	6.1%	Hardness: 260.1 MPa Elastic modulus: 4.7 GPa Good UV-blocking properties	[61]
Organosolv lignin	Dissolved in propylene carbonate	MDI [i/h ratio = 1]	Rigid and flexible foams	20%	95% shape recovery after cyclic compression	[62]
Industrial alkali lignin	Fractionation	Octamethylene diisocyanate [i/h ratio = 1]	Rigid foams	15%	Tensile strength: 0.9 MPa Elongation at break: >500%	[63]
Lignin from sawdust	Catalytic upstream biorefining	pMDI	Rigid foams	30%	Compressive strength: >0.25 MPa	[64]
Alkali lignin	Hydroxylation	TDI	Elastomers	22.2%	Tensile strength: 25.2 MPa Elongation at break: 500%	[65]
Hydrolyzed lignin	Acid-catalyzed phenolation	pMDI or HDI [i/h ratio = 0.95]	Films and adhesive	50%	Tensile strength: 47.6 MPa	[66]
Kraft lignin	Liquefaction	HDI [i/h ratio = 1]	Rigid foams	60%	Compressive stress: 86.80 kPa Compressive strain: 70% Thermal conductivity: 0.0401 W/mK	[67]
Enzymatic hydrolyzed lignin	Liquefaction	HDI	Rigid foams	NA	>99% oil–water separation Adsorption capacity: 98.2 mg/CuSO ₄ g	[68]
Lignin oligomers from sawdust	Reductive catalytic fractionation and oxypropylation	NA	NIPU films	17%	Tensile strength: 12.5 MPa Young’s modulus: 63 MPa	[69]
Black liquor lignin	Amination	NA	NIPU foam	NA	Higher hydroxyl number in amine-modified lignin	[70]
Enzymatic hydrolyzed lignin	Epoxidation	NA	NIPU film	40.36%	Tensile strength: 20.25 MPa Young’s modulus: 280 MPa Toughness: 5.51 MJ/m ³	[71]
Kraft lignin	Functionalization with APTES	NA	WPU films	0.5%	Tensile strength: 8.77 MPa Elongation at break: ~25%	[72]
Enzymatic hydrolyzed lignin	Solvent shifting	Isophorone diisocyanate (IPDI)	WPU emulsions	5%	Tensile strength: >25 MPa Young’s modulus: 322 MPa	[73]
Lignosulfonate	NA	IPDI	WPU composites	7%	Tensile strength: 5.57 MPa Antibacterial activity: 97.27% for <i>E. coli</i>	[74]

2.1. Unmodified Lignin-Based Polyurethanes

Chen et al. developed a lignin-based PU coating with anti-smudge and anti-UV properties in their study [61]. Lignin-based coatings (lig-PU-PDMS) were prepared using lignin as polyols, hexamethylene diisocyanate trimer (HDIT) as the crosslinking agent, and a small amount of monodimethylsiloxane (PDMS-OH) as the dewetting agent (Figure 2). The hardness (HIT) and elastic modulus (EIT) of lig-PU-PDMS coatings were measured by nanoindentation. When the PDMS-OH content increased from 0 to 5.0 wt.%, HIT decreased from 260.1 to 226.8 MPa, and EIT decreased from 4.7 to 4.1 GPa. After 400 h of UV aging, the HIT and EIT of lig-PU-PDMS (0 wt.%) increased by 16.9% and 16.1%, respectively, while those of lig-PU-PDMS (5.0 wt.%) increased by only 11.9% and 10.9%. Moreover, the coatings maintained good mechanical properties even after UV aging, with lignin protecting the PU from UV damage. The aromatic skeleton and conjugated structure of lignin provide the coatings with extraordinary UV-blocking capability and increase the strength of the PU coating. However, the study focused on a specific lignin type, indicating that further research may be necessary to adjust the process for other lignin sources.

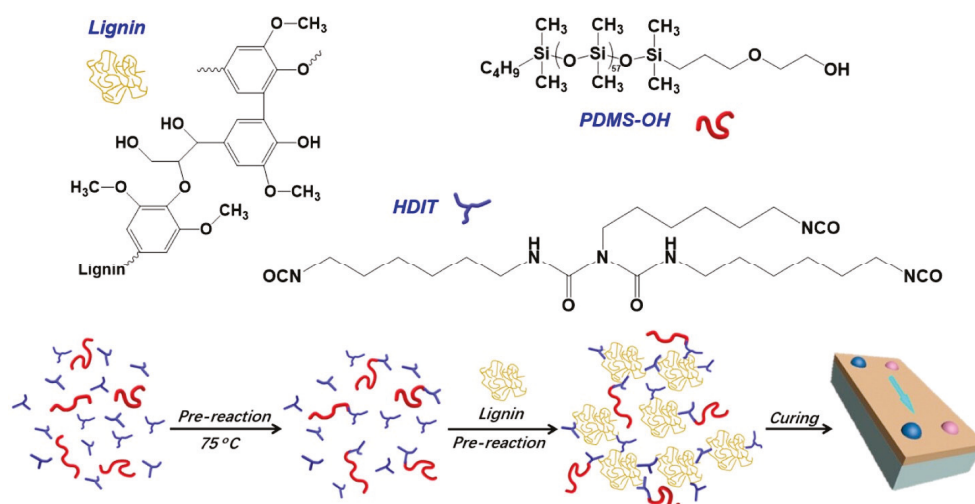


Figure 2. Preparation method for lignin-based PU coating (lig-PU-PDMS) [61]. Used with permission, copyright 2023 American Chemical Society.

In another study, Zhang and coworkers introduced a method to address the high viscosity of lignin, which limits its processability and application in lignin-based polyurethane foams (PUFs) [62]. Organosolv lignin was dissolved in green co-solvent (propylene carbonate) to reduce the viscosity and reacted with diphenylmethane-4,4'-diisocyanate (MDI) to form the lignin-based PUFs using a one-pot synthesis method (Figure 3). The lignin content varied from 20 to 80 wt.% and equal weights of propylene carbonate and lignin were used. Compared to mixtures without the co-solvent, those with the co-solvent exhibited lower viscosity, even at higher lignin content. Thermogravimetric analysis (TGA) showed increased thermal stability and higher char yield formation with lignin addition, attributed to its aromatic structure. Additionally, the lignin-based PUFs demonstrated enhanced hydrophobicity, with the water contact angle rising from 100 to 140 degrees as lignin content increased. Mechanical analysis indicated that, without the co-solvent, PUFs with low lignin content had slightly higher strength than the control PUF due to mechanical interlocking and hydrogen bonding between lignin and the matrix. However, higher lignin content reduced strength, as the brittle PU cells and non-uniform foam structure acted as rigid foams. In contrast, higher lignin content with the co-solvent resulted in greater

strength than the control PUF. The study successfully demonstrated the substitution of (up to 80 wt.%) petroleum polyol with unmodified lignin, preventing viscosity issues and maintaining favorable properties.

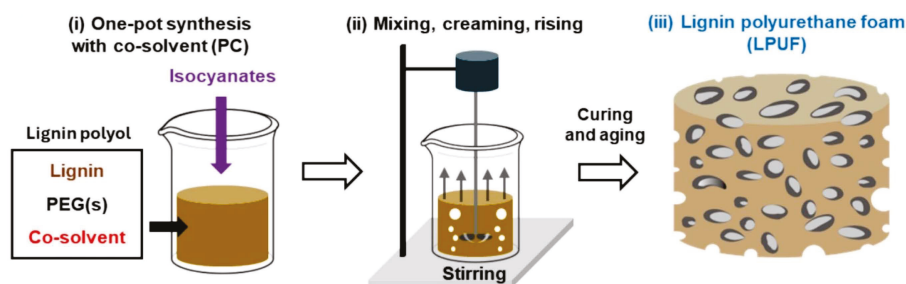


Figure 3. Illustration of the one-pot synthesis of lignin polyurethane foam using a co-solvent [62]. Used with permission, copyright 2024 Elsevier.

The inherent complexity and heterogeneity of lignin can lead to inconsistent performance in polyurethane applications when it is directly incorporated into the formulation without prior modification. To address this issue, Li et al. fractionated industrial alkali lignin using green solvents to study the effect of different molecular weight fractions on polyurethane foam (PUF) properties [63]. The dispersity and hydroxy content gradually decreased during fractionation, yielding lower molecular weight lignin in the initial steps. Fractionated lignin was used as a polyol source, with octamethylene diisocyanate as the isocyanate to synthesize PUFs (Figure 4). The impact of different lignin fractions was studied using 2 wt.% of lignin, and the substitution degree of lignin was evaluated using the lowest molecular weight fraction, which contained higher hydroxy group content. Lignin derivatives from low-molecular-weight fractions formed flexible PU, while the high molecular weight of lignin fractions formed rigid PU due to their high aromatic density. Lower molecular weight lignin improved mechanical strength, while higher molecular weight lignin enhanced thermal stability. When the substitution degree of lignin increased from 2% to 30%, elongation at break peaked at 834% with 5% substitution, then declined. Tensile strength increased with a 15% lignin substitution before decreasing with further additions.

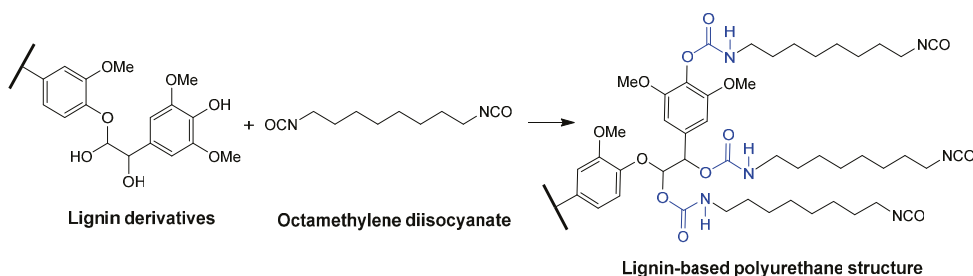


Figure 4. The reaction mechanism between lignin derivatives and octamethylene diisocyanate. Used with permission, copyright 2024 Elsevier.

In another study, lignin oligomers derived from poplar sawdust through catalytic upstream biorefining (CUB) were used as a partial polyol substitute for synthesizing rigid polyurethane foams (RPFs) [64]. The lignin oligomers were extracted from crude CUB oils produced with varying 2-propanol/water ratios (1/9, 3/7, 5/5, 7/3, and 9/1 *v/v*) in the pulping liquors. These oligomers, rich in hydroxyl groups, reacted with isocyanates to create the polyurethane networks. The chemical composition of the pulping liquor significantly influenced the properties of the lignin oligomers and, consequently, the physical characteristics of the RPFs. Increased substitution of lignin oligomers led to an increase in

apparent density but reduced compressive strength. Among all formulations, RPFs with 30% polyol substitution using lignin oligomers extracted with a 7/3 2-propanol/water ratio (R7/3) demonstrated the optimal balance, achieving the highest compressive strength and the lowest apparent density. Morphological analysis (Figure 5) revealed that these lignin oligomers, with their higher hydroxyl content, facilitated the formation of highly crosslinked nano-sized complexes, creating a stable polyurethane network that effectively supported the cellular foam structure.

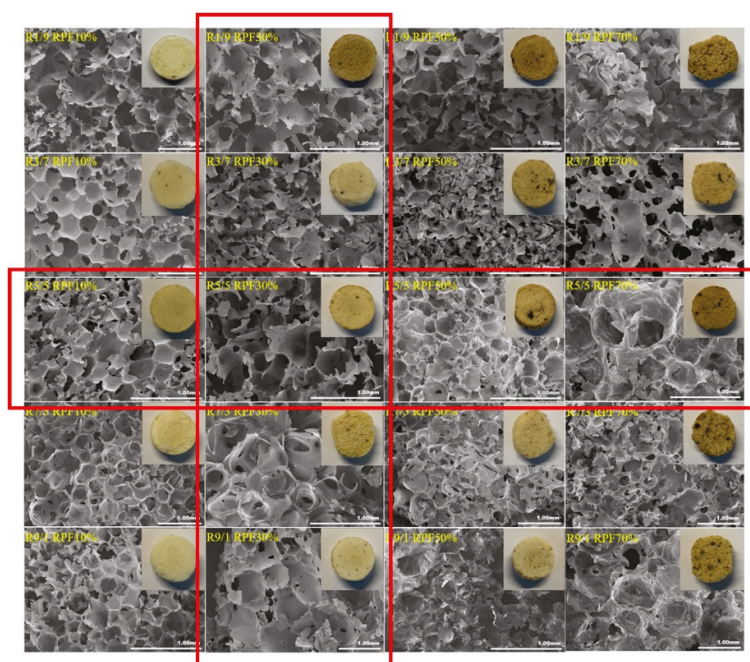


Figure 5. SEM images of RPFs synthesized using catalytic upstream biorefining (CUB) lignin oligomers as polyol substitutes [64]. Used with permission, copyright 2023 Elsevier.

2.2. Chemically Modified Lignin-Based Polyurethanes

Du et al. developed a lignin-based self-healing polyurethane elastomer (PUDA-L) by integrating hydroxylated-modified lignin, Diels–Alder (DA) bonds, and hydrogen bonds into the polymer matrix [65]. Hydroxylation modification of lignin increases its hydroxyl content, enhancing crosslinking density and improving mechanical properties. At the molecular level, hydroxylation increases the number of reactive $-OH$ groups on lignin, enhancing its ability to form urethane bonds with isocyanates. This leads to a denser crosslinked network, improving tensile strength and elasticity. The addition of DA diol as a chain extender promotes self-healing properties, while polycaprolactone (PCL) was selected as the soft segment due to its ability to enhance melting crystallization and self-healing efficiency (Figure 6). Figure 7 illustrates the self-healing mechanism of elastomers, which is driven by multiple molecular interactions. Lignin incorporation improves the thermal stability and rigidity of polyurethane, leading to higher glass transition (T_g) and energy storage moduli (E'). However, in DA-modified elastomers, the overall impact of lignin on thermal stability is less pronounced due to the strong crosslinking network. Mechanical testing showed that an optimal PCL–DA diol–TDI (toluene diisocyanate) ratio of 1:1:2.5 maximized tensile strength (25.2 MPa), while excessive or insufficient DA diol and higher lignin addition negatively affected mechanical performance. The optimized PUDA-L formulation achieved an elongation at a break of 500% and a self-healing efficiency of up to 100%, offering excellent durability, flexibility, and self-healing capabilities. The study provides valuable insights into the development of bio-based,

high-performance polyurethane elastomers for applications in innovative coatings, sensors, and flexible electronics.

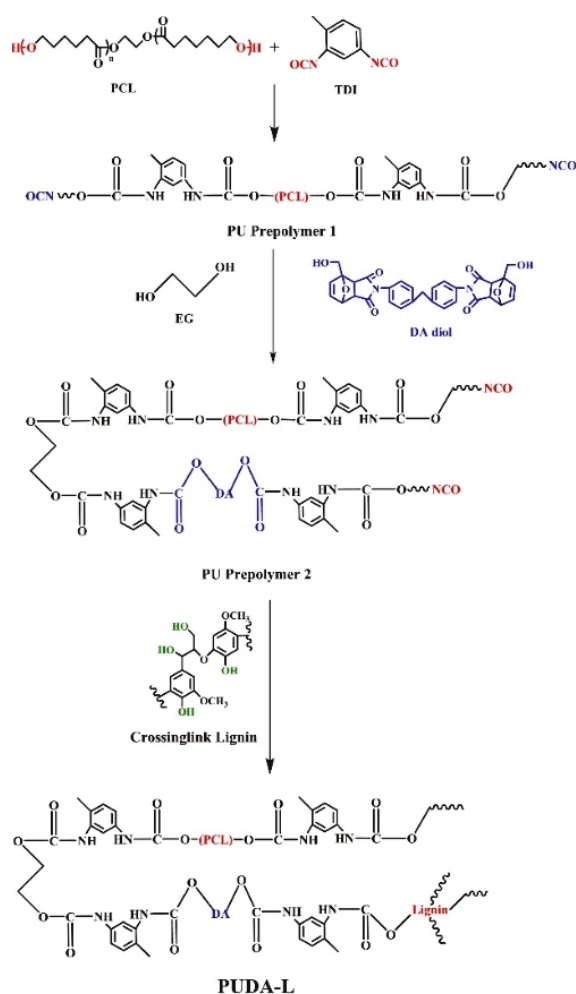


Figure 6. Preparation method of lignin self-healing polyurethane elastomer (PUDA-L) [65]. Used with permission, copyright 2023 Elsevier.

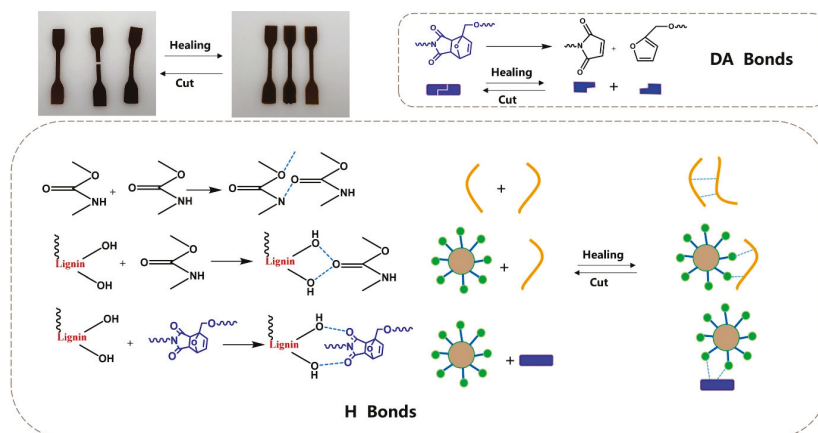


Figure 7. Self-healing mechanism of lignin-based self-healing polyurethane elastomer [65]. Used with permission, copyright 2023 Elsevier.

In another study, Falireas and coworkers employed an acid-catalyzed phenolation method (Figure 8) to modify lignin chemically, enhancing its reactive sites for polyurethane (PU) synthesis [66]. The acid-catalyzed phenolation increases the number of phenolic hydroxyl groups in lignin, enhancing its nucleophilicity and reactivity with isocyanate groups. This leads to more urethane bonds and higher crosslinking density in the polymer network. The enhanced mechanical and adhesive properties observed in pMDI-based systems can be attributed to strong interactions between the aromatic rings of phenolated lignin and pMDI, including π - π stacking and hydrogen bonding. This molecular interaction causes the superior performance of the phenolated lignin-based PU framework. Hydrolysis lignin (HL) was used as the lignin source for polymer preparation. A series of phenolated lignin-based polyurethane (PhLPU) films were formulated using phenolated lignin (0–60 wt.% of PhL) and polytetrahydrofuran as a polyol mixture, with either an aromatic poly[(phenyl isocyanate)-*co*-formaldehyde]. (pMDI) or an aliphatic hexamethylene diisocyanate trimer (HDI) as the isocyanate source. The study examined the significant impact of PhL concentration and the chemical structure of the isocyanate on the thermal stability and mechanical performance of PU films. Thermal analysis results showed that increasing the phenolated lignin content enhanced the thermal stability of the films, with HDI-based films exhibiting a higher maximum decomposition temperature compared to those made with pMDI. Mechanical performance testing revealed that PhLPU films with pMDI demonstrated superior strength characteristics compared to films with HDI trimer. The adhesive strength was evaluated using single-lap shear tests, with PhLPU films exhibiting comparable shear strengths to commercially available PU adhesives. Overall, the study highlights the effectiveness of the phenolation process in increasing the chemical reactivity of lignin, facilitating the development of high-performance PU films and adhesives, and contributing to the advancement of sustainable material solutions.

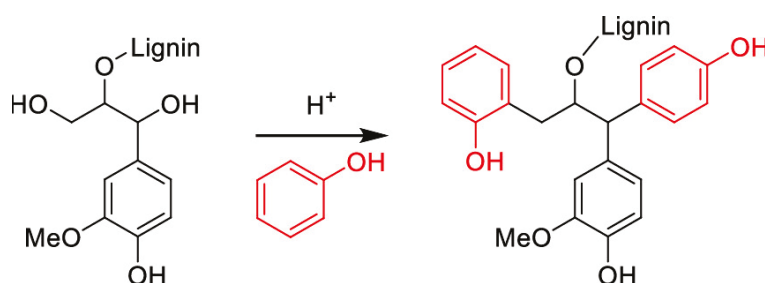


Figure 8. The replacement of aliphatic hydroxyl groups by ortho-/para-bound phenol through acid-catalyzed phenolation of lignin [66]. Used with permission, copyright 2024 American Chemical Society.

Liang et al. developed lignin-based polyols (LPs) by introducing hydroxyl groups into kraft lignin through liquefaction with polyethylene glycol (PEG). The PEG-mediated liquefaction involves acid-catalyzed transesterification between the ether linkages in PEG and the phenolic hydroxyl groups on lignin. This reaction yields lignin-polyether oligomers with enhanced hydroxyl group availability, improving solubility, molecular flexibility, and reactivity toward isocyanates during subsequent polyurethane formation. Increased hydroxyl content also promotes better crosslink density and phase dispersion within the resulting PU network. These LPs were then used to synthesize lignin-based polyurethane foams (LPUFs) by reacting them with hexamethylene diisocyanate (HDI), replacing 20–100 wt.% of conventional polyols (Figure 9) [67]. The inherent rigidity of lignin significantly enhanced the thermal and mechanical properties of the resulting foams. At 60% LP substitution, the foam exhibited a peak compressive stress of 86.80 kPa at 70% strain, which was approximately four times greater than the control polyurethane foam. This improvement was

attributed to the reinforcing effect of lignin's benzene ring structure. However, increasing LP content to 100% led to a decline in compressive stress (50.96 kPa), likely due to phase separation hindering optimal crosslinking. Additionally, the incorporation of lignin reduced the water contact angle and water absorption due to its hydrophobic nature and the reduced porosity of the foams. Biodegradation studies further demonstrated that LPUFs with 100% LP substitution degraded by 17.36% over 180 days, significantly improving compared to the 4.36% degradation observed in pure PUFs.

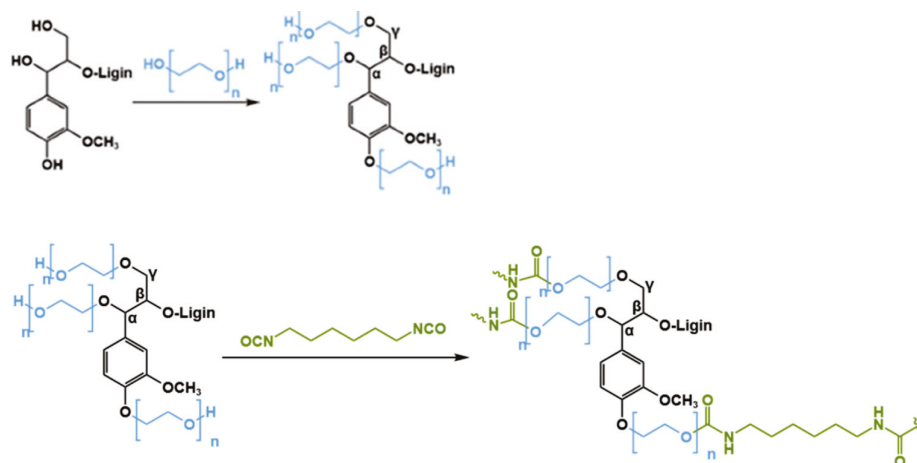


Figure 9. Lignin modification and preparation procedure of lignin-based polyurethane [67]. Used with permission, copyright 2024 American Chemical Society.

Oily wastewater poses significant environmental challenges and is produced across various sectors, including oil refining, food processing, automotive maintenance, shipping, construction, metalworking, and oil extraction [75,76]. Foams are one of the ideal solutions to separate oil from water due to their variable porosity and lightweight properties. Chen and coworkers enzymatically hydrolyzed lignin and liquefied it into a lignin-based polyol. Then, polyols were reacted with diisocyanate to produce synthesized lignin-based polyurethane foam (LPUF) [68]. The LPUF was further modified by in situ polymerization of dopamine on the foam surface under weakly alkaline conditions to deposit polydopamine (PDA) particles, followed by surface modification using phytic acid (PA) to prepare LPUF/PDA/PA foams (Figure 10). LUPF was degraded entirely in an alkali medium within 5 h, which was attributed to the presence of lignin. The LPUF/PDA/PA exhibited excellent superhydrophilicity with a water contact angle of 0° and underwater superoleophobicity with oil contact angles exceeding 150° , achieving over 99% oil–water separation efficiency for various mixtures while maintaining high performance after ten cycles. Additionally, it demonstrated good adsorption capacities for methylene blue (67.1 mg/g), rhodamine B (96.1 mg/g), and copper sulfate (98.2 mg/g) and could completely degrade under weak alkaline conditions after use. However, some limitations of LPUFs include challenges in scalability, durability, complex fabrication processes, limited versatility, potential environmental degradation, and chemical leaching, demanding further research to enhance their performance and sustainability.

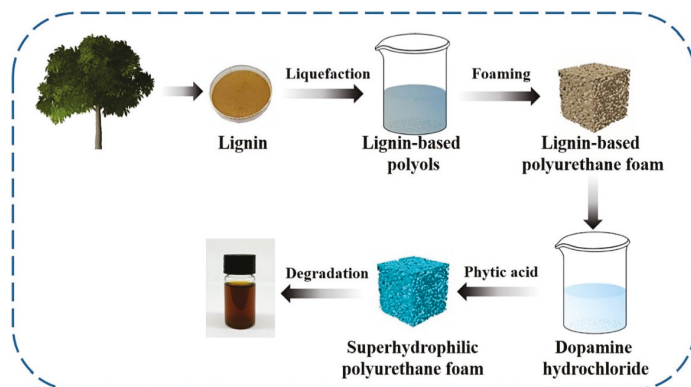


Figure 10. The preparation pathway of LPUF/PDA/PA and its degradation after usage in an alkali medium (after 5 h) [68]. Used with permission, copyright 2023 Elsevier.

2.3. Non-Isocyanate Polyurethanes (NIPUs)

Due to the high toxicity associated with isocyanates, there is a growing demand for developing and producing non-isocyanate polyurethanes (NIPUs) as a safer and more sustainable alternative to traditional PUs [77,78]. There are several methods to prepare NIPUs. Among them, aminolysis of cyclic carbonates (CCs) are more interesting because this approach involves the nucleophilic ring-opening reaction of CCs with amines, forming urethane linkages through a straightforward and non-toxic process. This process can be further enhanced by introducing five-membered cyclic carbonate groups (5CCs) into lignin or by depolymerizing lignin derivatives through glycidylation, followed by cycloaddition with CO_2 or oxyalkylation with glycerol carbonate to incorporate adjacent hydroxyl groups (Figure 11) [69]. Subsequently, transesterification with dimethyl carbonate allows the production of a series of NIPUs via the polyaddition of cyclic carbonate-functionalized lignin and diamines.

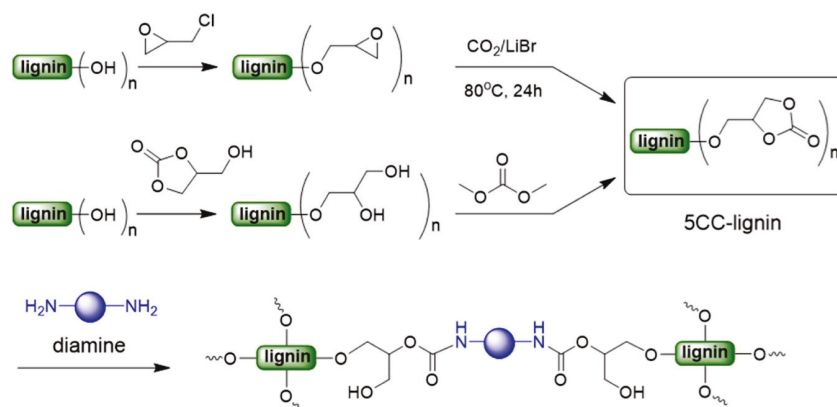


Figure 11. Non-isocyanate pathway to form polyurethanes from 5-membered cyclic carbonate functionalized lignin (5CC-lignin and amines.) [71]. Used with permission, copyright 2023 Elsevier.

Yang and coworkers proposed using lignin oligomers derived from reductive catalytic fractionation (RCF oil) as a substitute for NIPU production (Figure 12) [69]. Sawdust was used as a renewable polyol source to produce lignin oligomers. These oligomers were oxypropylated and modified with phthalic anhydride and epichlorohydrin to form a cyclic carbonate (5CC) intermediate (Figure 13). This intermediate was reacted with various amines to synthesize NIPUs. The amino group content and molecular size of amines significantly impacted mechanical properties, though they had a minimal effect on thermostability. Additionally, at the molecular level, oxypropylation of lignin introduces flexible polyether side chains via grafting propylene oxide onto the hydroxyl groups. This

significantly increases the number of aliphatic –OH groups, improving the reactivity of lignin with phthalic anhydride and epichlorohydrin for cyclic carbonate formation, and ultimately enhancing crosslinking density in the NIPU matrix. The flexible polyether chains contribute to improved toughness and tensile strength, while the rigid aromatic structure of lignin enhances thermal and UV resistance. The highest tensile strength (12.5 MPa) was achieved with polyether amine, while the highest Young's modulus (88 MPa) was obtained with triethyltetraamine. The isophorone diamine-crosslinked NIPU exhibited the lowest mechanical properties. Moreover, the tensile properties, heat resistance, and UV resistance of the NIPUs were enhanced by the rigid benzene ring and phenolic groups in lignin. Although the mechanical properties of NIPUs were comparable to traditional PU, further research is needed to explore their durability and performance under real-world conditions. Additionally, scaling up this synthesis method may present challenges in terms of cost and efficiency.

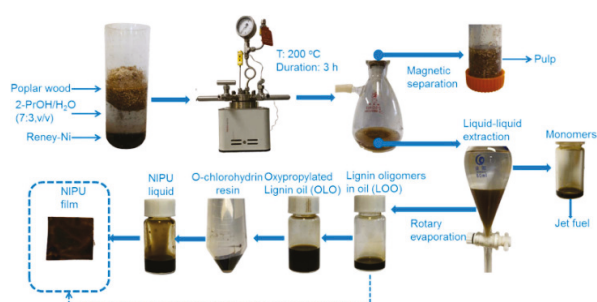


Figure 12. Preparation pathway of NIPU films from the lignin oligomers in the RCF oil [69]. Used with permission, copyright 2023 Elsevier.

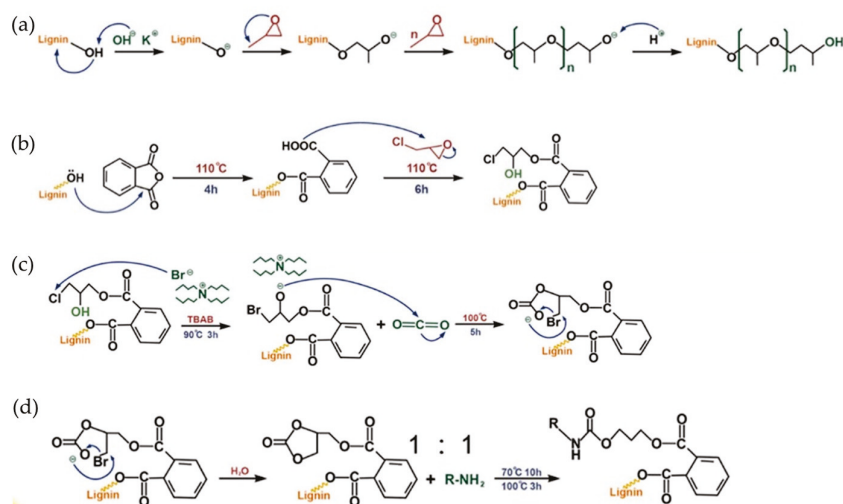


Figure 13. (a) The mechanism of oxypropylation reaction, (b) *o*-chlorohydrin resin formation (c) cyclic carbonation, and (d) NIPUs synthesis [69]. Used with permission, copyright 2023 Elsevier.

The previous method modified lignin with propylene carbonate to improve its solubility and reactivity. In the study by Najafi et al., lignin was initially modified with the amine, and the aminated product was next reacted with propylene carbonate (5CC) to enhance the hydroxy functionalities [70]. During the reaction, amination introduces nucleophilic amine groups onto the lignin backbone, which are then converted into hydroxyl groups through a ring-opening reaction with propylene carbonate. This two-step process significantly increases the hydroxyl number, thereby enhancing the lignin's reactivity toward cyclic carbonate–amine systems in NIPU synthesis. The increased hydroxyl functionality also

improves solubility and compatibility with other polyols. Furthermore, the reduced molecular weight of modified lignin may contribute to better dispersion and processability in polymer formulations. These structural changes explain the enhanced potential reactivity, although further studies are needed to correlate these modifications with final mechanical properties. In this work, lignin was extracted from raw liquor and then modified with diethylenetriamine (DETA) and ethylenediamine (EDA) to increase the number of functional groups and reactivity. The aminated lignin was further reacted with propylene carbonate using the Mannich reaction to prepare lignin-based polyols as an alternative to petroleum-based polyols to synthesize NIPU (Figure 14). This study did not address PU formation or the mechanical properties of the resulting polymers. However, evidence was provided through FT-IR analysis that the C-O bonds observed were due to urethane bonds in the modified lignin. GPC results revealed that lignin had been reduced to a lower molecular weight after modification, likely due to mechanical degradation during preparation. Additionally, the modified lignin-based polyol exhibited a much higher hydroxyl number (848–2220 mgKOH/g) than petroleum-based polyols, suggesting its strong reactivity potential. Therefore, further research is needed to apply this polyol in actual polyurethane production.

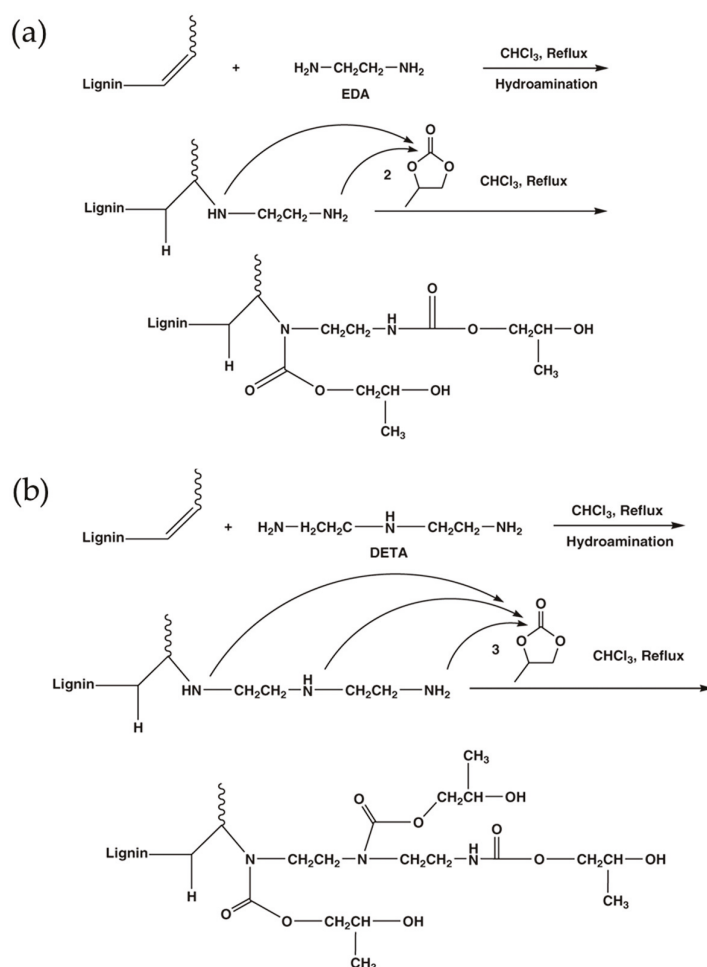


Figure 14. The reaction of (a) ethylene diamine (EDA) and (b) diethylenetriamine (DETA) with unsaturated regions of lignin, followed by the reaction of aminated lignin with propylene carbonate to produce lignin-based polyols [70]. Under a Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/> accessed on 27 April 2025).

Although five-membered cyclic carbonates (5CCs) can be used to synthesize lignin-containing NIPUs, the process is complex and requires expensive metal-based catalysts due to the slow reaction between 5CCs and amines. Additionally, 5CCs have lower reactivity, leading to slower curing times, incomplete reactions, and reduced mechanical and thermal performance, such as weaker tensile strength and lower thermal stability. To improve these limitations, potential solutions include using more reactive six-membered cyclic carbonates (6CCs), modifying 5CCs to enhance their reactivity and mechanical properties, or blending 5CCs with 6CCs to create hybrid systems that optimize material performance by leveraging the strengths of both structures.

Zhang and colleagues introduced a novel lignin/NIPU hybrid polymer (LNIPU) using bis(6-membered cyclic carbonate) (6CC) to address the limitations associated with 5CC. This example demonstrates how the structural rigidity of lignin influences toughness but limits self-healing, highlighting a critical trade-off in designing dynamic crosslinked networks with high-performance and reprocessability. Initially, amine-terminated non-isocyanate polyurethanes (amine-NIPUs) were synthesized from bis(6-membered cyclic carbonate) (6CC) and polyether amine (D-400) and then crosslinked with epoxidized enzymatic hydrolysis lignin (EEHL) at 0–50% epoxy group molar ratios to form LNIPU networks (Figure 15) [71]. Increasing lignin content in LNIPUs led to significant improvements in properties like storage modulus, glass transition temperature (T_g), and crosslink density, and notable increases in tensile strength, Young's modulus, and toughness. For instance, a material with 50% epoxy groups exhibited values of 20.25 MPa for tensile strength, 280.09 MPa for modulus, and 5.51 MJ/m³ for toughness, outperforming lignin-free versions. Adhesive strength also reached 24.5 MPa for bonding aluminum plates. Despite these gains, higher lignin content reduced elongation at break and self-healing efficiency, highlighting the need for optimized crosslinking strategies to improve overall material performance.

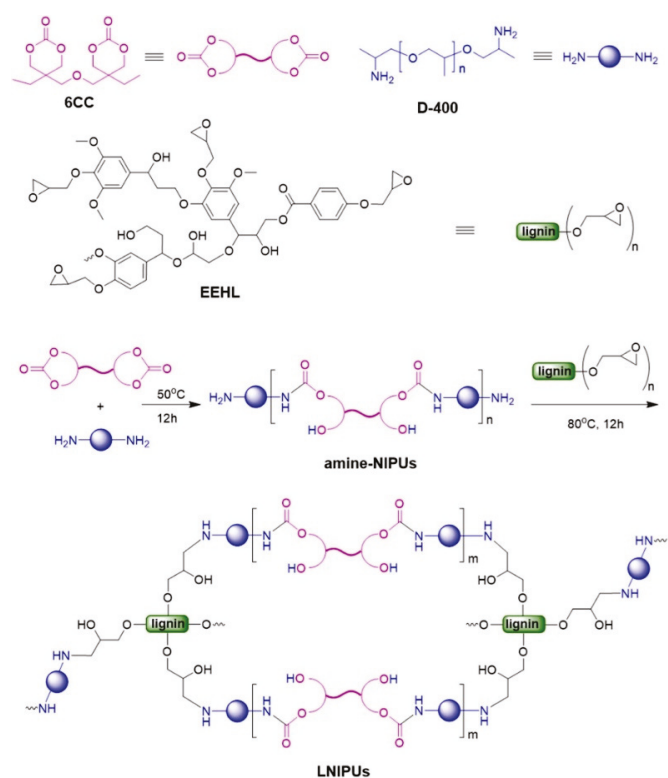


Figure 15. Illustration of the synthesis pathways for preparing LNIPUs [71]. Used with permission, copyright 2023 Elsevier.

2.4. Waterborne Polyurethanes (WPU)

Waterborne polyurethane (WPU) is an environmentally friendly polyurethane dispersed in water rather than organic solvents, thus reducing volatile organic compounds. Despite their significant advantages, WPUs encounter challenges related to mechanical performance, water resistance, drying times, and formulation complexity [72–74,79]. In response, Ng et al. developed a 3-aminopropyltriethoxy silane (APTES) functionalized kraft lignin-filled film to improve the reinforcing ability of WPUs [72]. Kraft lignin was functionalized by stirring it in a 2 wt.% APTES solution and subsequently mixed with WPU dispersion at varying proportions (0–2 wt.%) for comparison with unfunctionalized lignin. The films containing functionalized lignin demonstrated superior thermal stability compared to those with unfunctionalized lignin due to improved bonding. The optimal lignin loading was determined to be 0.5 wt.%, as higher concentrations resulted in agglomeration and reduced material properties. At this loading, functionalized lignin led to a 9% increase in ultimate tensile strength compared to pure WPU (from 8.05 MPa to 8.77 MPa), while unfunctionalized lignin yielded only a 0.3% improvement. Furthermore, films with functionalized lignin exhibited a lower Young's modulus and higher elongation at break, indicating enhanced flexibility and improved deformation resistance. This research demonstrates the potential of lignin as a reinforcement filler to enhance mechanical properties and thermal stability in WPUs; however, further investigations are necessary to address issues related to water resistance, drying times, and formulation complexity.

In another study by Fan and co-workers, WPU emulsions were developed using lignin nanoparticles (LNPs) derived from enzymatic hydrolysis lignin (EHL) [73]. The nanoscale dispersion of LNPs enhances interfacial bonding with the polyurethane matrix, contributing to better mechanical reinforcement and UV resistance without sacrificing flexibility—demonstrating the utility of nano-lignin as a multifunctional additive. A series of WPU composites (Figure 16) was formulated by varying the LNP content from 0 to 5 wt.%. Notably, the incorporation of 5 wt.% LNPs led to significant enhancements in the overall performance of the composites, particularly in terms of durability and UV resistance when compared to neat WPU. Kim et al. introduced an innovative approach to synthesizing WPU by incorporating lignin into a castor oil-based cationic waterborne polyurethane system [74]. This strategy remarkably reduced the solvent required for polymer synthesis by 50%. Lignin was incorporated in varying amounts (0–7 wt.%) to produce WPU composites (Figure 17). The results demonstrated that lignin improved mechanical properties, thermal stability, solvent resistance, UV resistance, and antibacterial activity. The composite containing 7 wt.% lignin exhibited a significantly higher tensile strength (5.57 MPa) than pure WPU (1.10 MPa). This data demonstrates that lignin acts not only as a passive filler but as an active reinforcing phase, contributing to load-bearing via π – π stacking and hydrogen bonding with the castor oil-based polyurethane segments. Furthermore, this composite displayed remarkable ethanol resistance and achieved 97.27% antibacterial activity against *Escherichia coli* (*E. coli*), compared to only 1.58% for the pure WPU.

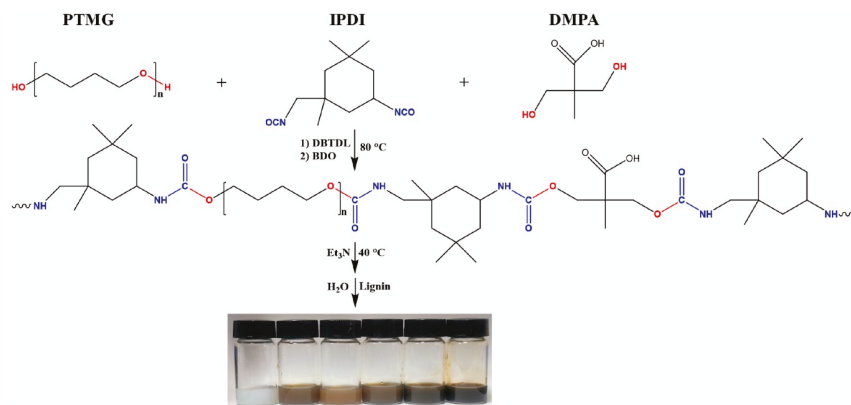


Figure 16. Preparation procedure of various lignin-based WPU emulsions [73]. Under a Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/> accessed on 27 April 2025).

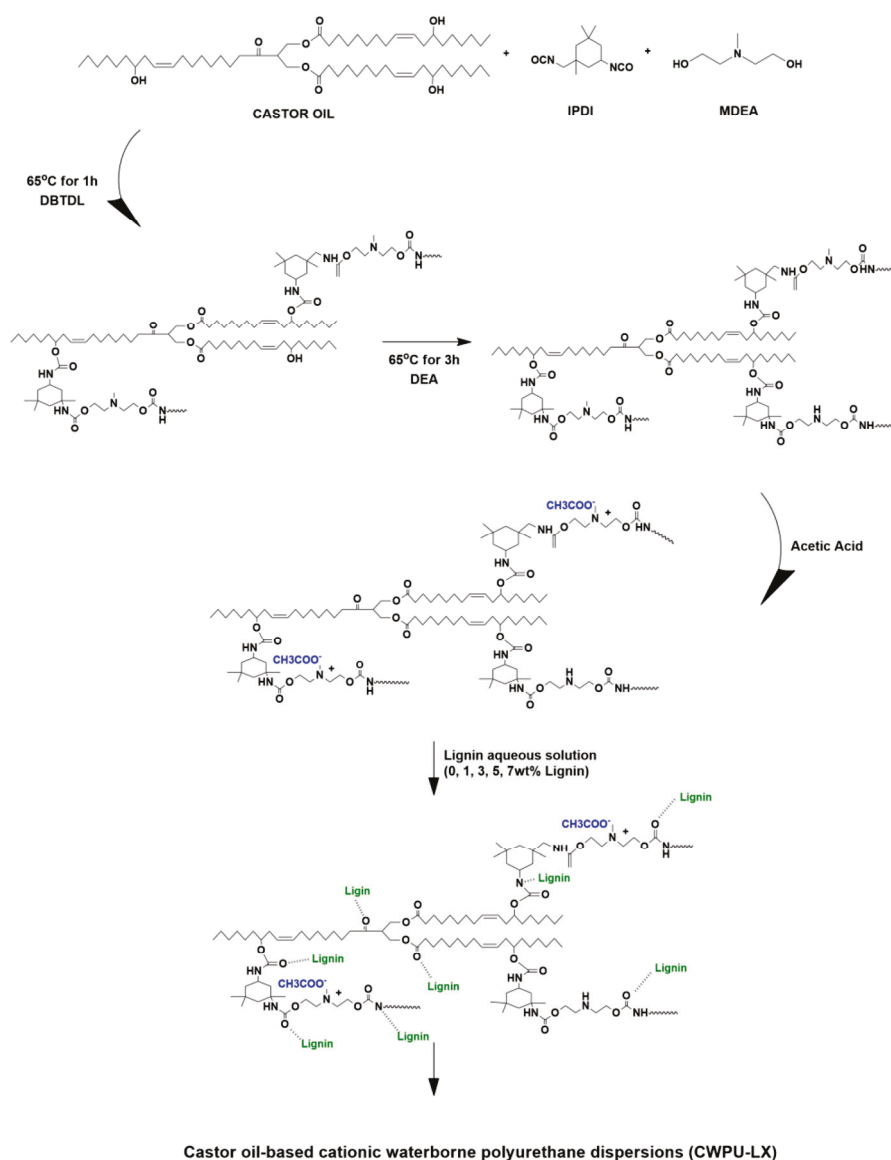
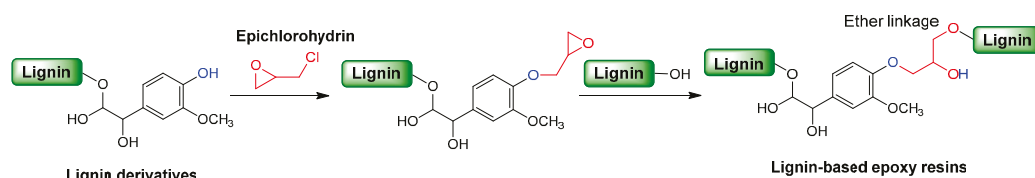


Figure 17. Schematic illustration of the preparation mechanism for castor oil-based cationic waterborne polyurethane incorporating lignin [74]. Under a Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/> accessed on 27 April 2025).

3. Lignin as a Precursor in Thermosets and Crosslinked Polymer Networks

3.1. Epoxy Resins

Epoxy resins are reactive pre-polymers that form crosslinked thermosets through curing reactions with various co-reactants, and they are widely used in applications such as coatings, adhesives, electrical insulation, and reinforced composites. Among them, the most commonly used epoxy resin reactant is diglycidyl ether of bisphenol A (DGEBA), which is synthesized from fossil-derived bisphenol A (BPA) [80,81]. However, the extensive use of BPA has raised significant environmental and health concerns, prompting the search for sustainable alternatives. Lignin is a promising substitute for BPA in the development of bio-based epoxy resins. Functionalization of lignin through glycidylation, typically via reaction with epichlorohydrin, introduces epoxy groups that enable the formation of crosslinked thermoset networks with ether linkages upon curing (Scheme 2). This section will discuss the use of lignin as a renewable feedstock to enhance the performance of epoxy resins while reducing dependence on petroleum-based polymers. Lignin types, modification methods, applications, and key properties for lignin-derived resins are summarized in Table 2.



Scheme 2. Synthesis pathways of lignin-based epoxy thermosets.

Table 2. Summary of thermoset and crosslinked polymer networks synthesized from lignin-based monomers and oligomers.

Lignin Type	Lignin Treatment/Modification Method	Resin Matrix	Application	Optimal Lignin wt. %	Reported Key Properties	Ref.
Enzymatic hydrolyzed lignin	Glycidylation and multi-carboxylation	Epoxied soya bean oil, citric acid	Epoxy thermosets	80%	Ultimate tensile strength: 33.2 ± 2.1 MPa Tensile modulus: 480.5 ± 41.7 MPa	[82]
Enzymatic lignin	Glycidylation and esterification	PEG 400 and base asphalt	Epoxy asphalt as a pavement material	20%	Ultimate tensile strength: 331.61 MPa Tensile strain: 382.4%	[83]
Industrial alkali lignin	Fractionation and glycidylation	Polyetheramine (JD400), diglycidyl ether (BADGE)	Epoxy thermosets	10%	Ultimate tensile strength: 5.42 ± 0.34 MPa Elongation at break: $255.32 \pm 3.16\%$	[84]
Black liquor lignin	Mannich reaction	Bisphenol A-based epoxy resin (E901)	Epoxy composite film	70%	Ultimate tensile strength: 30 MPa	[85]

Table 2. Cont.

Lignin Type	Lignin Treatment/ Modification Method	Resin Matrix	Application	Optimal Lignin wt. %	Reported Key Properties	Ref.
Kraft lignin (KL)	Ball milling (BMKL) Ultrasonication (NLH) Glycidylation (GKL)	Diglycidyl ether of bisphenol A (DGEBA), and curing agent aliphatic poly- oxypropylene α,ω -diamine (Jeffamine D-230)	Epoxy resin	3% KL	Ultimate tensile strength: 89.1 ± 5.7 MPa Strain at break: $6.3 \pm 0.4\%$	[86]
				6% BMKL	Ultimate tensile strength: 71.6 ± 4.3 MPa Strain at break: $5.9 \pm 0.4\%$	
				9% NLH	Ultimate tensile strength: 80.5 ± 6.1 MPa Strain at break: $7.4 \pm 0.6\%$	
				9% GKL	Ultimate tensile strength: 80.8 ± 1.5 MPa Strain at break: $6.8 \pm 0.3\%$	
Kraft lignin	Fractionation and glycidylation	DGEBA, and curing agent polyether amine (D-400)	Epoxy resin	60%	Ultimate tensile strength 14.7 ± 0.2 MPa Elongation at break $194.3 \pm 5.7\%$	[87]
Kraft lignin	Epoxidation	Amine-based NT-1515 curing agent, and Bamboo fibers	Epoxy resin	25% fiber addition	Storage modulus: 2.63 GPa Flexural strength: 116 MPa Flexural modulus: 5.69 GPa	[88]
Kraft lignin	Epoxidation	DGEBA, and tannic acid	Adhesive	5%	Shear strength: 10.57 MPa	[89]
Acidic lignin	Grafting silicon chains and etherification	Bisphenol F epoxy resin (DGEBF) and polyether amine (D230)	Epoxy resin composites (electronic packaging)	3%	Compared to neat EP: impact strength $\uparrow 16.25\%$, tensile $\uparrow 25.81\%$, bending modulus $\uparrow 16.21\%$, and bending strength $\uparrow 42.43\%$	[90]
Kraft lignin	Fractionation using a solvent system	Formaldehyde [p/a ratio = 1.7]	PF resin	25% of the soluble fraction	Bonding strength > 4 MPa	[91]
Alkaline lignin	Treated with the DES system	Phenol and formaldehyde [p/a ratio = 1.2]	Supercapacitor electrodes	30%	Capacitance: 112.4 F/g Energy density: 3.9 Wh/kg Power density: 125 Wk/g	[92]
Kraft lignin	Phenolation	Formaldehyde [p/a ratio = 0.5]	Wood adhesive	30%	Shear strength 3.41 MPa	[93]
Kraft lignin	Periodate Oxidation	Phenol and formaldehyde [p/a ratio = 3.84]	Wood adhesive	49% unmodified lignin	Dry shear strength: >2.75 MPa	[94]
Alkali lignin	Treated with phosphoric acid and hydrogen peroxide (PHP-lignin)	Phenol and formaldehyde [p/a ratio = 0.5]	Wood adhesive	PHP-lignin	Ultimate bonding strength 2.16 MPa	[95]

Zhou et al. developed fully bio-based epoxy thermosets using modified lignin (MGL) as a rigid crosslinker, combined with citric acid (CA) and epoxidized soybean oil (ESO) [82]. To enhance solubility, reactivity, and compatibility, lignin was chemically modified through glycidylation with glycidol to produce glycidylated lignin (GL), followed by maleation with maleic anhydride to introduce carboxyl groups, yielding MGL (Figure 18). In here, the glycidylation introduces epoxide groups that enhance compatibility and reactivity with the epoxidized soybean oil, promoting denser crosslinking. Maleation introduces carboxyl groups, which further increase hydrogen bonding and crosslinking potential with citric acid, thereby enhancing mechanical strength and rigidity. The thermosets were prepared by varying the MGL-COOH to CA-COOH ratios (L_{10}/C_0 , L_8/C_2 , L_5/C_5 , L_0/C_{10}) while maintaining a fixed epoxy-to-carboxyl molar ratio of 1:0.8 (Figure 19). Dynamic mechanical analysis (DMA) revealed an increase in glass transition temperature (T_g) from 17.1 °C (L_0/C_{10}) to 96.5 °C (L_{10}/C_0) with higher MGL content, indicating greater rigidity and crosslink density. Thermogravimetric analysis showed similar degradation temperatures at 5% weight loss ($T_{5\%}$) of 322–328 °C in nitrogen and 308–312 °C in air across all lignin-modified compositions. Mechanical testing demonstrated a rise in tensile strength from 0.6 MPa (L_0/C_{10}) to 33.2 MPa (L_8/C_2) and an increase in tensile modulus from 2.0 MPa to 480.5 MPa with higher MGL content. The highest elongation at break (55.1%) was observed for L_5/C_5 , while L_{10}/C_0 exhibited the lowest (9.3%). These thermosets displayed superior tensile strength compared to previously reported lignin-based systems and showed degradability in alkaline conditions due to ester bond hydrolysis. This result illustrates how glycidylation followed by maleation significantly increases the crosslinking efficiency and mechanical rigidity of lignin-epoxy networks, while preserving green degradability pathways, thereby balancing performance and sustainability.

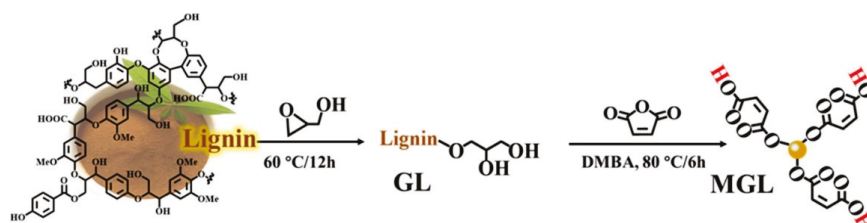


Figure 18. Lignin modification to synthesize glycidylated lignin (GL) and multicarboxyl lignin (MGL) [82]. Used with permission, copyright 2023 American Chemical Society.

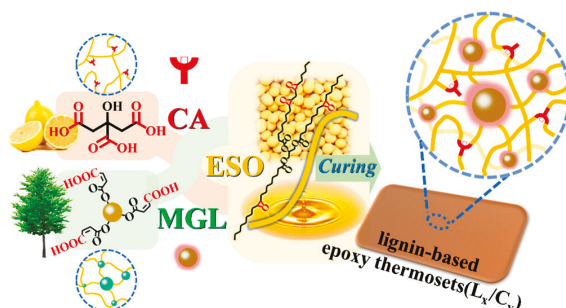


Figure 19. Preparation procedure for lignin-based epoxy thermosets (L_x/C_y) [82]. Used with permission, copyright 2023 American Chemical Society.

Conventional epoxy asphalt is a thermosetting material that is difficult to recycle [96]. Song et al. developed a lignin-based vitrimer system (ELV) to replace conventional epoxy asphalt, incorporating dynamic covalent ester-exchange bonds for recyclability [83]. Enzymatic lignin (EL) was reacted with epichlorohydrin (ECH) under alkaline conditions to obtain lignin epoxy resin (LER). EL was esterified with maleic anhydride (MA) to obtain

carboxylated lignin (EL-COOH). The lignin-based vitrimer system (ELV) was prepared by the Zn^{2+} catalyzed reaction of LER and EL-COOH in the presence of polyethylene glycol 400 (PEG 400). ELV (10–30 wt.%) was then introduced into base asphalt to produce different lignin-based epoxy asphalt systems (ELEA, Figure 20). ELEA exhibited improved high-temperature stability, with a higher softening point as ELV content increased. The glass transition temperature (T_g) remained stable (8–10 °C), ensuring good low-temperature performance. ELEA-20 (20 wt.% of ELV) showed comparable thermal stability to base asphalt (initial decomposition at 287 °C) and higher maximum decomposition temperature (470 °C vs. 454 °C). ELEA-20 met tensile strength (>1.5 MPa) and elongation at break (>200%) requirements for steel bridge deck pavement. Increasing ELV content enhanced tensile strength but reduced ductility. The dynamic covalent bonds in ELEA enable high-temperature restructuring, offering potential for recovery and recycling.

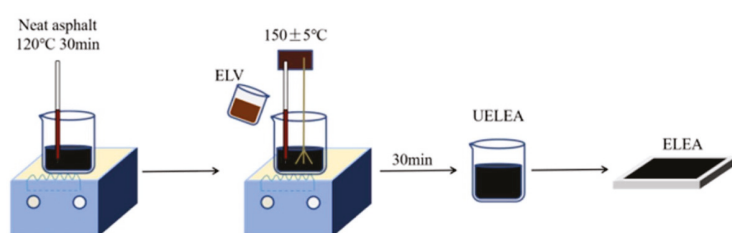


Figure 20. Illustration for the synthesis of lignin-based epoxy asphalt systems [83]. Used with permission, copyright 2023 Elsevier.

Zou and coworkers developed low-carbon, bio-based epoxy thermosetting materials using industrial alkali lignin [84]. Their approach highlights the advantage of fractionation for controlling molecular weight distribution, which directly impacts processability, epoxy functionalization efficiency, and mechanical consistency in the final resin network. To achieve better uniformity and lower molecular weight, the lignin was fractionated with acetone (F_{ASL}) and subsequently epoxidized with epichlorohydrin, introducing epoxy groups onto the lignin backbone (F_{ASLM}). This modification enhanced lignin's reactivity, enabling partial substitution of petroleum-based bisphenol A diglycidyl ether (BADGE) in epoxy resin formulations. The thermosetting resin was prepared by blending F_{ASLM} (5–10 wt.%) and BADGE in varying proportions, with polyetheramine (JD400) as the crosslinking agent (Figure 21). Thermal stability analysis revealed a significant improvement, with the degradation temperature ($T_{5\%}$) increasing from 200–230 °C for unmodified lignin to 270–290 °C for epoxidized lignin. The cured resins containing 5% and 10% F_{ASLM} demonstrated enhanced thermal properties, with $T_{5\%}$ reaching 315 °C and 289 °C, respectively, compared to pure BADGE. Mechanical properties also improved with lignin incorporation. The 5% F_{ASLM} resin exhibited a tensile strength of 4.6 MPa and an elongation at the break of 315.5%, outperforming pure BADGE (4.19 MPa, 161.16%). At 10% lignin content, tensile strength increased to 5.42 MPa, with elongation at break reaching 255.32%. However, at lignin concentrations above 10%, agglomeration issues were observed, and higher lignin content (above 20%) necessitated elevated curing temperatures, limiting its substitution potential. While epoxidation improved lignin's compatibility with BADGE, further optimization is needed to enhance interface compatibility and maximize lignin incorporation without compromising material properties.

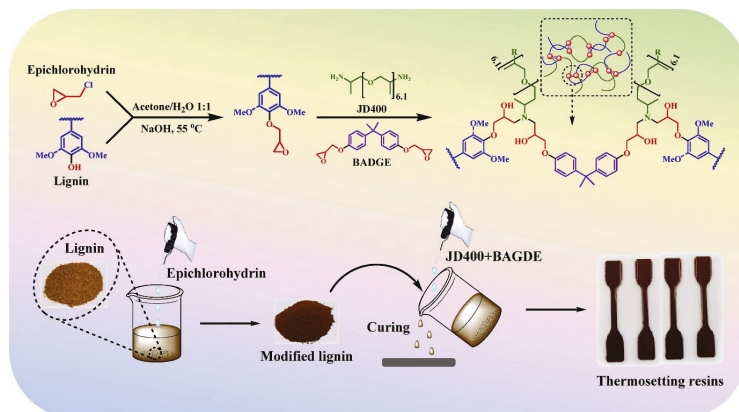


Figure 21. Schematic illustration of epoxidation using modified lignin [84]. Used with permission, copyright 2023 Elsevier.

Li and coworkers utilized black liquor lignin, a byproduct of the pulping industry, to develop lignin-based epoxy composite films [85]. Their strategy exemplifies a cost-effective valorization route, converting underutilized industrial byproducts into functional curing agents. The resulting composites reveal how aromatic rigidity and functional group density of modified lignin can be tuned to reinforce resin network architecture. The black lignin was modified via a Mannich reaction with phenol, formaldehyde, and diethylenetriamine to produce lignin-based phenolic amine (LPAA), which served as a curing agent for bisphenol A-based epoxy resin (E901). Phenolation introduces additional phenolic hydroxyl groups onto the lignin backbone, increasing both hydrogen-bonding capacity and reactive sites for subsequent Mannich-type amine functionalization. This increases the crosslinking density and intermolecular interactions within the epoxy network. Furthermore, the incorporation of phenol increases the aromatic content of lignin, resulting in a stiffer and more thermally stable network. LPAA was synthesized with varying mass ratios of black liquor lignin to phenol: 30:70, 70:30, and 100:0. The resulting LPAA and E901 were then combined in a 1:0.1 mass ratio to form the composite film (LPAAF, Figure 22). Thermal analysis showed an improvement in the degradation temperature at 5% weight loss ($T_{5\%}$) compared to the control sample without lignin, with increasing black liquor lignin content enhancing thermal stability. The tensile strength of LPAAF increased with lignin content, reaching a maximum of 30 MPa for LPAAF70 (70 wt.% black liquor lignin in LPAA). However, elongation at break decreased due to the rigid three-dimensional lignin network. Inorganic chemicals from black liquor lignin were well dispersed in the films, and future studies are required to assess the long-term stability and durability of the composites.

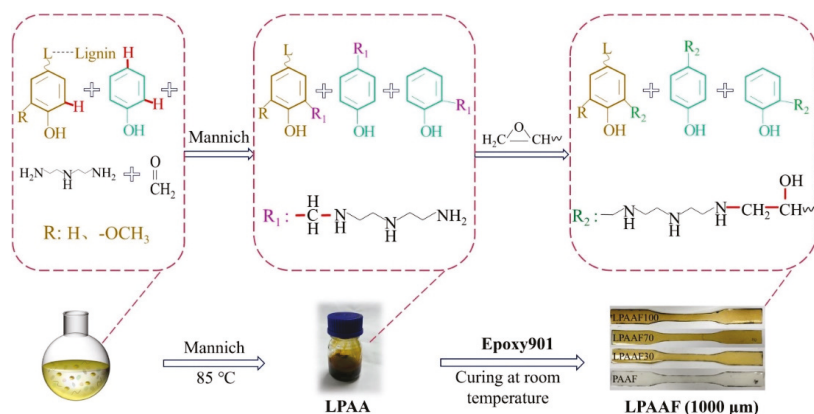


Figure 22. Preparation of lignin-based phenolic amine (LPAA) and epoxy resin (LPAAF) [85]. Used with permission, copyright 2023 Elsevier.

Pappa et al. explored the integration of kraft lignin (KL) into DGEBA-based epoxy resin systems to enhance sustainability and performance. Their work illustrates the importance of particle size and chemical compatibility. Nano-lignin incorporation preserved transparency and improved mechanical integrity, emphasizing that lignin processing form—micro vs. nano—has direct performance implications. KL was employed in various forms: as a direct additive, a partial replacement for the amine curing agent, and in a chemically modified form via glycosylation (GKL, Figure 23) [86]. Additionally, KL was used at both micro- and nanoscales, with nano-lignin (NLH) yielding transparent composites with notable improvements in thermal and mechanical properties. Glycidylated lignin enabled high substitution levels of bio-based epoxy content (up to 38 wt.%) while maintaining or enhancing composite performance. At 3 wt.% KL loading, tensile strength increased by 25% without compromising strain at break. Optimal improvements in tensile stress (3.1%), strain (35.2%), and stiffness (28.9%) were observed at 3 wt.% KL, while higher loadings (up to 9 wt.%) showed a trade-off with reduced strain but retained strength. Ball-milled KL (BMKL) composites showed moderate property enhancements even at 6 wt.% loading. Notably, NLH-based composites sustained or improved mechanical properties up to 30 wt.% loading, showing better stress, strain, and stiffness compared to the pristine polymer. GKL allowed substitution of up to 21 wt.% DGEBA (16.6 wt.% GKL content) with significant improvements in all mechanical metrics. These results highlight KL's versatility and effectiveness in enhancing epoxy systems, particularly when chemically modified or nanoscaled, making it a strong candidate for greener, high-performance thermoset composites.

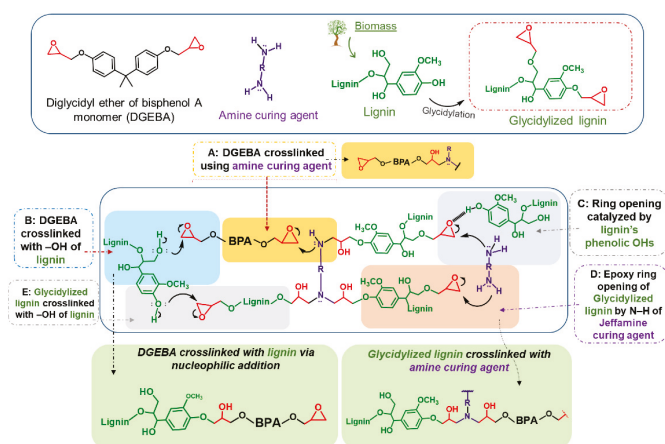


Figure 23. Proposed mechanism for crosslinking of lignin/DGEBA/diamine to prepare epoxy resins [86]. Used with permission, copyright 2023 Elsevier.

Xue and coworkers developed a lignin-based substitute for bisphenol A (BPA) for producing epoxy resin [87]. Kraft lignin was fractionated to produce low-molecular-weight (low-Mw) lignin, mainly consisting of syringaresinol (0–60 wt.%), to synthesize epoxy resins (Figure 24). The prepared epoxy resins with a 20% substitute amount exhibited the optimum properties. Thermal stability increased by 48%, and mechanical properties were significantly enhanced, with elongation improved by 239% and tensile stress by 144% compared to the BPA-based epoxy resin. The improved performance is attributed to the unique structural features of the lignin components: furan ring-opening of syringaresinol forming a flexible 3D crosslinked network, retention of conjugated C=C bonds in stilbene enhancing stress transfer, and Schiff base formation from aldoketones contributing to chain extension and flexibility (Figure 25).

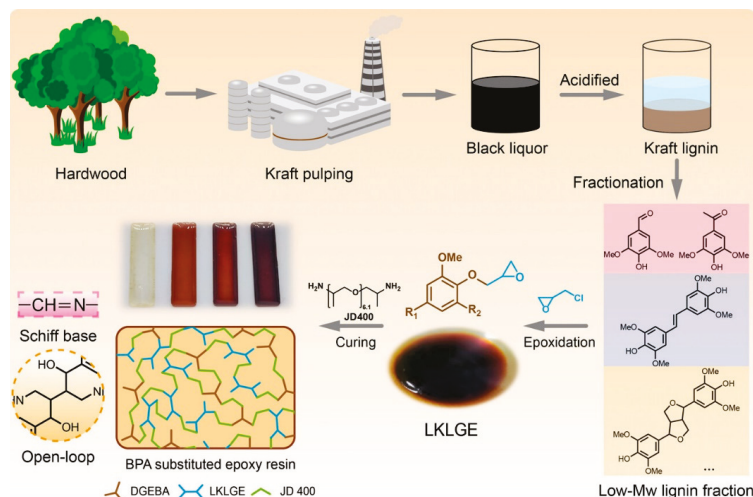


Figure 24. Preparation procedure of lignin-based epoxy resin using low-molecular-weight lignin glycidyl ether (LKLGE) [87]. Used with permission, copyright 2023 American Chemical Society.

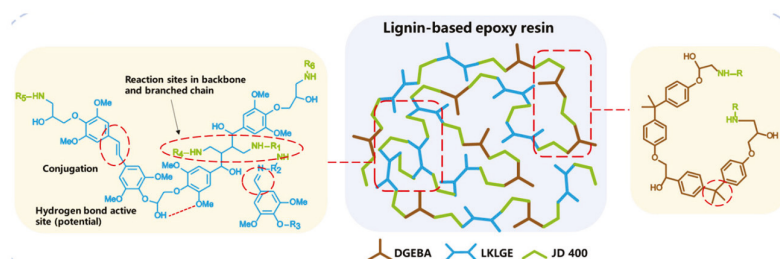


Figure 25. Toughening mechanisms in lignin-based epoxy resins and their impact on mechanical properties [87]. Used with permission, copyright 2023 American Chemical Society.

In another study, Bagheri and colleagues developed a bio-based epoxy resin to fully replace BPA [88]. In this approach, kraft lignin was epoxidized using a bio-derived epichlorohydrin (ECH) sourced from glycerol. The resulting resin was then used to impregnate unidirectional bamboo fibers, forming composite sheets for the fabrication of a water-based epoxy resin (Figure 26). During the reaction, epoxidation of kraft lignin introduces reactive epoxide rings onto the lignin structure, enhancing its ability to form covalent bonds during curing, which improves the network formation with curing agents and leads to better load transfer within the composite. The aromatic-rich structure of lignin promotes interfacial compatibility with bamboo fibers due to π - π and hydrogen-bonding interactions. When comparing the mechanical properties, the initial flexural strength of the lignin-based composite was lower than that of commercial epoxy, and the addition of 25 wt.% bamboo fibers significantly improved the flexural strength due to the strong interfacial bonding and natural compatibility between lignin and the bamboo fibers. Furthermore, the incorporation of fibers led to a notable increase in the flexural modulus, reinforcing the potential of this system to replace petroleum-based polymers.

Gavrilović-Grmuša et al. developed a bio-based epoxy adhesive for wood applications by incorporating lignin and tannic acid as reactive components [89]. Specifically, kraft lignin was chemically modified through epoxidation to partially substitute diglycidyl ether of bisphenol A (DGEBA), a conventional petroleum-based epoxy monomer (Figure 27). The modified resin formulations included lignin in concentrations ranging from 5 to 15 wt.%. Notably, the lower lignin content (5 wt.%) yielded the highest tensile shear strength (10.87 MPa), comparable to that of the unmodified resin. However, as the proportion of lignin increased, a gradual decline in adhesive strength was observed. This reduction was

attributed to excess lignin's limited dispersion and reactivity within the epoxy matrix. The structural similarity and chemical compatibility between lignin and tannic acid enhanced the mixture's homogeneity, thereby reducing lignin agglomeration and improving its interaction with the epoxy network.

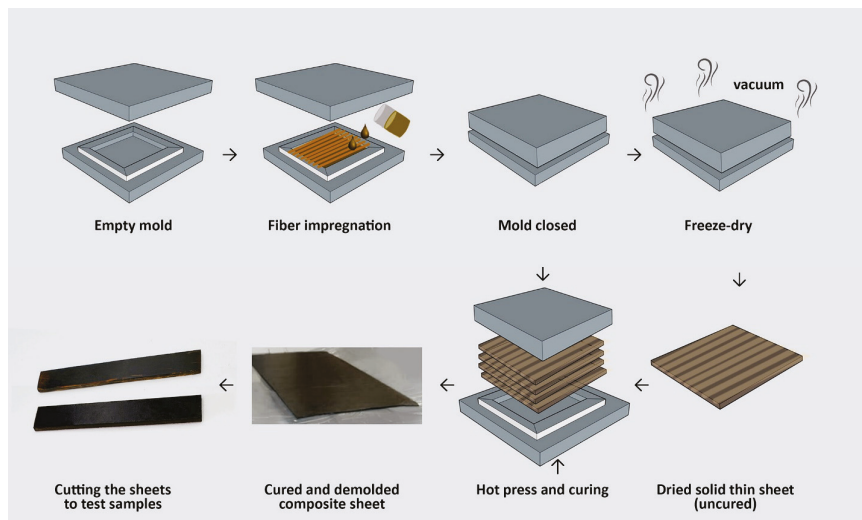


Figure 26. Schematic representation of the fabrication process for unidirectional, fully bio-based lignin epoxy/bamboo composites [88]. Under a Creative Commons Attribution (CC BY-NC-ND 4.0) license (<https://creativecommons.org/licenses/by-nc-nd/4.0/>).

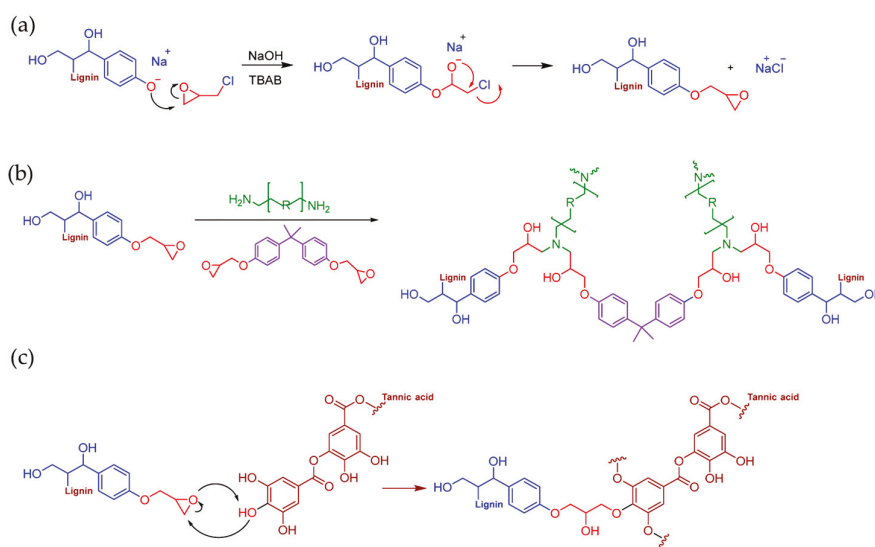


Figure 27. Proposed mechanism for (a) lignin epoxidation, (b) crosslinking of DGEBA, epoxy lignin, and amine hardener, and (c) crosslinking of epoxy lignin with tannic acid during curing [89]. Under a Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/> accessed on 27 April 2025).

Li et al. developed a lignin-based silicone-modified epoxy resin to improve the mechanical performance of epoxy resins, which are widely used in electronic packaging. The synthesis involved two main steps (Figure 28): first, flexible silicone chains were grafted onto lignin (0–9 wt.%) through a condensation reaction between the Si–H groups of polyhydromethylsiloxane (PHMS) and the hydroxyl groups of lignin. In the second step, the modified lignin was incorporated into the epoxy matrix via additional condensation between the lignin hydroxyl groups and the epoxy resin [90]. Optimal mechanical performance was achieved at 3 wt.% lignin loading, where impact strength increased by 16.25%,

tensile strength by 25.81%, bending modulus by 16.21%, and bending strength by 42.43% relative to neat epoxy resin. Moreover, elongation at break improved progressively with increasing lignin content, with a 37.86% enhancement observed at 3 wt.% lignin addition. Although higher lignin content reduced glass transition temperature and crosslink density, the modified resins still exhibited a desirable combination of toughness and strength.

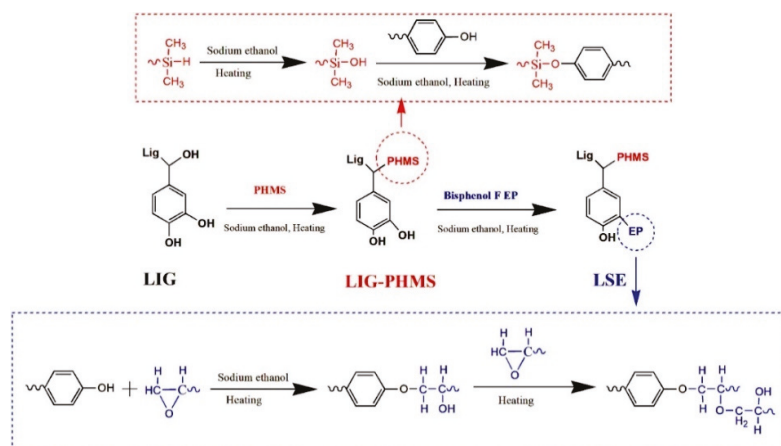
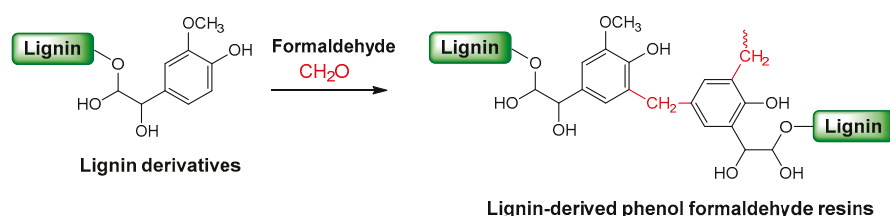


Figure 28. Synthesis of lignin-based silicone-modified epoxy resin [90]. Used with permission, copyright 2024 Elsevier.

3.2. Phenol-Formaldehyde Resins

Phenol-formaldehyde (PF) resins are widely utilized in wood adhesives, molding compounds, and insulation materials due to their excellent mechanical strength, water resistance, thermal stability, and flame retardancy [97,98]. Traditionally, these resins are synthesized from petroleum-derived phenol and formaldehyde. However, the use of these chemicals raises significant environmental and health concerns due to the toxicity of both phenol and formaldehyde. In response, lignin as a low-cost, renewable aromatic polymer has garnered significant attention as a sustainable alternative. Lignin's complex structure contains reactive phenolic and aliphatic hydroxyl groups, aldehydes, and carboxylic functionalities, which can participate in PF resin synthesis. Notably, the phenolic hydroxyl groups in lignin can undergo condensation reactions with formaldehyde, forming methylene bridges ($-\text{CH}_2-$) between aromatic units, thereby mimicking the structure of conventional PF networks (Scheme 3). This reactivity makes lignin a promising substitute for phenol in the development of eco-friendly phenolic resins. This section will highlight the role of lignin in various lignin-derived PF resin formulations, emphasizing its potential to reduce environmental impact while maintaining or enhancing resin performance.



Scheme 3. Synthesis of lignin-modified phenol-formaldehyde resins.

Rodrigues et al. investigated the development of lignin-based phenol-formaldehyde (LPF) resins using fractionated kraft lignin (KL) with lower molar mass fractions [91]. This example demonstrates how lignin fractionation is not merely a purification strategy, but a functional design tool that enhances solubility and reactivity, ultimately improving

adhesive bond strength and resin homogeneity. The lignin was extracted from *Eucalyptus urograndis* wood using ethyl acetate, resulting in both soluble and insoluble fractions. The study explored the effect of substituting 25% and 50% of phenol with fractionated lignin to produce eco-friendly LPF resins. The fractionation process notably increased the total hydroxyl content and solubility of lignin, which are critical parameters for enhancing resin performance. Thermal analysis demonstrated that the inclusion of lignin improved the thermal stability and increased the glass transition temperature (T_g) of the resins. Among the various formulations, resins containing 25% soluble lignin fractions, cured at 150 °C, exhibited adhesive properties comparable to pure phenol-formaldehyde (PF) resins without lignin substitution. Furthermore, the adhesive bond strength of the soluble fraction LPF resins was significantly higher than that of the insoluble fraction LPF resins and non-fractionated KL-based resins. This superior performance highlights the beneficial role of lignin fractionation in optimizing the properties of bio-based resins. Overall, the study successfully demonstrated that a small percentage of soluble lignin fractions can effectively replace phenol in commercial PF resins, contributing to the synthesis of environmentally friendly adhesive systems.

Li and coworkers deployed alkaline lignin with a deep eutectic solvent (DES) composed of *p*-toluenesulfonic acid and choline to prepare phenolic resin and porous carbon [92]. The depolymerized lignin was co-polymerized with phenol and formaldehyde to produce a lignin-based phenolic resin (DLPFC, Figure 29). A pure phenolic resin carbon (PFC) was also prepared similarly without lignin for comparison. The electrochemical performances of the prepared materials were obtained as supercapacitor electrodes. After the incorporation of lignin, DLPFC materials showed higher specific surface area, higher electrical conductivity, and better graphitization compared to pure resins. As a supercapacitor electrode, DLPFC exhibited higher specific capacitance (245.8 F/g at 0.25 A/g) than PFC (159.7 F/g) in a three-electrode system and had excellent cycling stability, retaining 97.6% capacitance after 10,000 cycles. In a two-electrode system, the DLPFC achieved an energy density of 3.9 Wh/kg at a power density of 125 W/kg, comparable to commercially available batteries.

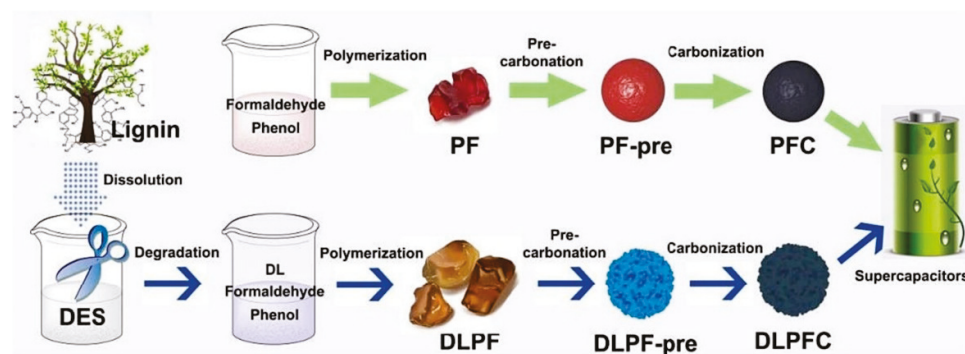


Figure 29. Schematic representation of lignin-based phenolic resin (DLPFC) preparation process and phenolic resin carbon (PFC) [92]. Used with permission, copyright 2023 Elsevier.

Galsino and coworkers used kraft lignin as a substitute for phenol in synthesizing phenol-formaldehyde (PF) resins [93]. Lignin was modified through a phenolation process to increase the reactivity of lignin and substituted phenol with varying proportions of phenolated kraft lignin (10–50 wt.%) to produce lignin-phenol-formaldehyde (LPF) resins. The LPF resins with phenol substitution above 20% (LPF30 and LPF20) showed superior shear strength compared to the control PF resin, and the highest shear strength was observed for the LPF30 resin (3.41 MPa). In TGA, the addition of kraft lignin reduced the thermal stability of the resins, resulting in lower decomposition temperatures and smaller

amounts of carbonaceous residues. The curing of lignin-based resins occurred at lower temperatures with higher enthalpies than the PF resin. Overall, the study demonstrates the potential of using kraft lignin as a sustainable substitute for phenol (up to 30% substitution) in phenol-formaldehyde resins for structural composite applications, highlighting the need for further optimization and investigation.

Bansode and coworkers explored the use of lignin as a substitute for phenol and formaldehyde in the preparation of phenol-formaldehyde (PF) wood adhesive resins [94]. Kraft lignin, derived from pine trees, was chemically modified using sodium periodate oxidation to introduce aldehyde functionalities (Figure 30). The oxidized lignin was then employed to partially or fully replace formaldehyde in synthesizing bio-based novolac PF (BNPF) resins. In the study, unmodified lignin was used to replace phenol, while oxidized lignin served as a formaldehyde replacement to produce BNPF resins. The tensile shear strengths of the resulting BNPFs were compared to those of commercially available novolac PF resins. Results showed that lignin-based PF resins exhibited superior dry adhesion strength compared to commercial PF resins. However, the wet adhesion strength of lignin-based resins was lower, likely due to the hydrophilic nature of lignin. Additionally, the curing temperature of the BNPF resins was reduced when formaldehyde was replaced with oxidized lignin. This reduction in curing temperature is attributed to the higher reactivity of periodate-oxidized lignin compared to formaldehyde when reacting with phenol or lignin.

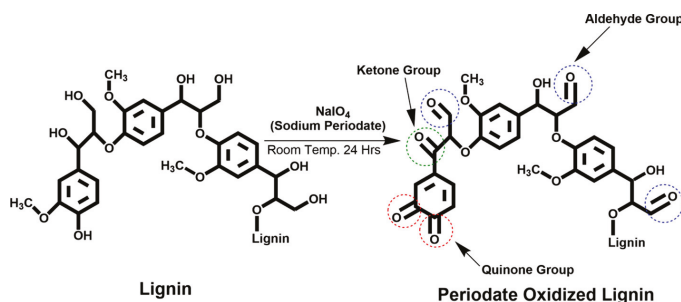


Figure 30. Schematic representation of the periodate oxidation of softwood lignin, targeting aromatic and aliphatic hydroxyl groups [94]. Used with permission, copyright 2023 American Chemical Society.

Zhao et al. developed lignin–phenol-formaldehyde (LPF) resins using unmodified lignin derived from phosphoric acid and hydrogen peroxide (PHP) pretreated biomass waste and evaluated their performance as wood adhesives [95]. The PHP-lignin-based LPF (PLPF, Figure 31) resins were compared with those synthesized from conventional alkali lignin (ALPF). Particleboards incorporating 5–15 wt.% LPF resin exhibited good dimensional stability and solvent resistance. However, the presence of resin adhesives hindered enzymatic hydrolysis, reducing cellulose-to-glucose conversion to 18.3%. An alkali pretreatment step significantly improved glucose conversion up to 57.8%, offering a promising detoxification strategy for recycling lignocellulosic composites. In adhesive performance, the bond strength of lignin-derived resins met the requirements for first-grade plywood (≥ 0.7 MPa), although the ultimate bonding strength of PLPF (2.16 MPa) and ALPF (1.59 MPa) was lower than that of pure PF resin (3.09 MPa). This reduction was attributed to the lower reactivity of lignin due to fewer reactive sites, higher molecular weight, and steric hindrance. Notably, PLPF outperformed ALPF, likely due to the higher hydroxyl content of PHP-lignin enhancing its reactivity with formaldehyde.

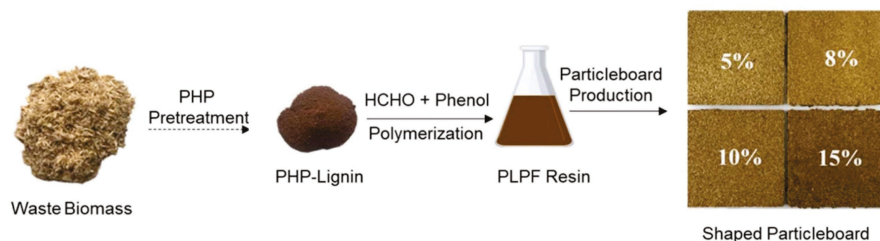
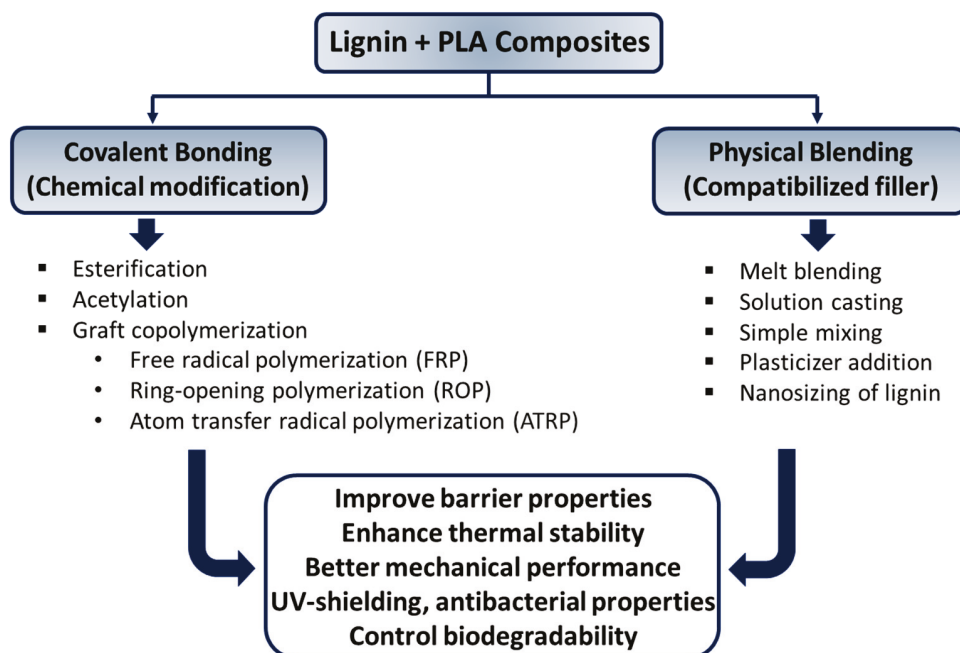


Figure 31. Synthesis of phosphoric acid and hydrogen peroxide pretreated lignin–phenol–formaldehyde resins (PLPF) and particleboards [95]. Used with permission, copyright 2024 Elsevier.

4. Polylactic Acid (PLA) Composites

Poly(lactic acid) (PLA) is a versatile aliphatic linear thermoplastic biodegradable polymer derived from fully renewable sources such as wheat, corn, rice, and sweet potatoes. It offers numerous advantages, including renewability, sustainability, biocompatibility, and compostability, making it an attractive candidate for eco-friendly materials. PLA production involves low energy consumption and generates minimal greenhouse gas emissions, further enhancing its appeal for various applications, including 3D printing [99,100]. However, PLA also exhibits several drawbacks, such as poor gas and water barrier properties, limited toughness, low glass transition temperature, hydrophilicity, and challenges with dispersion when blended with other components [101]. These limitations hinder its widespread use in commercial applications. PLA is often blended with natural additives like lignin and fibers to address these issues, to enhance its thermal stability, water barrier performance, crystallization behavior, mechanical strength, antimicrobial properties, and degradability (Scheme 4). This section explores recent advancements in the development of biodegradable composites, focusing specifically on incorporating lignin into PLA to improve its performance characteristics (Table 3).



Scheme 4. Strategies for incorporating lignin into polylactic acid (PLA) systems.

Table 3. Summary of the lignin-based PLA polymers.

Lignin Type	Lignin Treatment Method	Monomer/ Polymer Matrix	Application	Optimal Lignin Details	Reported Key Properties	Ref.
Commercial lignin	NA	Lauryl methacrylate	Composite films	5 wt.% Lg-g-PLMA	Strain at break: 8.1% Toughness: 245.6 MJ/m ³	[102]
Kraft lignin	Ethyl acetate extraction and methylation	Glycidol	PLA films	10 wt.%	Elongation at break: 297.7% Toughness: 39.92 MJ/m ³ Blocked 99.52% of UV-A	[103]
Alkali lignin	NA	PLA granules	PLA films and sunscreen	90 wt.%	Stronger UV shielding, in the UV-A range	[104]
Softwood kraft lignin	Fractionation, followed by ultrasonication (SLNs)	Poly(L-lactic acid) (PLLA)	Films	1 wt.%	Enhanced crystallization rate, crystallinity, and nucleation density with SLNs	[105]
Commercial lignin	Etherification with EGDE or PEGDE	PLA	Packaging material	5 phr with EGDE	O ₂ barrier property: 58.3% Tensile strength: >60 MPa	[106]
Lignin from corncob refinery residues	Alkali pretreatment	Epoxidized natural rubber/PLA	Disposable tableware, packaging materials and toys	20 wt.%	Tensile strength: 45.31 MPa Young's modulus: 2.36 GPa	[107]
Kraft lignin	Grafting 2-ethylhexyl acrylate (e-Lignin)	PLA	3D printing	2.5 wt.% with e-Lignin	Tensile strength: >60 MPa Young's modulus: >3 GPa Toughness: >2 MJ/m ³	[108]
Kraft lignin and kraft nano-lignin	NA	PLA, L-Lactide	Films	0.5 wt.% nano-lignin	Hardness: 265 MPa Elastic modulus: 6098 MPa	[109]
Alkali lignin	Laccase enzymatic degradation (EL)	PLA	3D printing	1.25 wt.% with EL	Tensile strength: >40 MPa Elastic modulus: 2.2 GPa	[110]
Soda lignin from Beech sawdust	Steam-explosion pretreatment and modified with PEG	PLA	3D printing	10 wt.% PEG-modified lignin	Tensile strength: >60 MPa Elongation at break: >6% Elastic modulus: >3.5 GPa	[111]
Commercial lignin	Urethanization of lignin with HDI	PLA	Films	14 wt.%	Tensile strength: 36.5 MPa Elongation at break: 110% Elastic modulus: 5.56 GPa	[112]
Alkali lignin	Etherification	PLA	Composite films	3 wt.%	Tensile strength: >25 MPa Young's modulus: 1.3 GPa	[113]
Alkali lignin	Fractionation and Dopamine-grafting (OL@DA)	PLA	Composite films	0.5 wt.% of OL@DA	Tensile strength: >50 MPa Elongation at break: 14% Impact strength: ~16 kJ/m ³	[114]

Shi and coworkers developed wholly bio-based composites by grafting lignin with lauryl methacrylate (LMA), a renewable plant-based fatty acid monomer, through free-radical polymerization to produce lignin graft copolymers (LG-g-PLMA) [102]. These copolymers (0–20 wt.% of LG-g-PLMA) were subsequently blended with polylactic acid (PLA) to create the composites. The long hydrophobic side chain of LMA was designed to improve the hydrophobicity, flexibility, and compatibility of lignin with PLA, while lignin was anticipated to contribute UV barrier properties to the composites. The incorporation of LG-g-PLMA significantly enhanced the crystallinity of PLA, increasing from 6.42% to 17.46%. When the LG-g-PLMA loading was below 9%, the thermal stability of the

composites showed a slight improvement compared to pure PLA. However, the glass transition temperature (T_g) of the composites decreased with increasing LG-g-PLMA content. At a 5% LG-g-PLMA loading, the composites demonstrated a 42% increase in elongation at break and a 36% increase in tensile toughness compared to pure PLA. While the tensile strength of the composites declined from 55.6 MPa (pure PLA) to 26.5 MPa at 20% LG-g-PLMA loading, the composites exhibited a strong UV barrier effect, nearly completely blocking UV light at low LG-g-PLMA concentrations. The successful grafting of LMA onto the lignin via free-radical polymerization enhanced both the hydrophobicity and thermal stability of lignin. This modification also improved its compatibility with PLA and strengthened interfacial adhesion. As a result, the composites exhibited improved mechanical properties even at low LG-g-PLMA loading.

Kim et al. addressed the inherent brittleness and poor UV resistance of PLA by incorporating chemically modified lignin, leveraging cationic ring-opening polymerization (CROP) techniques to enhance material performance [103]. The modification involved ethyl acetate-extracted lignin (EL) subjected to CROP using tetrahydrofuran (THF) and glycidol (GLY) in the presence of a boron trifluoride (BF_3) catalyst, resulting in EL_C and EL_MC (methylated EL) variants (Figure 32). The methylation of EL was specifically designed to regulate the hydroxyl content, optimizing compatibility with the PLA matrix. The CROP reaction was carried out to promote homopolymerization under starvation of the initiator (oxirane), leading to the formation of long polyether chains grafted onto the lignin core. The introduction of these polyether chains aimed to improve the compatibility and interfacial adhesion between the hydrophobic lignin and the non-polar PLA matrix. To fabricate lignin/PLA films, EL, EL_C, and EL_MC were individually dissolved in chloroform along with PLA, followed by solvent casting to produce films designated as EL_F (EL/PLA), EL_CF (EL_C/PLA), and EL_MCF (EL_MC/PLA). The glass transition temperatures (T_g) of the films followed a descending trend from EL_MCF to EL_CF, EL_F, and PLA, with the reduced T_g of the lignin-polyether (LPE)/PLA films indicating a plasticizing effect that enhanced the flexibility of the PLA matrix. Thermal stability (T_{max} , maximum weight loss temperatures) was highest for EL_MCF (395.86 °C), followed by EL_F (365.84 °C) and EL_CF (357.2 °C). EL_MCF showed superior mechanical properties with the highest elongation at break (297.7%) and toughness (39.92 MJ/m³) compared to EL_CF (242.07% and 28.19 MJ/m³) and neat PLA (72.58% and 18.41 MJ/m³). The tensile strength hierarchy was PLA (30.88 MPa) > EL_CF > EL_MCF > EL_F, with EL_F demonstrating poor mechanical properties. Additionally, the lignin/PLA films demonstrated remarkable UV-blocking capabilities. The EL_MCF film achieved outstanding UV protection, blocking 99.52% of UVA and 88.95% of UVB rays without significantly compromising transparency. These enhancements in mechanical performance and UV resistance underscore the potential of chemically modified lignin to expand the applicability of PLA in industries requiring durable and UV-stable materials.

Yu et al. introduced lignin's surface activity to stabilize reverse (water-in-oil) emulsions through interfacial interactions with polylactide (PLA) in the organic phase [104]. Unmodified alkali lignin was used to stabilize reverse (water-in-oil) emulsions through interfacial interactions with PLA in an organic, continuous phase (chloroform). Lignin was dispersed in the 1 M NaOH aqueous phase (up to 30 wt.%), while PLA was dissolved in chloroform (up to 10 wt.%). The aqueous lignin and PLA solutions were mixed in various ratios to form emulsions using ultrasonication (Figure 33). The emulsions were cast onto glass plates or coated onto hydrophilic substrates (glass or paper) to create composite films. The lignin-PLA interactions at the oil-water interface were critical for achieving long-term emulsion stability. Uniform composite films with lignin loadings as high as 95% (with PLA being the balance) were obtained by emulsion casting and coating onto hydrophilic sub-

strates. The lignin–PLA films demonstrated enhanced UV-blocking properties, especially in the UV-A region, compared to a commercial sunscreen, while maintaining transparency. The presence of PLA in the continuous matrix of the film imparted water resistance to the coated hydrophilic substrates.

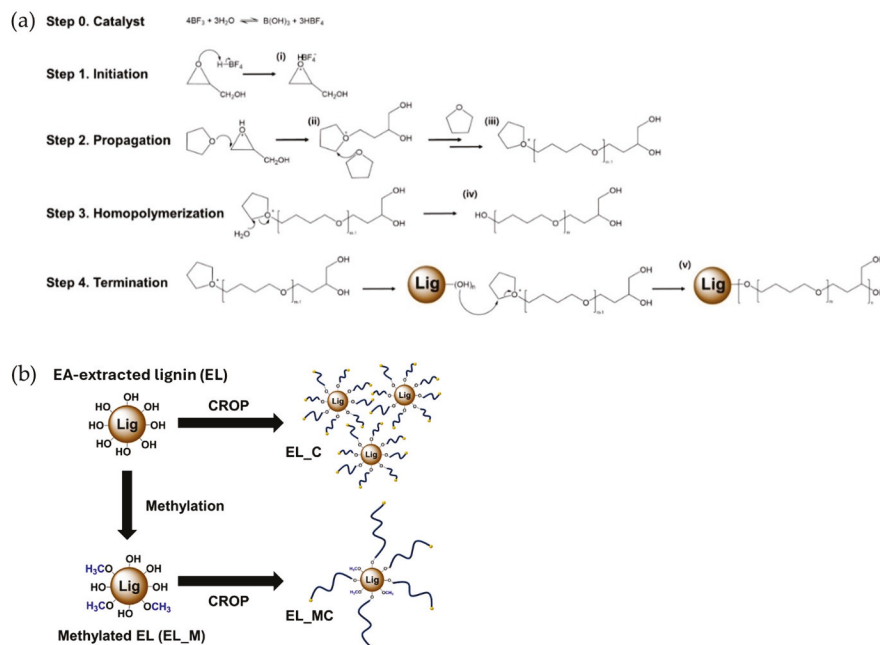


Figure 32. (a) The proposed mechanism of cationic ring-opening polymerization (CROP) reaction and (b) illustration for the preparation of EL_C and EL_MC [103]. Used with permission, copyright 2023 Elsevier.

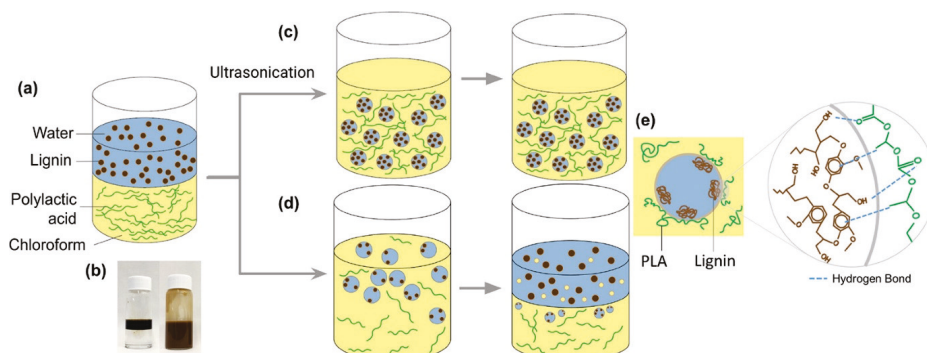


Figure 33. Unstable water-in-oil emulsion to stable dispersion using lignin and PLA. (a) Schematic of separate oil and water phases with their constituents before emulsification. (b) Mixture appearance before and immediately after sonication. (c) Stable W/O emulsion with aqueous lignin droplets suspended in a continuous PLA organic phase. (d) Unstable W/O emulsion showing droplet coalescence and phase separation due to insufficient lignin or PLA. (e) Close-up of a droplet interface with adsorbed lignin and PLA molecules, highlighting proposed intermolecular interactions [104]. Used with permission, copyright 2023 American Chemical Society.

Banpean et al. investigated the impact of lignin nanoparticles (SLNs) and microparticles (SLMs) on the crystallization behavior of poly(L-lactic acid) (PLLA) [105]. Softwood kraft lignin was fractionated with dichloromethane (DCM) to produce DCM-insoluble lignin for SLMs, while SLNs were obtained by further fractionating SLMs with acetone, followed by ultrasonication and centrifugation (Figure 34). Composite films with 1 wt.% of either SLNs or SLMs were prepared via solution casting. Both additives enhanced PLLA's crystallization rate, crystallinity, and nucleation density, with SLNs performing better due

to their smaller size. The PLLA/SLN composite required less energy for nuclei formation and had lower folding surface energy than neat PLLA and PLLA/SLM composites. The glass transition temperature (T_g) of PLLA remained unaffected by the addition of SLMs and SLNs.

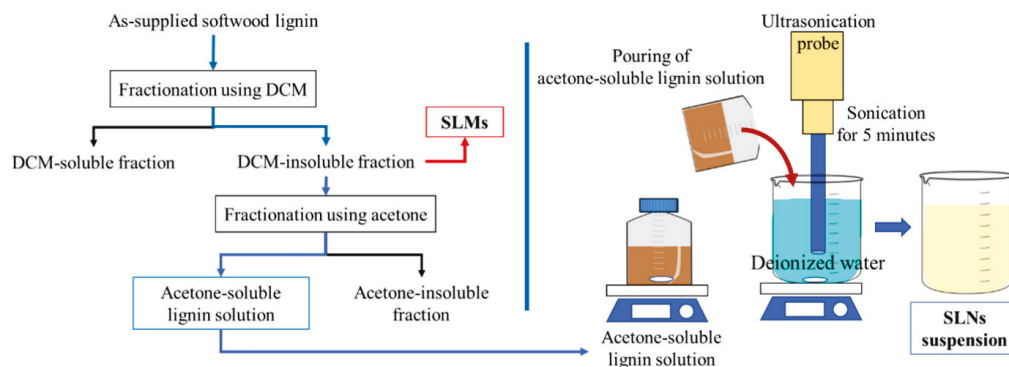


Figure 34. Preparation procedure for lignin nanoparticles (SLNs) and microparticles (SLMs) [104]. Used with permission, copyright 2023 Springer Nature.

Shakoor Shar et al. used low-viscosity epoxy resins, ethylene glycol diglycidyl ether (EGDE), and poly(ethylene glycol) diglycidyl ether (PEGDE) as compatibilizers to enhance lignin dispersion in the PLA matrix (Figure 35) [106]. These epoxies form chemical linkages or hydrogen bonds with PLA and lignin, improving compatibility and interfacial adhesion. PLA/lignin bio-composites were prepared with 1, 3, and 5 phr lignin loadings. Lignin addition increased PLA's onset degradation temperature by up to 15 °C and improved crystallinity. EGDE and PEGDE reduced the glass transition, crystallization, and melting temperatures, indicating their compatibilizing and plasticizing effects. Oxygen barrier properties were significantly enhanced, with PLA/EGDE/5LG and PLA/PEGDE/5LG showing up to 58.3% and 57.3% enhancement, respectively. Tensile strength observed only a modest increase (~15%), and compatibilizers did not significantly boost tensile strength. The composites demonstrated enhanced thermal stability, crystallinity, and oxygen barrier properties, showing promise for green packaging, though biodegradability was not assessed. This enhancement is attributed to a reduction in micro- and nanoscale porosity and the formation of a more tortuous diffusion path due to homogeneous dispersion of lignin domains within the polymer matrix. These effects collectively suppress oxygen permeability by limiting molecular transport, consistent with barrier improvements reported in lignin-polyester and lignin-PLA systems [115].

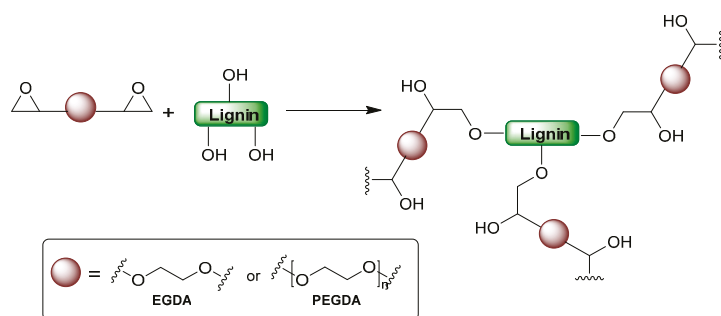


Figure 35. Chemical illustration of the reactivity of ethylene glycol diglycidyl ether (EGDE) and poly(ethylene glycol) diglycidyl ether (PEGDE) with lignin.

Poor mechanical properties limit the applications of PLA/lignin composites due to poor compatibility and low interfacial adhesion. Ou and coworkers addressed this by using epoxidized natural rubber (ENR) as a reactive compatibilizer, enabling the formation of interfacial crosslinking copolymers through reactions between ENR, PLA, and lignin during melt processing (Figure 36) [107]. The addition of ENR reduced interfacial gaps, blurred phase boundaries, and enhanced the elastic response, demonstrating improved compatibility. PLA/lignin/ENR composites were produced by melt compounding at 170 °C with a fixed PLA-to-lignin ratio (80:20) by changing the ENR content (1–10 wt.%). Increasing ENR content raised the glass transition temperature (T_g) of the composites due to reduced free volume and chain mobility. Mechanical testing showed that tensile strength and elongation at break for PLA/lignin (80/20) without ENR were 45.31 MPa and 2.9%, respectively. Tensile strength with 1 wt.% ENR increased by 15% to 52.03 MPa, and elongation at break improved by 77% to 4.6%. While higher ENR content (10 wt.%) further enhanced elongation at break to 20.1%, it reduced tensile strength to 38.37 MPa. Overall, ENR effectively enhanced the mechanical performance of PLA/lignin composites.

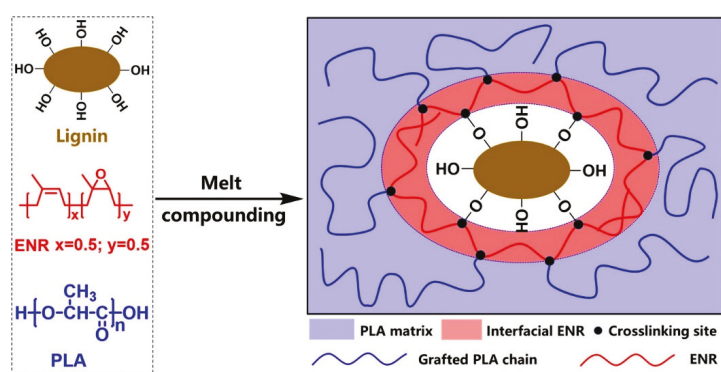


Figure 36. Mechanisms of interfacial reactions in preparing epoxidized natural rubber (ENR) compatibilized PLA/lignin composites [107]. Used with permission, copyright 2023 Elsevier.

Ding and coworkers developed PLA/lignin biocomposites for 3D printing by chemically modifying the lignin through grafting 2-ethylhexyl acrylate (EHA) to produce modified lignin (e-Lignin, Figure 37) [108]. The grafting improved interfacial interactions between lignin and PLA, enhancing dispersion and mechanical properties. The low melt viscosity of e-Lignin facilitated thermo-rheological relaxation, promoting PLA molecular chain mobility and making composites suitable for fused deposition modeling (FDM) 3D printing. Different PLA/e-Lignin specimens were prepared, adding 0–15 wt.% of e-Lignin to assess the properties. The addition of e-Lignin showed lower glass transition temperatures (T_g) and improved thermal stability, with higher decomposition temperatures ($T_{5\%}$) and maximum weight loss temperatures (T_{max}). Although the storage modulus (E') was lower than pure PLA below 50 °C, indicating reduced stiffness, higher $\tan \delta$ peaks suggested enhanced molecular chain mobility. While tensile strength and modulus decreased, the elongation at break and toughness significantly improved. The 10 wt.% e-Lignin composites showed the highest toughness (3.84 MJ/m³) and impact energy (6.36 KJ/m²) compared to pure PLA (1.16 MJ/m³, 2.12 KJ/m², respectively) in 3D-printed specimens. The enhanced toughness and impact energy were due to the synergistic effects of plasticization and the bridging effect of e-Lignin (Figure 38). The composites maintained suitable melt viscosity for 3D printing, with improved interlayer adhesion due to better interfacial diffusion. However, the long-term performance and aging behavior of the 3D-printed composites need to be assessed.

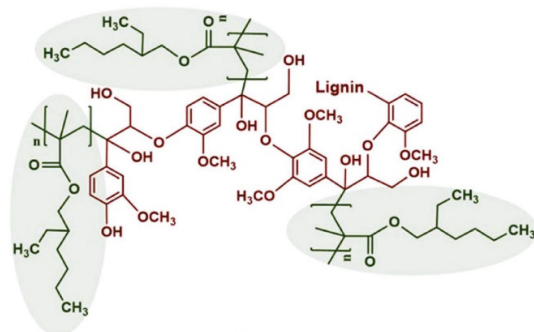


Figure 37. The predicted structure of e-Lignin by grafting 2-ethylhexyl acrylate (EHA) onto lignin [108]. Used with permission, copyright 2023 Elsevier.

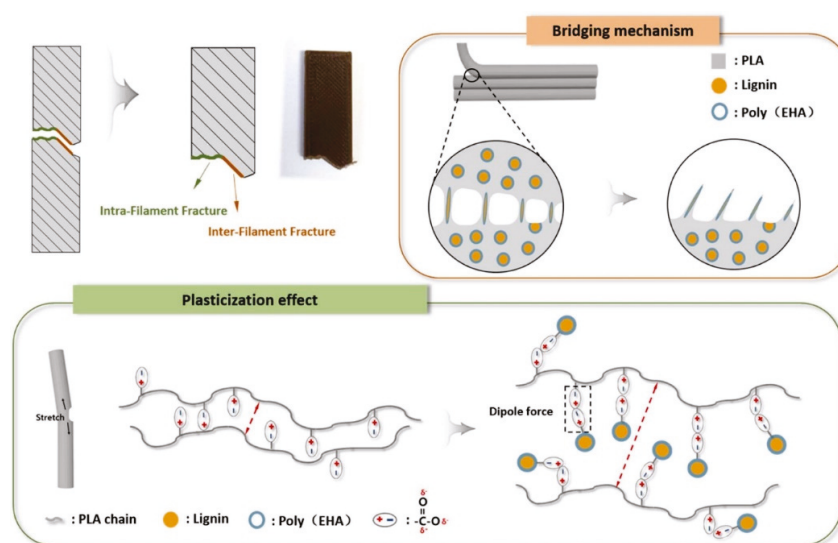


Figure 38. Proposed mechanism for the toughening effect in 3D-printed PLA/e-Lignin composites [108]. Used with permission, copyright 2023 Elsevier.

Makri et al. developed PLA composites incorporating lignin using two distinct techniques: conventional melt-mixing and in situ ring-opening polymerization (ROP) with reactive processing [109]. Composites containing 0.5 wt.% lignin or nano-lignin were investigated, with the in situ ROP method demonstrating a reduction in reaction time as catalyst content increased. Thermal analysis revealed that even at low lignin concentrations, the reactive processing method significantly influenced the thermal transitions of PLA. In the comparison of mechanical properties, the addition of 0.5 wt.% lignin or nano-lignin to neat PLA reduced the stress at break and elastic modulus, while elongation at break remained unaffected. Compared to conventional melt-mixing, where poor lignin dispersion and aggregation resulted in weakened properties, the in situ ROP method achieved superior mechanical performance due to enhanced lignin dispersion and improved interfacial bonding, maintaining or enhancing the material's mechanical properties.

Murillo-Morales et al. investigated the use of enzymatically modified lignin (EL) as a nucleating agent to enhance the mechanical properties and biodegradability of polylactic acid/thermoplastic polyurethane (PLA/TPU) blends for 3D printing applications (Figure 39) [110]. Alkali lignin (AL), extracted from *Bacillus ligniniphilus*, was partially degraded using a laccase enzyme to produce EL. PLA/TPU blends with varying ratios (90:10, 85:15, and 80:20) were prepared, incorporating AL and EL in different concentrations (1.25–5.00 wt.% based on PLA weight). The lignin-based thermoplastic composites were extruded into filaments and processed using fused deposition modeling (FDM) for 3D

printing. Notably, EL addition increased the elasticity modulus and elongation at break compared to AL filaments. The highest elasticity modulus (2.177 GPa) was observed with a low EL content (1.25 wt.%), representing a 239% increase compared to the control (0.9121 GPa). Additionally, the presence of EL led to a more moderate decrease in the molar mass than AL filaments, maintaining viscosity while enhancing elasticity. Overall, the incorporation of EL in PLA/TPU blends significantly improved the mechanical properties of the 3D-printed composites, providing tunable performance, a wood-like color, and enhanced biodegradability.

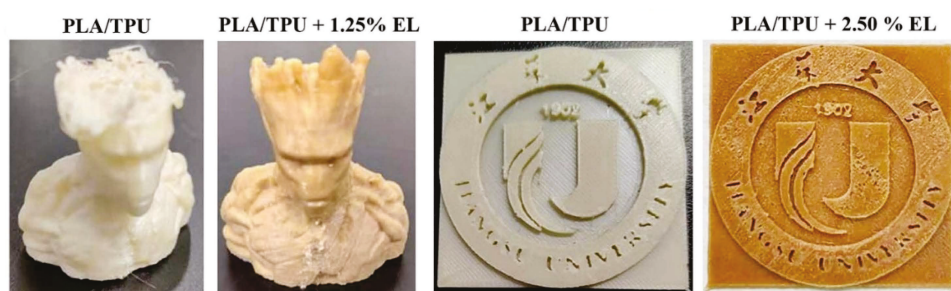


Figure 39. Representative images of routine FDM 3D-printed objects using a PLA/TPU blend alone and a PLA/TPU blend incorporating bacterial enzymatically modified lignin (EL) [110]. Used with permission, copyright 2023 Elsevier.

Ju et al. proposed a strategy to enhance the mechanical and thermal properties of polylactic acid (PLA) biocomposites by incorporating polyethylene glycol (PEG)-modified lignin as a functional filler [111]. Soda lignin and PEG-modified lignin were prepared from beech wood via steam blasting pretreatment, followed by two processes: alkali delignification and acid-catalyzed PEG solvolysis (Figure 40). The incorporation of PEG-modified lignin improved the heat resistance of the PLA matrix, enhancing its applicability in thermally demanding environments. Compared to pure PLA, the incorporation of unmodified soda lignin (PLA-L composites) showed a progressive reduction in tensile strength and elongation at break with increasing lignin content (Figure 41). This decline in mechanical properties is attributed to lignin's tendency to disrupt the continuity of the PLA matrix, inhibiting the formation of a uniform, long-range polymer phase. However, the tensile modulus increased, with PLA-L30 exhibiting an 18.6% higher modulus than neat PLA. In contrast, composites containing PEG-modified lignin (PLA-PL) demonstrated significantly improved mechanical properties. PLA-PL10 composites exhibited a 22% higher tensile strength than PLA-L10, demonstrating the compatibilizing effect of PEG chains. Furthermore, PLA-PL30 showed a 26.4% increase in tensile strength and a 78.9% increase in elongation at break compared to pure PLA, indicating that up to 30 wt.% of PEG-modified lignin can enhance both strength and flexibility, improving the mechanical performance.

Beniwal and co-workers investigated the improvements in mechanical strength, thermal resistance, and oxygen barrier performance of polylactic acid (PLA) composites through the incorporation of lignin functionalized with hexamethylene diisocyanate (HDI) to form lignin-grafted polyurethane (L-g-HDI) structures (Figure 42) [112]. The lignin was chemically modified via a heterogeneous reaction in dimethyl sulfoxide, introducing urethane linkages through HDI-improved compatibility with the PLA by enhancing hydrogen bonding and intermolecular interactions between the lignin and polymer matrix. When blended with PLA, the crosslinked lignin significantly enhanced the mechanical properties of the resulting composites, particularly in terms of tensile strength and elongation at break, due to the interactions. Optimal mechanical performance was observed at 14 wt.% L-g-HDI loading, with a remarkable 231% increase in tensile strength, elongation at break reaching 37.8%, and a sixfold increase in Young's modulus compared to neat PLA. However, further

increases in filler content (16 wt.%) led to diminished mechanical performance due to filler agglomeration and disruption of the polymer phase continuity. In addition to mechanical enhancements, the incorporation of L-g-HDI resulted in increased crystallinity, improved thermal stability, and enhanced barrier and water-resistance properties.

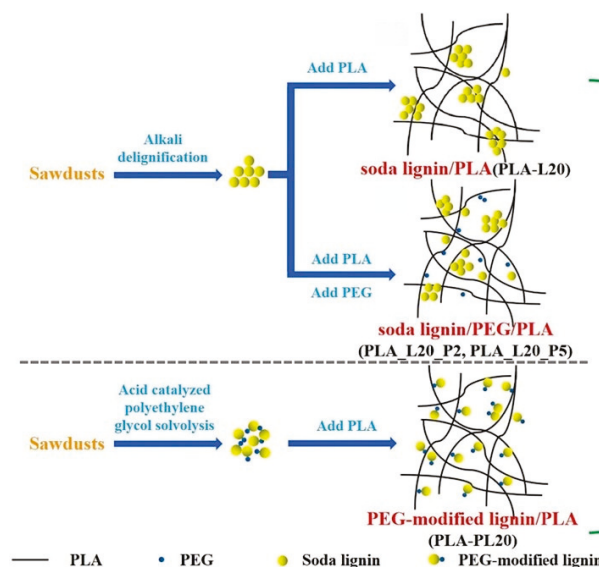


Figure 40. Different methods of polyethylene glycol (PEG) incorporation for the preparation of lignin/PLA composites [111]. Used with permission, copyright 2024 Elsevier.

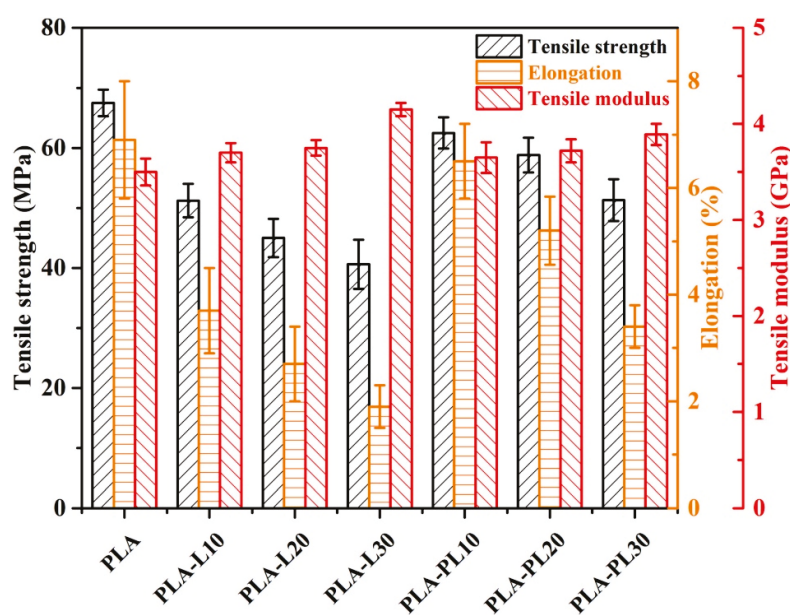


Figure 41. Mechanical properties of neat PLA, PLA composites with unmodified soda lignin (PLA-L), and PLA composites with PEG-modified lignin (PLA-PL) [111]. Used with permission, copyright 2024 Elsevier.

Li et al. developed a sustainable and efficient approach to modify lignin for enhanced performance in poly(lactic acid) (PLA) biocomposites. A mild hydroxyl-yne click reaction was employed to simultaneously functionalize both phenolic and aliphatic hydroxyl groups in alkali lignin (ALK) under low-temperature conditions without generating byproducts (Figure 43) [113]. In here, the hydroxyl-yne click reaction covalently attaches vinyl ether groups to both phenolic and aliphatic hydroxyls in lignin, increasing crosslink density and enhancing compatibility with the PLA matrix. This functionalization restricts polymer

chain mobility, thereby improving mechanical strength and modulus. Additionally, the introduced vinyl ether groups contribute to strong UV absorption due to their conjugated unsaturated bonds, indicating excellent UV shielding and stability. Incorporation of 3 wt.% of the modified ALK into PLA resulted in notable improvements in the mechanical properties of the resulting biocomposite films. Specifically, the yield strength, breaking strength, and Young's modulus increased by 114.2%, 30.7%, and 250.2%, respectively. Furthermore, the introduction of vinyl ether linkages through the click reaction reported excellent UV shielding (100% absorption) and anti-UV aging properties. Even at a low loading of 1 wt.% ALK, the biocomposites exhibited high visible light transmittance (84.3%) alongside remarkable UV stability. Moreover, after 3 h of UV irradiation, the composite maintained superior mechanical performance, with yield strength and Young's modulus increasing by 209% and 86%, respectively.

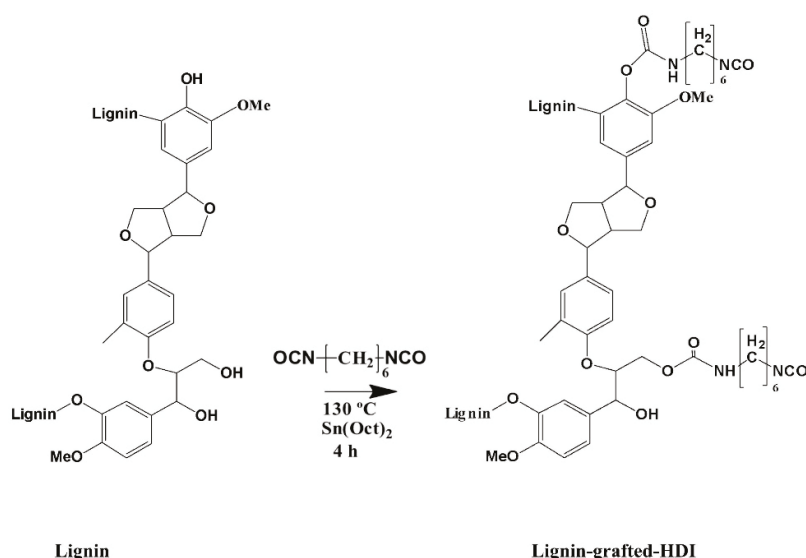


Figure 42. Schematic representation of the preparation method for lignin-grafted polyurethane (L-g-HDI) via the reaction of lignin molecules with hexamethylene diisocyanate (HDI) [112]. Used with permission, copyright 2024 Elsevier.

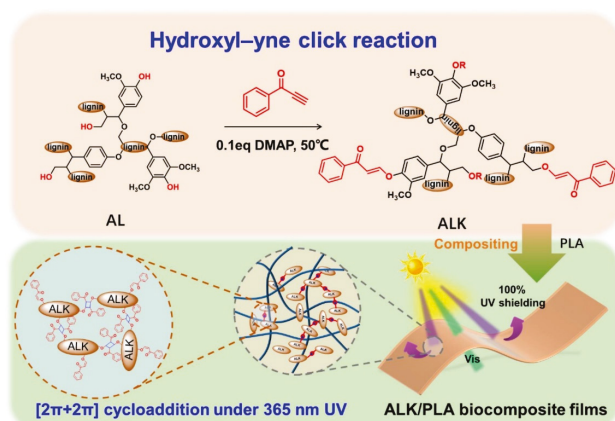


Figure 43. Schematic representation of the preparation of lignin derivatives via the hydroxyl-yne click reaction, enabling the development of UV-shielding and anti-aging alkali lignin/poly(lactic acid) (ALK/PLA) biocomposite films [113]. Used with permission, copyright 2024 Elsevier.

Cao et al. developed a novel bio-based modifier, OL@PDA, to address key limitations of polylactic acid (PLA), such as brittleness, low thermal stability, and vulnerability to hydrolysis. OL@PDA is synthesized by grafting low-molecular-weight lignin (OL) with

polydopamine (PDA), combining the structural benefits of lignin's aromatic rings with the adhesive and compatibility-enhancing properties of PDA (Figure 44) [114]. Incorporating OL@PDA into PLA led to significant performance enhancements. Specifically, the OL@PDA0.5/PLA composite (containing 0.5 wt.% of PDA) exhibited a 14% increase in tensile strength and a 42% increase in elongation at break, along with notable improvements in flexural and impact strength (Figure 45). These enhancements were attributed to improved interfacial compatibility and uniform nanofiller dispersion, which facilitated stress dissipation and triggered a transition from brittle to ductile fracture behavior. Thermal analysis revealed elevated decomposition temperatures, indicating enhanced thermal stability due to stronger interactions between OL@PDA and the PLA matrix. Additionally, OL@PDA significantly reduced UV transmittance from 65.8% (pure PLA) to less than 5%, providing effective UV shielding. While the presence of hydrophilic groups in PDA slightly lowered the contact angle, the overall hydrophobicity remained suitable for industrial use. These findings highlight OL@PDA as an effective and environmentally friendly approach to enhancing the multifunctionality of PLA-based materials.

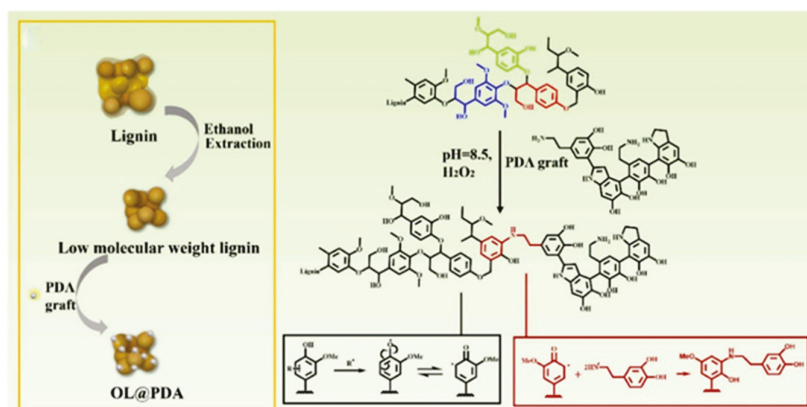


Figure 44. Schematic illustration of the preparation of dopamine-modified lignin (OL@PDA) via the grafting of polydopamine onto low-molecular-weight lignin (OL) [114]. Used with permission, copyright 2024 Elsevier.

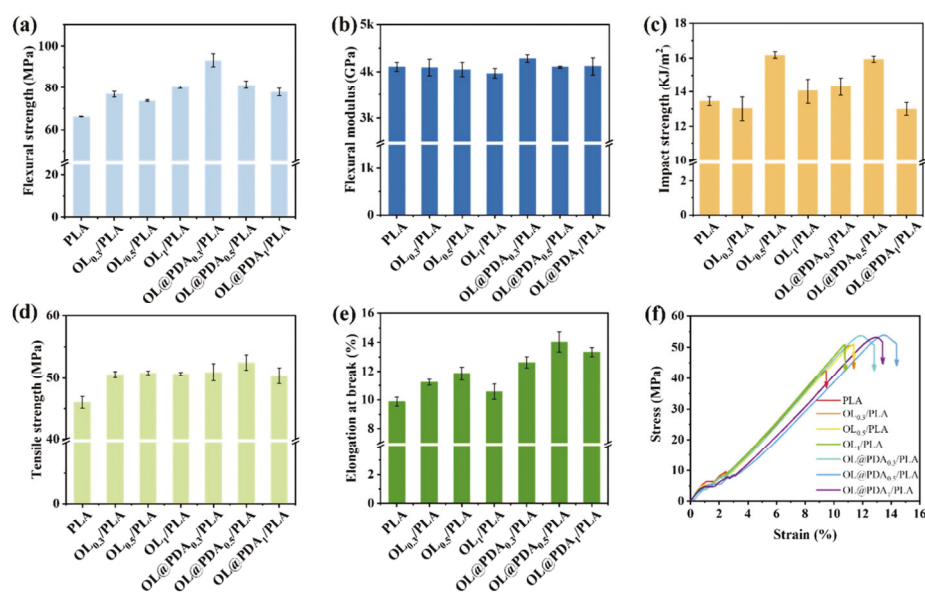
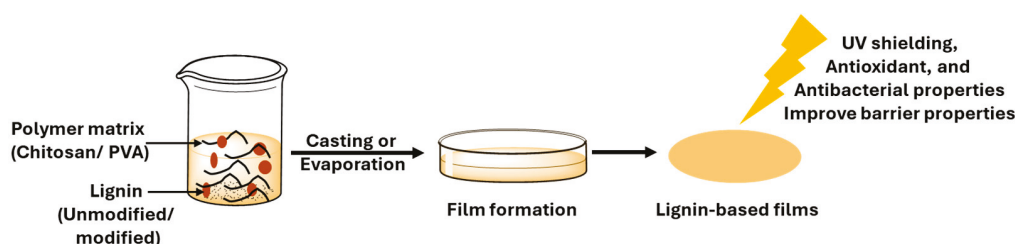


Figure 45. Comparison of mechanical properties between pure PLA and OL@PDA/PLA composites: (a) flexural strength, (b) flexural modulus, (c) impact strength, (d) tensile strength, (e) elongation at break, and (f) tensile stress–strain curves [114]. Used with permission, copyright 2024 Elsevier.

5. Other Strategies for Lignin Incorporation into Materials

5.1. Polymers with Covalently Attached Lignin

Non-biodegradable materials significantly contribute to long-term environmental pollution and waste accumulation. Currently, packaging materials account for nearly half of the total municipal solid waste. Therefore, identifying biodegradable alternatives for plastic films is crucial. One promising approach is the incorporation of unmodified or chemically crosslinked (e.g., via aldehyde or epoxy linkers) lignin into biodegradable plastics through blending or chemical crosslinking. Films can be formed via casting or solvent evaporation. In these systems, lignin not only enhances the mechanical strength and gas barrier properties of food packaging films but also imparts valuable functionalities such as antioxidant, antibacterial, and UV-blocking capabilities (Scheme 5) [116–119].



Scheme 5. Formation of lignin-based films and coatings via blending or chemical crosslinking.

Olonisakin and coworkers prepared a biodegradable polymer using lignin and tannic acid as fillers to modify poly(butylene adipate-co-terephthalate) (PBAT) [120]. Initially, lignin and tannic acid (Ta) were microencapsulated and mixed with epoxidized soybean oil (ESO) to form ESO-Lig-Ta (Figure 46), and the different amounts of ESO-Lig-Ta (0–3 wt.%) were then incorporated into the PBAT matrix to form PBAT-ESO-Lig-Ta composite film. Microencapsulation prevents premature aggregation of lignin and tannic acid, ensuring their uniform distribution within aqueous or polymeric matrices. It also enables controlled release functionality, improves interfacial adhesion, and protects phenolic hydroxyl groups from oxidative degradation—benefits that are particularly relevant in biomedical and barrier applications where component stability and homogeneity are critical. The modification involved the reaction between the phenolic groups of lignin and tannic acid with the epoxy rings of ESO, forming ether linkages in the ESO-Lig-Ta network, and a stable composite was formed by reacting ESO with the end groups of PBAT chains. ESO-Lig-Ta fillers improved the tensile strength, thermal stability, barrier properties, and biodegradability of the PBAT films. The loading of 3 wt.% ESO-Lig-Ta enhanced the UV-blocking ability by completely blocking UV-B radiation and reduced water vapor and oxygen transmission rates compared to neat PBAT, making them suitable for food packaging applications. The presence of lignin and tannic acid accelerated the biodegradation process through the hydrolysis of ester bonds to improve the sustainability of the film. Tensile strength values increased for 1 wt.% (7.27 MPa) and 2 wt.% ESO-Lig-Ta (6.55 MPa) compared to neat PBAT (3.13 MPa), and elongation at break decreased with increasing ESO-Lig-Ta loading compared to neat PBAT. For real-world applications of this film, further research may need to assess its long-term durability under varying packaging environments, such as changes in humidity, temperature, and mechanical stresses during handling and transportation.

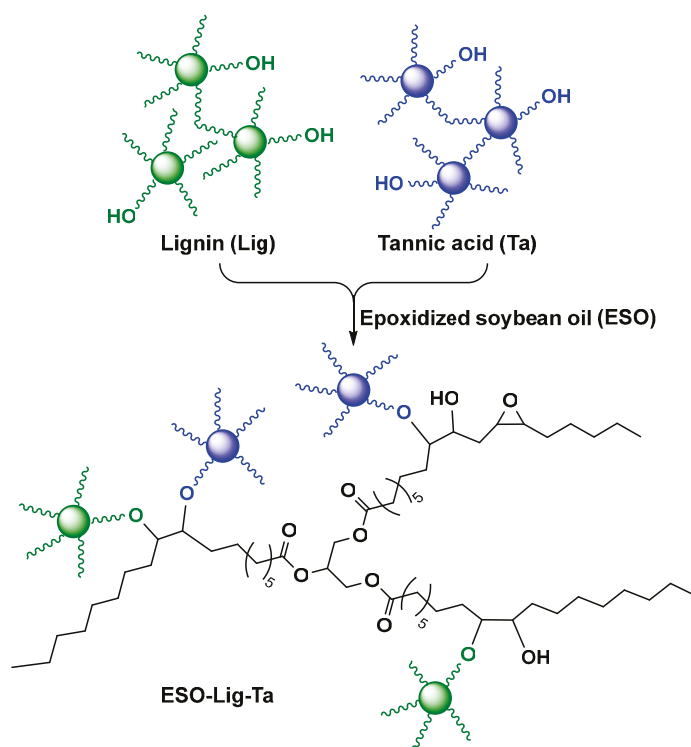


Figure 46. Schematic representation of the preparation of ESO-Lig-Ta by modifying lignin with tannic acid and epoxidized soybean oil.

Waqas Ali Shah and co-workers examined the preparation and properties of biodegradable films produced by grafting alkali lignin (AL) and enzymatically modified alkali lignin (EMAL) onto keratin using laccase and dye-decolorizing peroxidase enzymes [121]. Keratin and lignin components were dissolved separately, mixed with glycerol, cast into films, and dried before further analysis. The visual appearance of films is represented in Figure 47. The incorporation of EMAL improved the tensile strength, elongation at break, and thermal stability, and acted as an antimicrobial and antioxidant in the films. The films with EMAL modification showed greater tensile strength (14.8 ± 1.8 MPa) and elongation at break ($23.7 \pm 0.3\%$). The films exhibited good antibacterial properties against *Staphylococcus aureus* and *E. coli* and high antioxidant activity. Overall, the study introduced an approach for lignin valorization to produce sustainable biomaterial films with improved properties.

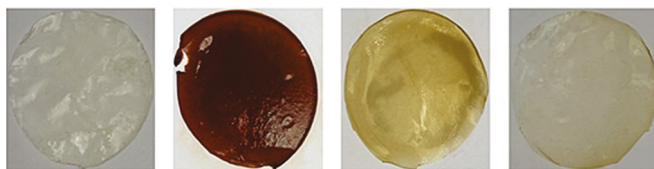


Figure 47. Visual appearance of keratin film, alkali lignin film, decolorized/ modified lignin by laccase, and decolorized/modified lignin by dye-decolorizing peroxidase (left to right) [121]. Under a Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/> accessed on 27 April 2025).

The production and refining of petroleum-derived polymers contribute to environmental degradation, including high carbon emissions and the depletion of non-renewable resources. Bio-based fillers, derived from renewable resources, are essential to reduce the reliance on petroleum-based raw materials [122]. Baniasadi and coworkers successfully developed sustainable, high-performance bio-composites using alkali lignin particles [123]. These lignin particles were surface-modified by grafting *n*-octadecyl isocyanate

molecules onto the lignin hydroxyl groups via a urethane reaction (Figure 48a). The copolyamide matrix (PA11coPA1210) was synthesized by copolymerizing castor oil-based monomers (11-aminoundecanoic acid and sebacic acid) with a petroleum-based monomer (1,12-diaminododecane) (Figure 48b). Following the surface modification of lignin particles, these were incorporated (10–50 wt.% of modified lignin) into the copolyimide matrix to develop a low-melting-point bio-based film. The results showed a significant increase in storage modulus with higher lignin content, indicating improved component compatibility, mechanical properties, and bio-content through tailored lignin surface chemistry and copolymer composition. Future work would be needed to optimize lignin modification processes by exploring alternative bio-based monomers and non-toxic compounds to enhance the performance and sustainability of these composites.

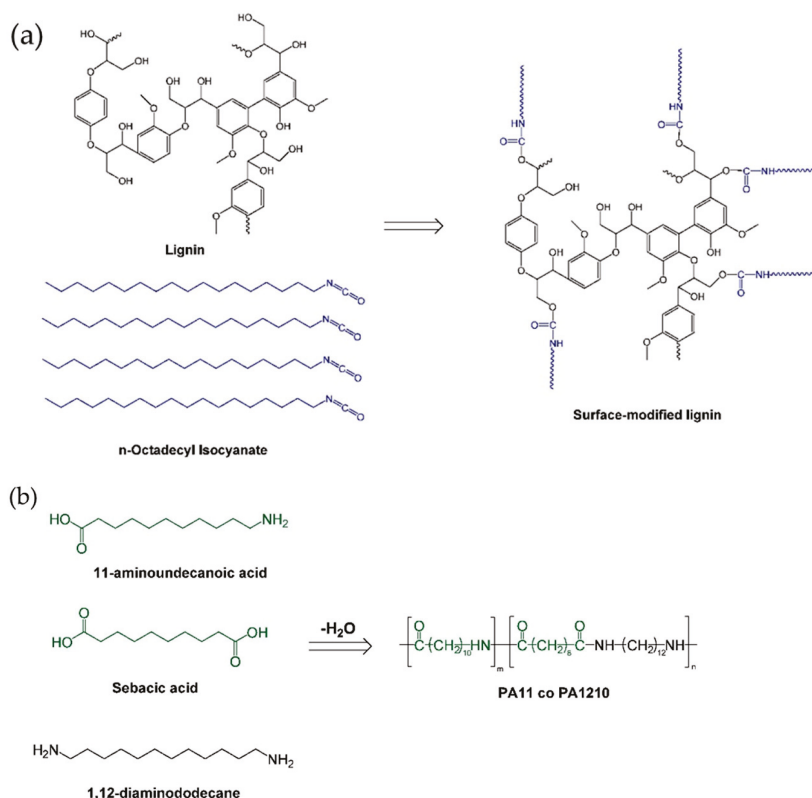


Figure 48. (a) Schematic representation of the surface-modified lignin preparation and (b) copolymerization of castor oil-based and petroleum-based monomers to form the copolyimide matrix [123]. Under a Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/> accessed on 27 April 2025).

Tian and their research group modified lignin through a cyanoethylation reaction to make it water-soluble. Cyanoethylation is an etherification reaction where phenolic hydroxyl groups on lignin are substituted with cyanoethyl groups, improving water solubility due to disruption of hydrogen bonding between lignin fragments (Figure 49) [124]. This was completed under mild conditions using acrylonitrile. The cyanoethylated lignin (CEL) was then used to modify poly (vinyl alcohol) (PVA) as the polymer matrix. CEL acted as both a biomimetic bonding adhesive and a functional additive. It was coated onto cellulose nano-whiskers (CNWs) to form a multi-functional building block with a mussel-inspired layered structure. This CEL@CNW building block was blended with an aqueous PVA solution to create a liquid mulching film suspension. CEL improved the thermal stability of the PVA composite films by decreasing the maximum thermal decomposition rate compared to pure PVA or PVA/CNW films, and the tensile strength of the PVA/CEL@CNW

films decreased as the percentage of CEL@CNW increased. Specifically, the tensile strength decreased from 33.6 MPa for pure PVA to 25.2 MPa for PVA/10% CEL@CNW. Overall, CEL modification improved the compatibility of lignin (typically insoluble) with the PVA matrix, including various performance properties like thermal stability, mechanical strength, and barrier abilities due to aromatics and phenolic functionalities in lignin.

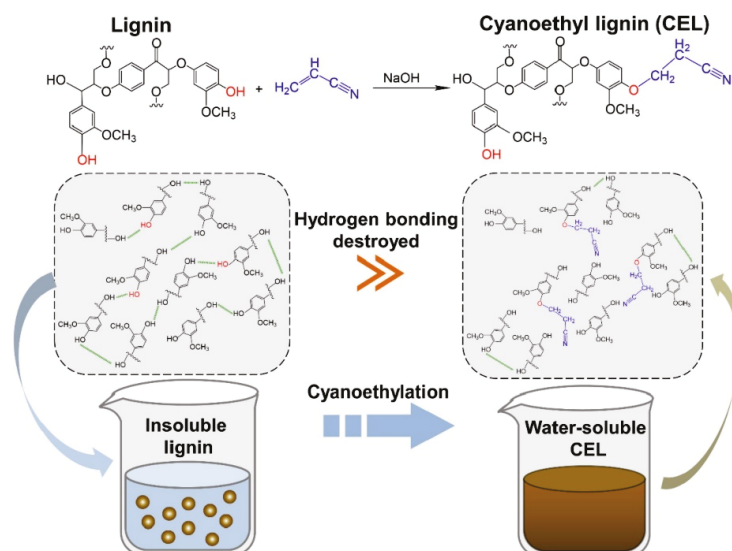


Figure 49. Reactions in the formation of cyanoethyl lignin (CEL) and possible mechanisms of water solubility of CEL [124]. Used with permission, copyright 2023 Elsevier.

Xu and coworkers designed a silver nanoparticle (AgNP) doped chitosan/sodium lignin sulfonate/polypyrrole (p₁i₁) film with antibacterial and endotoxin adsorption properties. The films were fabricated using a one-step electrodeposition method to produce a chitosan/Ag/polypyrrole film, followed by electrostatic layer-by-layer self-assembly of lignin sulfonate (Figure 50) [125]. Lignin sulfonate (LS), a negatively charged polyelectrolyte, was introduced through electrostatic interactions with the positively charged chitosan. The self-assembly of LS increased the film's negative zeta potential and hydrophilicity. The adsorption experiments found that the electrostatic interactions between chitosan and endotoxins played a significant role in removal, with efficiencies up to 85–87% within 1 h. Overall, the film with LS showed the inhibition of *E. coli* and *S. aureus* bacteria and rapid adsorption of endotoxins released from decomposed bacteria, indicating that the addition of LS improved antibacterial effects.

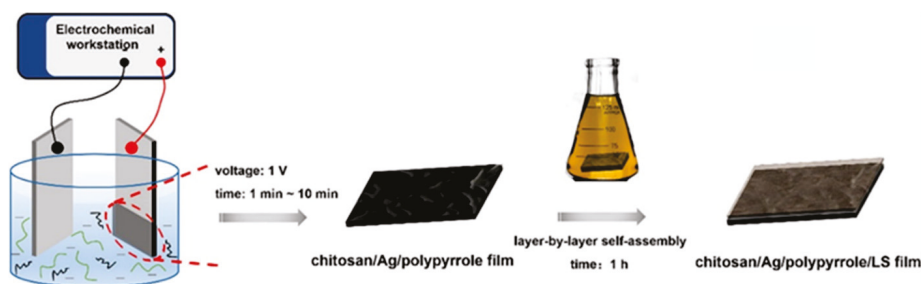


Figure 50. The synthetic pathway of silver nanoparticle (AgNP) doped chitosan/sodium lignin sulfonate/polypyrrole (p₁i₁) films [125]. Used with permission, copyright 2023 Elsevier.

This study investigated the use of a ternary deep eutectic solvent (TDES) based on benzyl trimethylammonium chloride, formic acid, and maleic acid for the pretreatment of radiata pine biomass (Figure 51) [126]. The TDES removed 87.80% of lignin and 89.15% of hemicellulose while preserving most cellulose. The pretreated material was then mechanically fibrillated to produce lignin-containing cellulose nanofibrils (LCNFs). The LCNF films showed good mechanical properties, thermal stability, and barrier properties against oil and air, indicating potential applications as packaging materials. The LCNF-130 (treated at 130 °C) film achieved the highest tensile strength at 52.0 MPa and exhibited superior ductility with an elongation at break of 8.4%, attributed to its lower lignin content. LCNF films with reduced lignin content also demonstrated increased elastic modulus, suggesting improved stiffness and resistance to deformation. Overall, the TDES pretreatment conditions improved the tensile strength, elongation at break, and elastic modulus of the LCNF films.

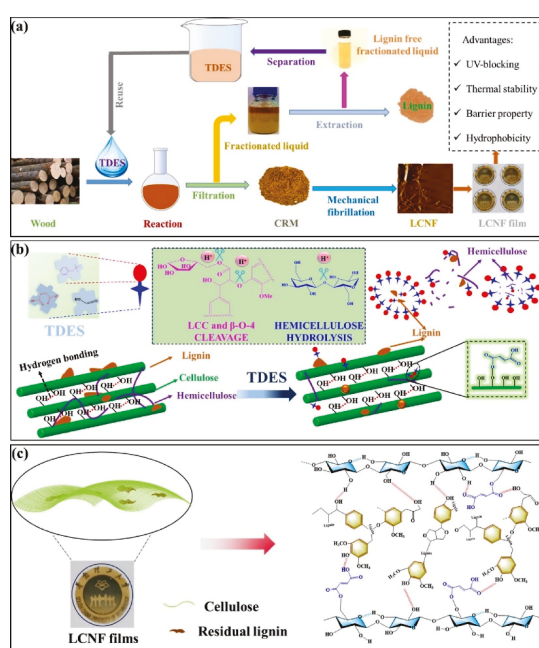


Figure 51. (a) Schematic illustration of TDES pretreatment process, (b) pretreatment mechanisms of TDES on radiata pine, and (c) the structural linkages between lignin and cellulose in the LCNF [126]. Used with permission, copyright 2023 Elsevier.

Sustainable composite biofilms, which incorporate lignin nanoparticles (LNPs) into a starch matrix, were developed by Sun and coworkers to enhance the mechanical, thermal, optical, and antioxidant properties of films used in food packaging. LNPs were produced from Moso bamboo lignin using a two-step pretreatment and self-assembly process, and starch/LNP composite films were made using solution casting by adding different concentrations (0–5 wt.%) of LNPs (Figure 52) [127]. The modification after adding LNPs significantly improved the composite film's tensile strength (48.9 MPa) and elastic modulus (2288.8 MPa) compared to the pure starch film. Figure 53 illustrates the distribution of LNPs in the starch matrix, forming hydrogen bonds with good compatibility and physical reinforcement with starch to improve properties. Additionally, the composite films exhibited excellent UV shielding. They significantly delayed the oxidative deterioration of soybean oil during storage by lowering its peroxide, saponification, and acid values, indicating their potential application in food packaging.

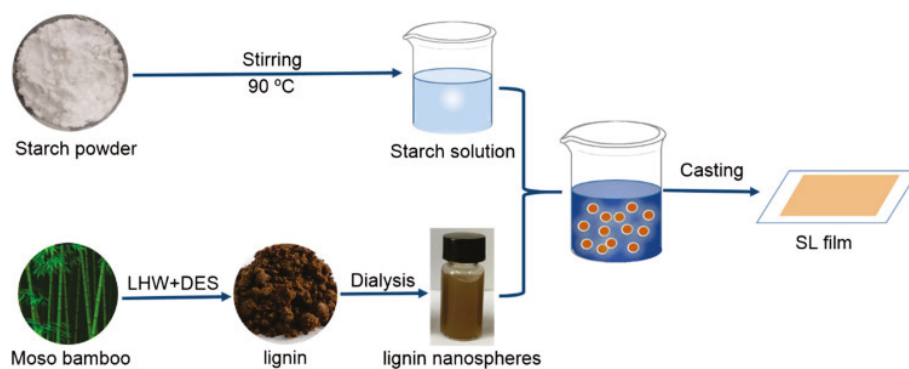


Figure 52. The preparation pathway of the starch/LNPs composite film [127]. Under a Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/> accessed on 27 April 2025).

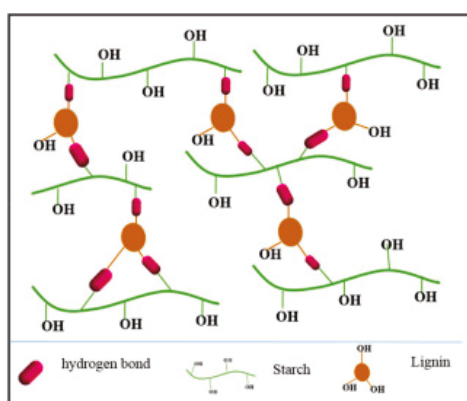


Figure 53. The schematic illustration of the interaction between LNPs and starch matrix [127]. Under a Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/> accessed on 27 April 2025).

In their study, Rodrigues et al. prepared thermoplastic starch (TPS) films with additions of different fractions of kraft lignin (KL) to enhance their properties after exposure to ultraviolet C (UV-C) light [128]. Using ethyl acetate as a solvent, KL from *Eucalyptus* wood was fractionated into insoluble (INS) and soluble (SOL). Films were prepared by mixing the lignin fractions (4 wt.% KL, SOL, INS) into a TPS matrix. The films were exposed to UV-C (254 nm) light for 432 h and analyzed before and after exposure. Figure 54 represents the preparation and characterization of KL-modified TSP films. SOL-modified TSP films exhibited the highest photoprotection and stabilizing effect as a UV blocker in the TPS matrix due to higher phenolic content. The maximum force of SOL-modified TSP (14.37 N) was improved compared to pure TSP (5.58 N) after exposure to UV, indicating the enhancement of crosslinking between hydroxy functionalities in lignin. Overall, SOL-modified TSP films showed minimal structural changes, better thermal stability, higher crystallinity, and improved mechanical properties after UV exposure compared to other films.

Pham and co-workers created a chitosan film incorporating lignin, obtained as a byproduct from the black liquor generated during the cellulose isolation process from rice straw without further modification. The different concentrations of lignin (0–8 g/L) were combined with a 2% (*w/v*) solution of chitosan (a biopolymer derived from shrimp shells) to form chitosan/lignin composite films via a simple solution casting method [129]. The addition of lignin to the chitosan films improved the UV-blocking ability (100% UV light blocking even at low lignin concentrations), antioxidant activity (DPPH radical scavenging activity increased from 56% for pure chitosan to over 91% with lignin), and antimicrobial

activity against *E. coli* and *C. albicans*, and reduced water vapor permeability compared to pure chitosan films. This enhancement is attributed to the intrinsic UV-absorbing capacity of lignin's conjugated aromatic structures, which strongly absorb in the 280–340 nm range. Additionally, lignin's phenolic hydroxyl groups act as free radical scavengers, disrupting UV-induced oxidative chain reactions and thereby stabilizing the chitosan matrix against photodegradation. The films with 2 g/L lignin concentration showed the highest tensile strength of 16.637 N/mm² compared to 6.578 N/mm² for the pure chitosan film. Conversely, a lower lignin content (1 g/L of lignin) exhibited a higher elongation at break than pure chitosan, which decreased with increasing lignin loading. Furthermore, high lignin incorporation (8 g/L lignin) showed the highest Young's modulus of 676.578 N/mm² compared to pure chitosan film (13.986 N/mm²), indicating that lignin acts as a rigid filler, stiffening and reinforcing the chitosan matrix. Overall, low-to-moderate lignin loadings enhanced tensile strength and elongation, while high lignin concentrations increased stiffness but compromised the tensile properties of the chitosan films due to possible agglomeration effects.

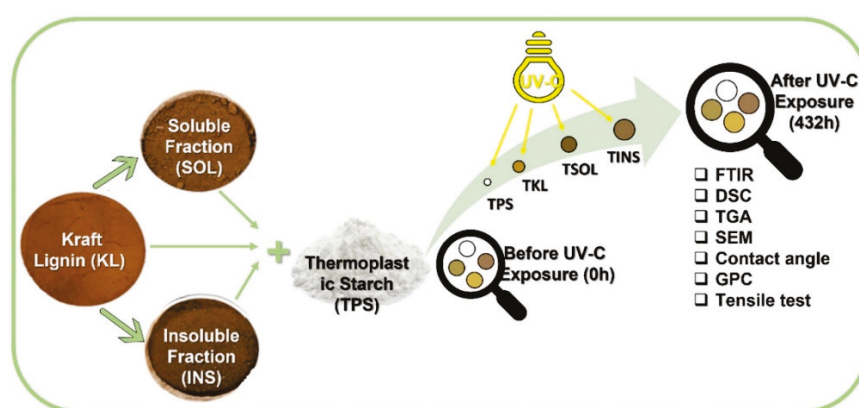


Figure 54. Preparation and characterization of KL-modified TSP films [128]. Used with permission, copyright 2023 Elsevier.

Ou and researchers modified lignin through phenolation and employed the resultant lignin nanoparticles (LNPs) to reinforce both cellulose nanofibril (CNF) composite films as well as polyvinyl alcohol (PVA) films. The kraft lignin (KL) was reacted with phenol under acidic conditions to increase phenolic hydroxyl group content and reduce lignin's molecular weight. The anti-solvent precipitation method was used to convert phenolated lignin to LNPs, and different amounts of LNPs (0–30 wt.%) were mixed with CNFs to prepare LNP/CNF films (Figure 55) [130]. LNP/PVA films were prepared by mixing 5 wt.% LNPs and 5 wt.% of PVA. The strengthening effect of introducing phenolated LNPs (P-LNPs) into CNF films is shown in Figure 56, indicating intermolecular hydrogen bonds. The formation of more hydrogen-bonding interactions between LNPs and hydroxyl groups in CNFs or PVA exhibited a high dispersity of LNPs, and significantly improved tensile strength (~190 MPa), toughness (~15 MJ/m³), and UV-shielding properties (~99%) of LNP-inserted films compared to the neat CNF/PVA films or composites with unmodified lignin.

In another study, Gao et al. developed a lignin-modified film through the amination of lignin. Lignin was aminated via the Mannich reaction to produce aminated lignin (aL) and complexed with Fe(III) by impregnating it in a Fe(NO₃)₃ solution to form the aL/Fe(III) complex [131]. Then, the complex was blended with polyvinyl alcohol (PVA) in different ratios (0:10, 1:9, 1:4, 3:7, 2:3, and 1:1) in an aqueous solution, and the mixture was cast into a film named (aL/Fe(III)/PVA). The tensile strength of the aL/Fe(III)/PVA (3:7) film was increased to 3.6 MPa compared to pure PVA (3.3 MPa). Elongation at break and fracture resistance also increased compared to PVA film by 107.8% and 21.9%, respectively.

During the reaction, the introduced amino groups on lignin enhance hydrogen bonding and interfacial interactions with the hydroxyl-rich PVA matrix, while Fe(III) chelation forms coordination crosslinks that improve structural integrity. This dual crosslinking network results in enhanced tensile strength and elongation. Overall, the synergistic effects between the lignin, PVA, and the iron chelation enhanced the performance of the final material, including mechanical, UV-blocking, hydrophobicity, and slow-release properties compared to pure PVA.

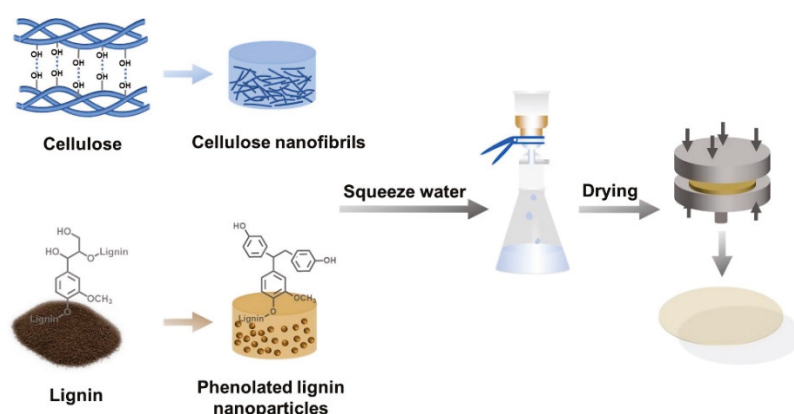


Figure 55. The schematic diagram for preparing LNP/CNF composite film [130]. Used with permission, copyright 2023 Elsevier.

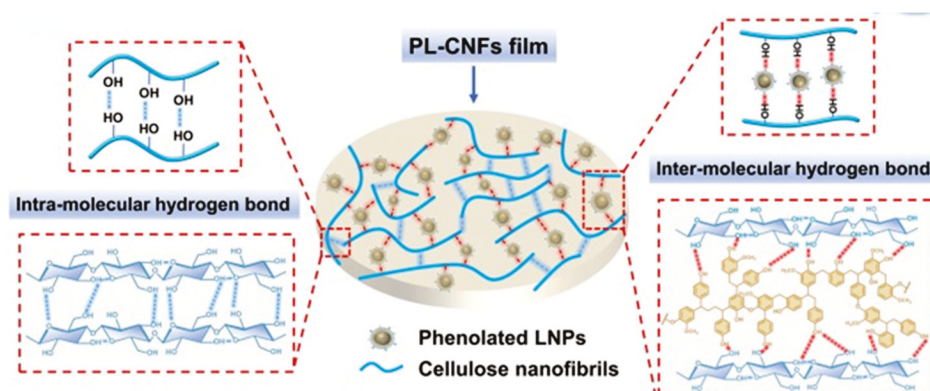


Figure 56. The proposed strengthening effects of phenolated LNP (P-LNPs/CNF) films exhibiting the hydrogen bonds [130]. Used with permission, copyright 2023 Elsevier.

Jiang H. and coworkers reported sustainable packaging material using biodegradable lignocellulose. As an initial step, deep eutectic solvent (DES) pretreatment (a mixture of choline chloride and lactic acid) was employed at a designated temperature and time to improve the solubility of lignin-containing cellulose (CL), varying lignin contents (14.1–21.2 wt.%) [132]. The modification facilitated the dissolution in the $\text{AlCl}_3/\text{ZnCl}_2$ aqueous system, reducing the water demand for the dissolution process (Figure 57). The dissolved CL samples were regenerated as lignocellulose films (FCLs) by casting and solvent removal. Due to their lignin content, the FCL films exhibited excellent UV-blocking, hydrophobic, mechanical strength, and natural degradation properties. The primary thermal decomposition for all samples occurred between 250 and 350 °C, and CL films with 14.1–15.6% lignin contents showed higher tensile strengths.

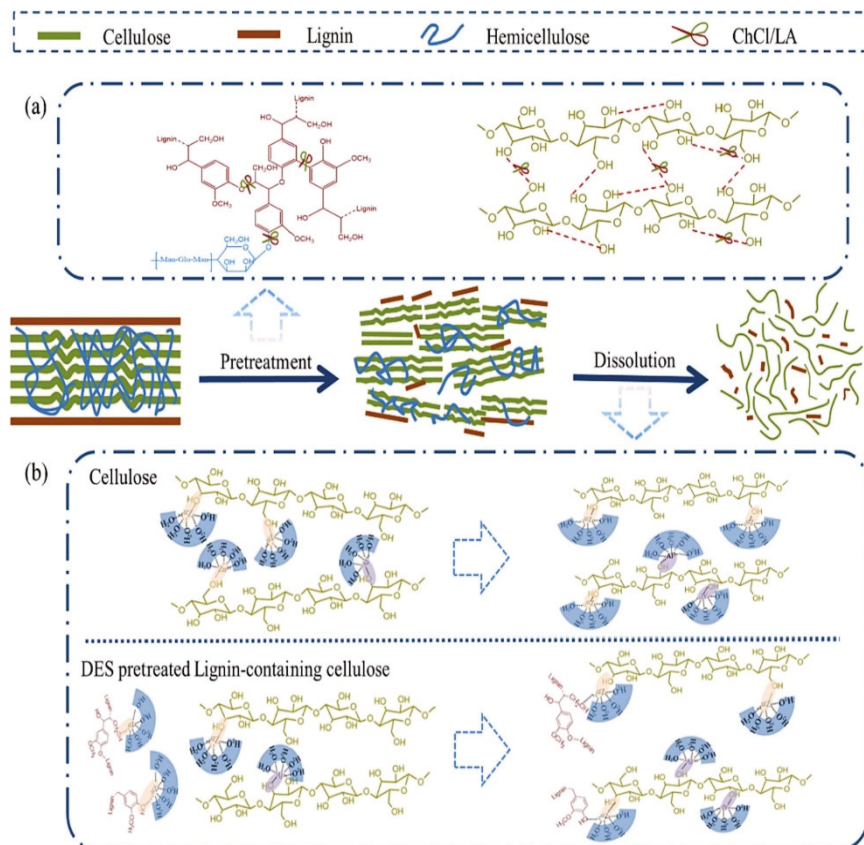


Figure 57. Descriptive diagram for DES pretreatment on lignin and cellulose (a), dissolution process of cellulose and (b) lignin-containing cellulose after DES pretreatment in $\text{AlCl}_3/\text{ZnCl}_2 \cdot 3\text{H}_2\text{O}$ solutions [132]. Used with permission, copyright 2023 Elsevier.

In another study, Zolfaghari et al. developed an eco-friendly biopolymeric film derived from potato crop residues as a sustainable alternative to conventional plastic food packaging [133]. Lignin was extracted using the organosolv method, achieving a high recovery yield of 73.5 wt.% under optimized conditions. The extracted lignin was incorporated into a starch matrix to form biocomposite films, and their physicochemical properties were compared with those of neat starch films. Notably, the addition of 1 wt.% lignin increased thermal stability by 25% and antioxidant properties by 78%, while reducing oxygen permeability by 87%. Furthermore, films containing 3 wt.% lignin exhibited the lowest water vapor transmission rate, indicating superior barrier performance and increased resistance to moisture penetration. Mechanical characterization (Figure 58) revealed that the incorporation of 1 wt.% lignin significantly improved Young's modulus and maintained comparable ultimate tensile strength relative to the neat starch films. These enhancements were attributed to strong intermolecular interactions between the lignin and starch hydroxyl groups. However, increasing lignin content beyond 1 wt.% resulted in a decline in tensile properties, likely due to the inherent brittleness of lignin disrupting the polymer network. Overall, films containing 1 wt.% lignin demonstrated the most optimized performance, showing considerable potential for use in sustainable food packaging.

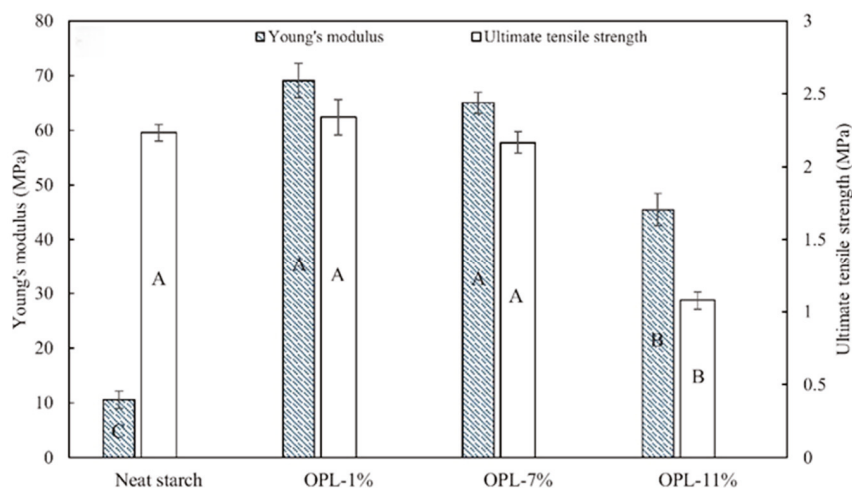


Figure 58. Mechanical characterization data of neat starch and the lignin-modified bio-based films [133]. Different letters (A, B and C) indicate significant differences at $p < 0.05$. Used with permission, copyright 2024 Elsevier.

Warale and co-workers fabricated novel poly(vinyl alcohol) (PVA) films by incorporating silver-embedded clay and organosolv lignin via a solution casting approach [134]. A series of composite films was prepared by varying the weight percentage (0–5 wt.%) of lignin-functionalized silver nanoparticles with halloysite nanotubes (Lig/AgNP-HNTs). With increasing Lig/AgNP-HNTs content, the films exhibited enhanced thermal and mechanical stability compared to neat PVA. As illustrated in Figure 59, the film containing 5 wt.% Lig/AgNP-HNTs exhibited the highest tensile strength (28.56 MPa) and elongation at break (59%), while the film with 3 wt.% addition showed a higher Young's modulus. Furthermore, compatibility tests suggested that these lignin-modified films hold promise as potential wound-healing materials, pending further validation through pre-clinical and clinical evaluations.

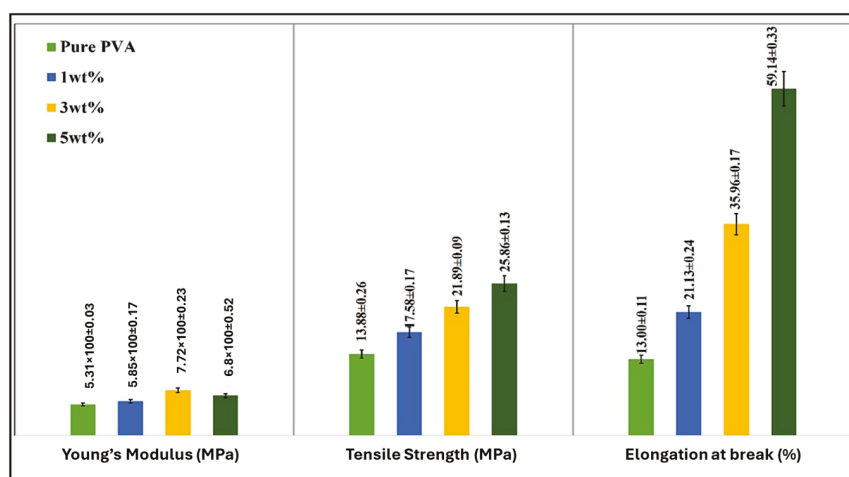


Figure 59. Mechanical characterization data of the prepared lignin-modified films [134]. Used with permission, copyright 2023 Elsevier.

Domínguez-Robles and researchers developed wound dressing (medical) materials with antioxidant and antimicrobial properties by incorporating curcumin (CUR) and lignin (LIG) into poly(caprolactone) (PCL), a biocompatible polymer (Figure 60) [135]. D-panthenol (DPA) was also included in the formulation of composite materials due to its skin regenerative ability, and materials were manufactured using semi-solid extrusion (SSE) 3D

printing without solvents. These 3D-printed dressings sustained DPA and CUR release for 4 and 35 days, respectively. The presence of LIG and CUR provided antioxidant properties to the 3D-printed dressings, as confirmed by the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. The 3D-printed materials showed a marked resistance to the adherence of *Staphylococcus aureus*, with substantial reductions of up to 89.9% and 98.9% after 4 h and 24 h of incubation, respectively. The combination of CUR and DPA exhibited the most significant values for cell viability and proliferation in in vitro studies. In vivo, wound-healing studies in Wistar rats showed that the dressings containing CUR and DPA exhibited marked improvement at any stage of the treatment process.

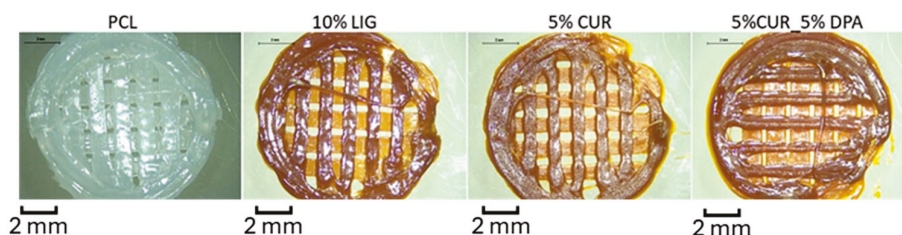


Figure 60. Light microscopic images of the 3D-printed wound dressings containing different proportions (*w/w*) of all the compounds (Scale bar is 2 mm) [135]. Under a Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/> accessed on 27 April 2025).

Surface bionics and interfacial chemistry advancements have led to the development of specially designed wettability materials, enabling efficient oil–water separation. Superhydrophobic and superoleophilic materials, when combined with lignin, demonstrate exceptional capabilities in removing oil, dyes, and other organic impurities from water [136–141]. Recently, titanium carbide-based separation materials ($\text{Ti}_3\text{C}_2\text{T}_x$) have been utilized in wastewater treatment. However, their limited functionality restricts their effectiveness in treating complex oily wastewater. Modifying the surface is one of the best solutions to overcome this concern. Wang et al. functionalized the surface of $\text{Ti}_3\text{C}_2\text{T}_x$ with enzymatic hydrolysis lignin (EHL), which increased its hydrophilicity and stability through secondary bonds formed by its oxygen-containing groups on the surface of the $\text{Ti}_3\text{C}_2\text{T}_x$ MXene [136]. Figure 61 exhibits the preparation pathway of EHL-functionalized $\text{Ti}_3\text{C}_2\text{T}_x$ (EHL- $\text{Ti}_3\text{C}_2\text{T}_x$). Films were applied to the surface of the PU sponge and mixed cellulose ester (MCE) membrane to prepare the EHL- $\text{Ti}_3\text{C}_2\text{T}_x$ @PU sponge and EHL- $\text{Ti}_3\text{C}_2\text{T}_x$ @MCE membrane, respectively. According to the results, the EHL- $\text{Ti}_3\text{C}_2\text{T}_x$ @PU sponge efficiently separated various oil–water mixtures (>99.5%) with a permeation flux of up to $321 \text{ L m}^{-2} \text{ h}^{-1}$ in the case of a cyclohexane/water mixture and showed excellent chemical stability in corrosive environments. Moreover, the EHL- $\text{Ti}_3\text{C}_2\text{T}_x$ @MCE membrane selectively removed cationic dyes, achieving rejection rates of 99.1% for malachite green and 98.1% for methylene blue, with ~72% permeation flux compared to pure water. Overall, the super-hydrophilic micro-scale pore structure in functionalized PU significantly improved oil–water separation efficiency. The enhanced interactions and considerable interlayer distance in functionalized MCE membranes yielded highly effective dye removal (Figure 62). Further research would be needed to overcome the potential fouling, challenges in scalability, selective removal of specific contaminants, and uncertain long-term stability in real-world wastewater treatment.

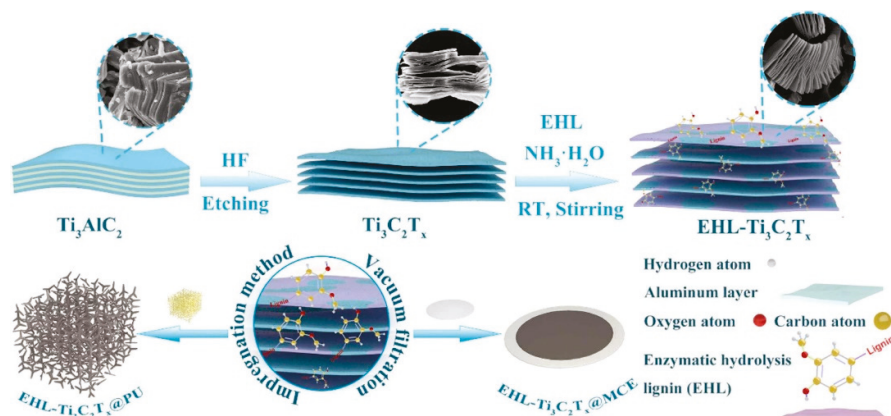


Figure 61. Preparation processes of EHL- $\text{Ti}_3\text{C}_2\text{T}_x$, EHL- $\text{Ti}_3\text{C}_2\text{T}_x$ modified PU sponge, and MCE membrane [136]. Used with permission, copyright 2023 Elsevier.

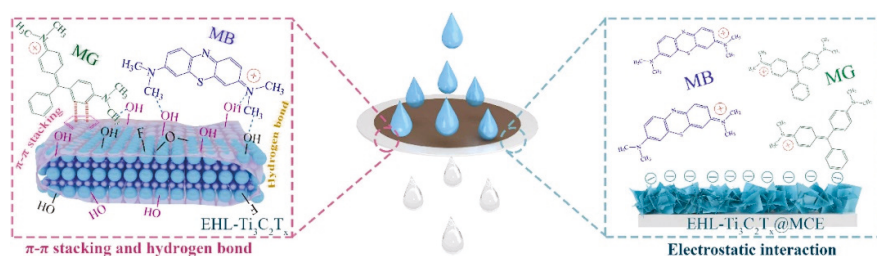


Figure 62. The different mechanisms involved in dye separation using EHL- $\text{Ti}_3\text{C}_2\text{T}_x$ and EHL- $\text{Ti}_3\text{C}_2\text{T}_x$ @MCE membrane [136]. Used with permission, copyright 2023 Elsevier.

In another study, Huang et al. developed a magnetic, superhydrophobic coating using lignin [137]. Hydrothermally treated lignin was synthesized by combining with Fe_3O_4 through a combination of solvent displacement and hydrothermal processing. These particles served as the primary building blocks, while nanocellulose crystals (CNCs) and titanium dioxide (TiO_2) acted as reinforcing agents. Polydimethylsiloxane (PDMS) was used as an adhesive matrix. The coating was applied on a PU sponge using a simple one-step spray or dip-coating method. The resulting material exhibited remarkable durability, enduring up to 87 abrasion cycles due to the synergistic strengthening effect of lignin, CNC, and TiO_2 . In addition to its excellent UV resistance, the coating exhibited remarkable oil absorption capabilities. When applied to a PU sponge, it achieved an absorption capacity ranging from 17.17 to 24.78 g/g for various oils. Moreover, the magnetic properties of the material allowed the sponge to be easily retrieved using a magnet.

Yu and coworkers developed highly phenolic lignin nanoparticles (HP-LNPs) that exhibit excellent antioxidant properties and UV resistance. A green and economical strategy was used to modify industrial lignin, and a mild and efficient enzymatic treatment with laccase was employed to prepare the HP-LNPs (Figure 63) [142]. The alkaline treatments with urea and hydrogen peroxide (H_2O_2) were used to enhance the antioxidant properties and reduce the visible light absorption of the LNPs. Modification of lignin enhanced its desirable properties, and the HP-LNPs exhibited excellent antioxidant properties, with DPPH and PTIO radical scavenging rates of 76.12% and 51.50%, respectively. The whiteness value of the HP-LNPs increased from 43.50 to 56.39, making them suitable as natural anti-UV filters in sunscreens and demonstrating to directly extend the shelf life of fruits like bananas by about 7 days, showing their potential as a food preservative. The prepared HP-LNPs have good potential for industrial applications in bio-based adhesives, sunscreens, and biomedicine due to their favorable antioxidant and UV-blocking properties.

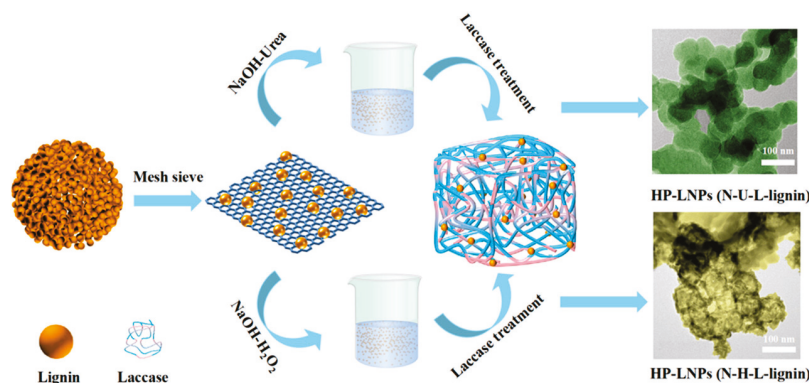


Figure 63. Schematic illustration for the preparation of HP-LNPs [142]. Used with permission, copyright 2023 American Chemical Society.

Lignin has been increasingly explored as a renewable component in adhesive formulations due to its aromatic structure, which enhances bonding strength and provides a sustainable alternative to petroleum-based resins. Zhan and coworkers synthesized a high-performance, sustainable lignin-based composite adhesive by leveraging the benefits of a deep eutectic solvent (DES) to modify the lignin structure and enable its effective reaction with furfural as a replacement for phenol and formaldehyde. DES (Choline chloride/urea with K_2CO_3) and alkaline lignin were reacted in a 2:1 optimum mass ratio for modification of lignin, and furfural was added for the preparation of lignin–furfural adhesive (DLF) [143]. After that, the adhesive, obtained after a 6-h reaction, was applied to a paulownia plate for the adhesion performance test. The adhesive strength of the modified composite achieved 4.54 MPa at room temperature, while hot-pressed at 0.13 MPa, and at $-30\text{ }^\circ\text{C}$, the strength increased by 16.74% compared to room temperature. Tensile strength properties were studied using balsa wood, and DLF–balsa wood composites showed significantly higher strength (3.61 MPa) than plain balsa wood (1.73 MPa). In addition to enhanced mechanical properties, the lignin-based composite adhesive exhibited considerably improved thermal and flame-retardant properties compared to traditional petroleum-based adhesives and coatings.

Wu and coworkers developed a lignin-based copolymer as a promising sustainable alternative to petroleum-based thermoplastic elastomers and adhesives [144]. Organosolv lignin was modified to synthesize a lignin macromolecular initiator (OL-Br) by esterification of lignin hydroxyl groups with 2-bromoisobutyryl bromide (BiBB). Afterward, two biomass-derived monomers, lauryl methacrylate (LMA) from plant oil and isobornyl methacrylate (IBOMA) from pine sap, were grafted onto the lignin macroinitiator via ARGET ATRP (activators regenerated by electron transfer for atom transfer radical polymerization) to form lignin-*graft*-poly(isobornyl methacrylate-*co*-lauryl methacrylate) copolymers (OL-*g*-P(IBOMA-*co*-LMA)), Figure 64). Different mass ratios of lignin and IBOMA were used to prepare various elastomers. The thermal stability and the glass transition temperature (T_g) of the copolymers were increased with the addition of lignin. Moreover, the molar ratio of IBOMA to LMA in the copolymers also influenced their thermal and mechanical properties. Increasing the IBOMA content led to higher glass transition temperatures and improved tensile properties. The OL-*g*-P(IBOMA-*co*-LMA) copolymers exhibited adhesive properties on various substrates, such as glass, metal, plastic, and paper, making them potential all-biomass adhesives with UV absorption abilities.

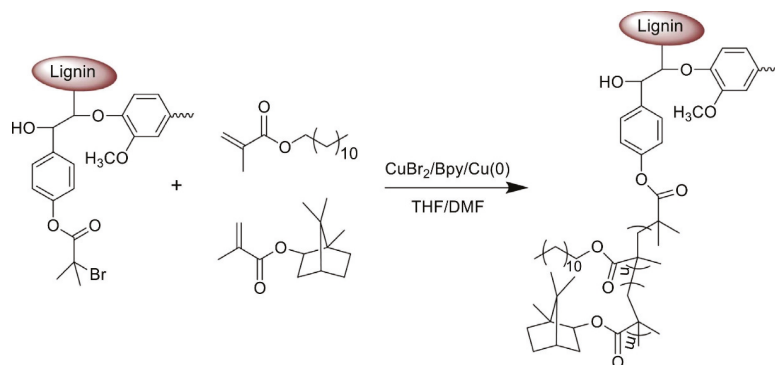


Figure 64. Synthesis of OL-g-P(IBOMA-co-LMA) copolymers [144]. Used with permission, copyright 2023 Elsevier.

In another study, Ou and co-workers developed lignin-graft copolymers [Lignin-graft-poly(*n*-butyl acrylate-co-acrylic acid)], via reversible addition–fragmentation chain transfer (RAFT) polymerization (Figure 65) [145]. These lignin-based materials exhibited good thermal stability and tunable glass transition temperatures (T_g). The mechanical properties, including tensile strength, elongation at break, Young's modulus, and adhesive properties, can be readily adjusted by varying the *n*-butyl acrylate (BA)/acrylic acid (AA) monomer ratio and lignin content. A higher BA content resulted in lower T_g due to increased chain mobility, while grafted copolymers generally showed higher T_g values than their linear counterparts owing to restricted chain flexibility. At a lignin content of 7.7 wt.%, the copolymer demonstrated exceptional stretchability with low tensile stress, whereas 11.8 wt.% lignin incorporation yielded the highest tensile strength (14.0 MPa) with moderate elongation (510%), emphasizing the reinforcing role of lignin. Additionally, the 7.7 wt.% lignin-containing sample exhibited the highest adhesion strength (8.7 MPa), surpassing that of neat samples due to the physical crosslinking effect of lignin. Strong adhesion was also observed on various substrates, particularly wood, aluminum, and copper, highlighting the material's potential for broad adhesive applications.

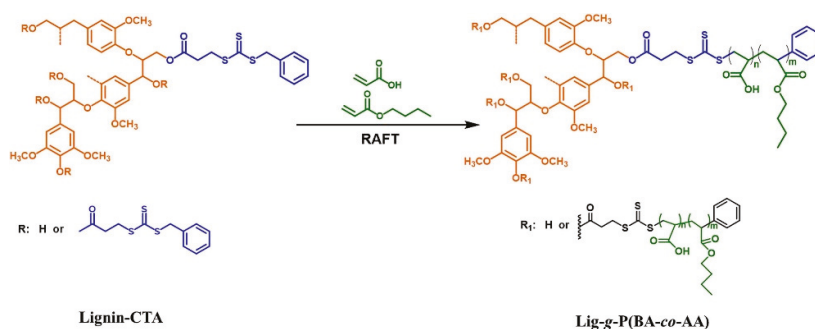


Figure 65. Preparation of Lignin-graft-poly(*n*-butyl acrylate-co-acrylic acid) copolymer [145]. Used with permission, copyright 2023 Elsevier.

5.2. Lignin as Filler or Compatibilizer in Blends and Composites

Additive manufacturing, also known as 3D printing, has emerged as a state-of-the-art technique for fabricating complex, customizable structures across a wide range of materials, including polymers, ceramics, and metals. More recently, the 3D printing of lignin-based composites has garnered significant interest due to lignin's abundance, renewability, and potential to replace fossil-derived polymers while enabling tailored mechanical and functional properties [146,147]. A novel 3D scaffold was introduced by Silva-Barroso et al. to overcome challenges such as poor mechanical strength, limited bioactivity, and inadequate cellular integration in bone tissue regeneration [148]. In the study, sodium alginate (SA) and

lignin (LG) were mixed in different ratios (25–50 wt.% of LG) to form the polymer matrix and reacted with tricalcium phosphate (TCP) to prepare TCP/LG/SA scaffolds through the layer-by-layer deposition method. The macroscopic images (Figure 66a) showed a color change in the 3D scaffolds from white to light brown with lignin addition, and SEM images (Figure 66b) revealed an irregular, rough surface, providing more anchorage points that enhance bioactivity, protein adsorption, and cell adhesion. The addition of 33.3 wt.% lignin improved the mechanical resistance of the scaffolds, resulting in a 15% increase in compressive strength compared to TCP/SA scaffolds without lignin. Moreover, TCP/LG/SA composites improved the wettability of the 3D-printed TCP/SA composite scaffolds while maintaining good biocompatibility and osteogenic potential, supporting the use of lignin in developing 3D scaffolds for bone tissue regeneration applications. However, further in vivo evaluation of TCP/LG/SA scaffolds is essential to validate their potential for clinical applications.

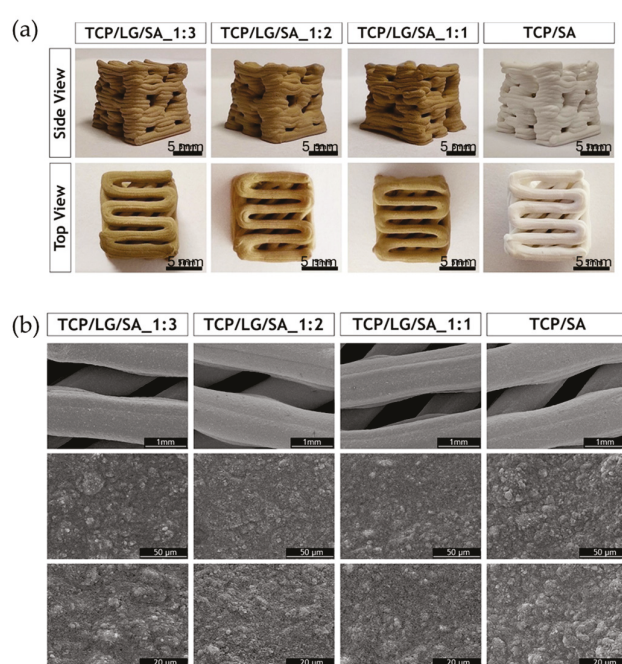


Figure 66. (a) Representative macroscopic images of the different scaffolds (side and top views) and (b) SEM images showing the morphology and surface topography of the 3D scaffolds produced at different magnifications [148]. Used with permission, copyright 2023 Elsevier.

Norouzi and co-workers developed electrospun scaffolds using lignin-modified PLGA [poly(L-lactide- ϵ -caprolactone)] and PLGA/Fibrin blends, both of which are biocompatible and biodegradable copolymers [149]. Plant-derived alkali lignin and animal-derived fibrin were used to prepare various scaffold formulations. The scaffold formulations included a 10% solution of pure PLGA, along with 10% polyblend solutions combining PLGA with either fibrin or lignin in ratios of 9:1, 8:2, and 7:3. Additionally, 10% polyblend solutions incorporating PLGA, fibrin, and lignin were prepared in ratios of 7:2:1, 6:2:2, and 5:2:3. Pure PLGA scaffolds exhibited the highest tensile strength (117.56 MPa) while the addition of lignin, fibrin, or fibrin/lignin to PLGA decreased the tensile strength of the scaffolds. PLGA/10% lignin scaffolds showed the second-highest tensile strength (70.52 MPa), and increasing lignin content in these scaffolds reduced their tensile strength from 70.52 MPa to 52.70 MPa and their elongation at break from 87.08% to 20.83%. Similarly, increasing lignin in PLGA/Fibrin/Lignin scaffolds decreased their tensile strength from 64.46 MPa to 27.38 MPa, Young's modulus from 8.85 MPa to 5.68 MPa, and elongation at break from 28.29% to 20.83%, indicating their potential as appropriate candidates for engineering

various tissues. In vitro biological responses of the human Adipose-derived Stem Cells to the scaffolds were studied. However, more comprehensive studies and a full understanding of the long-term performance are required for the targeted applications.

Bitumen (asphalt), a binder in pavement materials, was modified by Su et al., adding lignin to improve its aging resistance and thermal-oxidative stability [150]. Lignin-modified bitumen samples were prepared by adding different percentages of lignin (0–15% by mass) to the base bitumen. The modification was performed through physical blending, where lignin was added to the molten bitumen and mixed using a high-speed shear mixer (Figure 67). Dynamic shear rheology (DSR) tests were conducted to find complex shear modulus, phase angle, rutting factor, and complex modulus aging index (CAI) at different temperatures (58 °C, 64 °C, and 70 °C) before and after short-term aging. The complex shear modulus and rutting factor values of lignin-modified bitumen before and after aging were higher than those of unmodified bitumen, indicating improved elastic recovery, deformation, and rutting resistance. The CAI values of lignin-modified bitumen were lower than those of pure bitumen at all temperatures, suggesting better resistance to short-term thermal-oxidative aging. TGA data revealed that the initial decomposition temperature of lignin-modified bitumen was lower due to the decomposition of lignin earlier during the thermal-oxidative aging process. Lignin acts as a “sacrificial agent” helping slow down bitumen aging. However, further studies on low-temperature cracking resistance and moisture susceptibility are required for lignin-modified bitumen before its application as a road construction material.

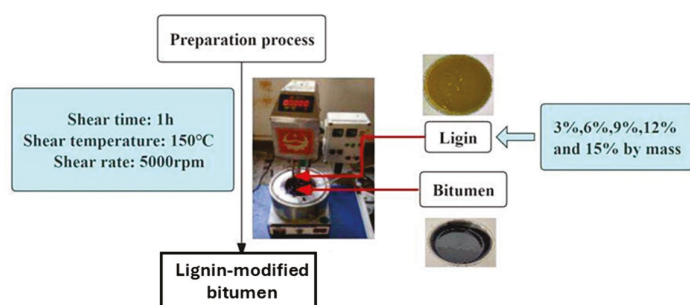


Figure 67. Experimental plan of the study [150]. Used with permission, copyright 2023 Elsevier.

Rezazad Gohari et al. investigated the use of kraft lignin as a modifier for bitumen [151]. Kraft lignin, derived from softwood (0–20 wt.% of lignin), was added to a PG 58S-28 bitumen using two blending protocols (Figure 68). The properties of the lignin-modified bitumen, produced with a mechanical mixer (at 1000 rpm) and a high-shear mixer (at 5000 rpm), were then evaluated. When processing pure bitumen, the mechanical mixer method resulted in a higher final viscosity than the high-shear mixer due to the loss of volatile compounds (Figure 69). DSR analysis at 58 °C, 64 °C, and 70 °C showed that the mechanical mixer method yielded higher complex shear modulus and rutting factor values, which further increased with lignin addition. TGA data indicated improved thermal stability with lignin incorporation, while the mechanical mixer method exhibited higher thermal decomposition than the high-shear mixer. Lignin content and processing conditions had a minimal impact on T_g , but T_g remained lower than that of virgin bitumen. Overall, the study highlights the influence of lignin loading and processing conditions on the lignin-modified bitumen.

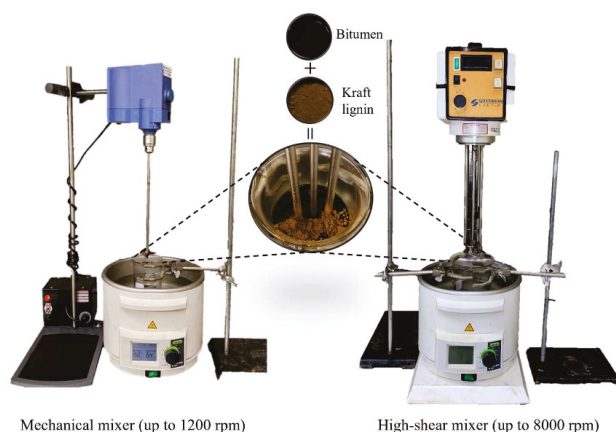


Figure 68. Blending kraft lignin and bitumen using a mechanical mixer (MM) and high-shear mixer (HSM) in the oil bath [151]. Under a Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/> accessed on 27 April 2025).

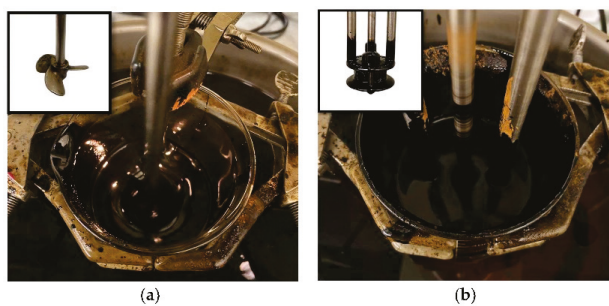


Figure 69. Comparison between (a) the mechanical mixer (MM) and (b) the high-shear mixer (HSM) during the blending process [151]. Under a Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/> accessed on 27 April 2025).

Antioxidants such as *N*-(1,3-dimethylbutyl)-*N'*-phenyl-*p*-phenylenediamine (6PPD) are combined with rubber to produce automotive tires [152]. 6PPD is a toxic compound that has the critical drawback of being utilized as an antioxidant. As a solution, Chung et al. introduced amine-functionalized lignin (AL) as an eco-friendly antioxidant to replace 6PPD [153]. Kraft lignin (KL) was chemically modified via amination with diethylene-triamine (DETA) and formaldehyde, grafting primary and secondary amine groups to enhance the radical scavenging ability of lignin (Figure 70). The AL particles (0–4 phr) were incorporated into natural/butadiene rubber (NB) using an internal mixer, followed by sulfur crosslinking and compression molding. Compared to 6PPD and KL, AL-modified rubber exhibited superior thermal and ozone aging resistance, indicating improved antioxidant properties. AL also participated in the vulcanization reaction, promoting sulfur activation, increasing the curing rate, and enhancing crosslink density. Notably, NB/AL (2 phr) showed a 28% higher crosslink density than NB/6PPD. In mechanical testing, AL-modified rubber outperformed 6PPD and KL, with a higher 50% modulus value, suggesting improved stiffness and elasticity. After thermal aging at 120 °C for 24 h, NB/AL (2 phr) retained more tensile strength (36.2% decrease) and elongation (46.9% decrease) than 6PPD and KL-modified rubber, likely due to a balance of crosslinking hardening and chain scission softening. Overall, amine-functionalized lignin improved both the antioxidant and mechanical properties of rubber, demonstrating its potential as a sustainable alternative to 6PPD. However, further environmental testing and long-term performance evaluation are necessary for broader industrial applications.

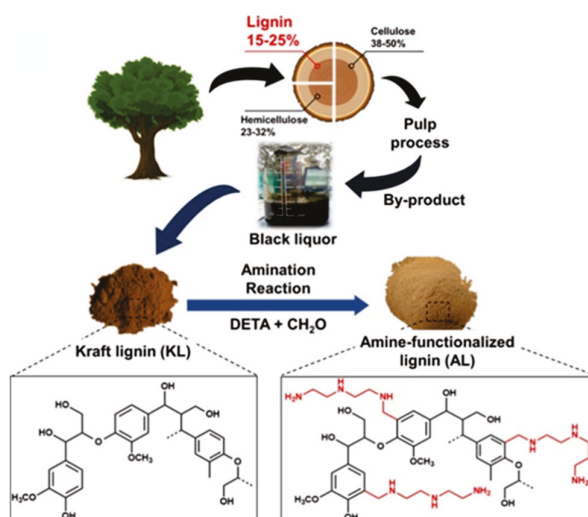


Figure 70. Schematic illustration of the process used to fabricate AL via the amination reaction [152]. Used with permission, copyright 2023 American Chemical Society.

Laanesoo et al. developed a novel approach to bio-based high-performance polymers by combining isosorbide, a rigid bicyclic diol derived from glucose, with lignin-derived phenyl aromatic groups such as vanillic, syringic, and benzoic acids [154]. Methacrylate monomers were synthesized via esterification of isosorbide with these lignin derivatives and subsequently polymerized through free radical polymerization to produce aromatic polymethacrylates (PArIMAs, Figure 71). The resulting polymers exhibited high glass transition temperatures (T_g) ranging from 80 to 168 °C and demonstrated thermal stability up to 268 °C, attributed to the rigidity of the isosorbide core and the restricted chain mobility from the aromatic side groups. Dynamic rheology studies confirmed the materials' excellent shear stability, with constant complex modulus and viscosity maintained over time and at elevated temperatures up to 200 °C. These findings indicate that the lignin-derived polymethacrylates are thermally stable, resistant to degradation, and melt-processable, making them promising candidates for sustainable high-performance plastic applications.

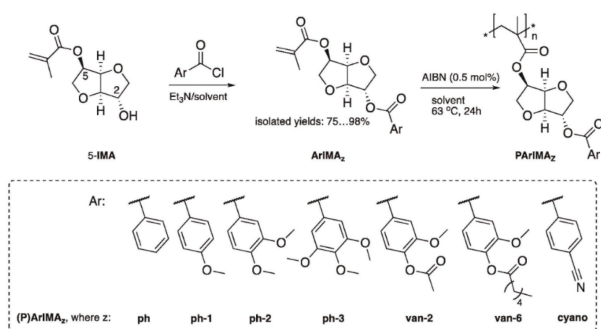


Figure 71. Synthesis and polymerization of isosorbide methacrylates substituted with different lignin derivatives with phenyl groups (IMA: isosorbide methacrylates, Ar: the aromatic side group in lignin) [154]. Under a Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/> accessed on 27 April 2025).

The environmental impact of fossil-based energy has intensified the demand for clean and sustainable alternatives. Triboelectric nanogenerators (TENGs) offer a promising solution by harnessing sustainable energy sources for electricity generation [155]. Nanthagal et al. demonstrated the use of lignin as a triboelectric active material in natural rubber (NR)-based TENGs [156]. Lignin was depolymerized using ultrasound irradiation in a basic solution, producing micrometer-sized particles with increased polar functional groups to

enhance lignin's performance (Figure 72). These particles were incorporated into NR and NR grafted with polyacrylamide (NR-g-PAM) composites, where PAM acted as a stabilizer, improving dispersion and interfacial adhesion. The controlled coprecipitation of NR latex and lignin dispersion facilitated uniform integration. The incorporation of lignin significantly enhanced TENG power output, with NR/lignin and NR-g-PAM/lignin achieving 500% and 1300% improvements, respectively, compared to NR TENGs. However, TGA analysis showed no notable improvement in thermal stability. The mechanical properties of the composites played a crucial role in TENG performance. Under the glassy state, NR/lignin exhibited a lower storage modulus (E'), indicating that lignin behaved as a diluent due to agglomeration and low interfacial area. In contrast, NR-g-PAM/lignin showed a significant increase in E' , demonstrating a reinforcing effect that enhanced charge transfer efficiency and mechanical durability. Future work could explore surface modifications or crosslinking strategies to improve lignin dispersion further and enhance thermal stability, broadening its potential applications in next-generation TENGs.

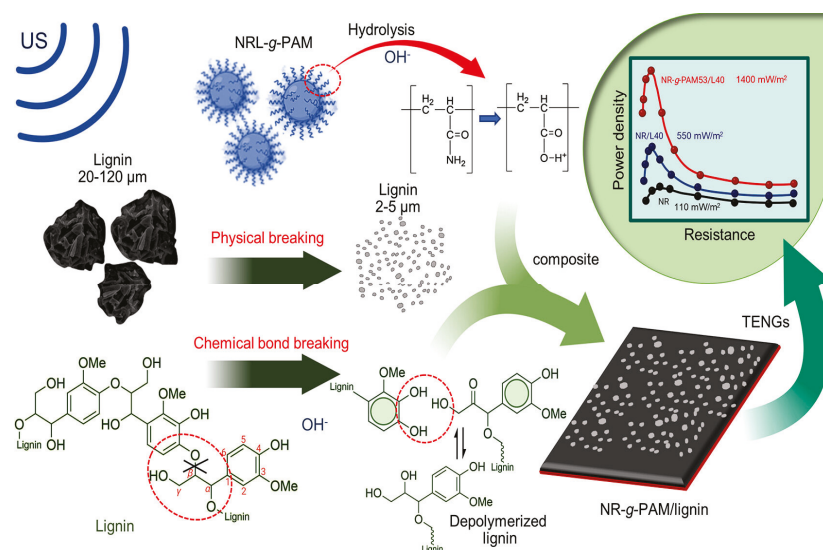


Figure 72. Schematic demonstration of the ultrasound-induced transformation of the lignin to a triboelectric-active material for NR-based TENGs [156]. Used with permission, copyright 2023 American Chemical Society.

Silica is commonly used as a reinforcing filler in polymers to enhance mechanical properties. However, its tendency to agglomerate and disperse poorly within the rubber matrix presents challenges. To address this, Chowdhury and coworkers partially replaced silica with lignin in a solution-grade styrene-butadiene rubber (SSBR) compound, optimizing the formulation for tire tread applications [157]. The Taguchi method was used to optimize the compound, considering factors such as SSBR vinyl content, silanization temperature, silica–lignin ratio, and silane coupling agent (SCA) dosage. Lignin incorporation improved the thermal stability of the rubber due to its high molecular weight aromatic fractions, leading to elevated decomposition temperatures. However, when silica replacement exceeded 20%, re-agglomeration of filler particles occurred, resulting in inferior dispersion. The reinforcement index (RI), calculated as the ratio of modulus at 300% elongation to 100% elongation, was used to assess filler effectiveness. The optimized compound, formulated with 58% vinyl SSBR, 160° C silanization temperature, a 40:10 silica–lignin ratio, and 8–10% SCA dosage, exhibited a higher RI, indicating better reinforcement and improved tensile properties compared to other compositions. Despite these promising results, long-term aging and durability studies are needed to fully establish lignin as a viable replacement for synthetic fillers in tire formulations.

Qiu et al. developed lignin/SiO₂ nano-hybrid fillers to reinforce natural rubber (NR) composites. Lignin was modified via the Mannich reaction with diethylamine and formaldehyde, producing diethylamine-grafted lignin (DL), which was then hybridized with SiO₂ to form DLSi composites (Figure 73) [158]. The grafted diethylamine (18.55 wt.%) enabled lignin to ionize under acidic conditions, promoting strong interactions with negatively charged SiO₂ and facilitating the formation of nano-hybrid particles. The DLSi1 hybrid (DL/SiO₂ = 1:2) exhibited enhanced hydrophobicity, increasing the water contact angle from 64.9° to 91.1°. In NR composites, 20 wt.% of the total filler was replaced by DLSi1, and vulcanization was carried out at 151 °C (Figure 74). NR composites incorporating DLSi1 and carbon black (CB, 40 phr) demonstrated a storage modulus comparable to NR-CB composites in dynamic mechanical analysis (DMA) tests, indicating strong rubber–filler interactions. Additionally, the lower $\tan\delta_{\max}$ in NR-DLSi1 suggested that the hybrid filler restricted the viscoelastic relaxation of NR. Mechanical testing showed that NR-DLSi1 had a tensile strength (23.7 MPa), 100% modulus (1.8 MPa), and elongation at break (565%) comparable to NR-CB (24.2 MPa, 1.8 MPa, and 536%, respectively), demonstrating that DLSi1 could replace 20% of CB without compromising performance. NR-DLSi1 also outperformed NR composites modified with SiO₂ alone, highlighting the reinforcing effect of the hybrid filler due to its nano-size, uniform dispersion, and strong interfacial interactions within the NR matrix.

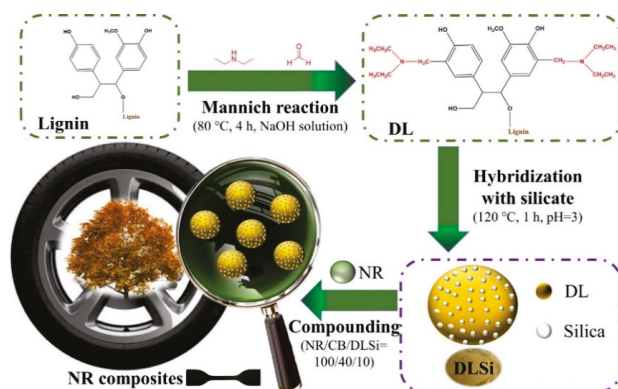


Figure 73. The schematic diagram for lignin modification and composite preparation [158]. Used with permission, copyright 2023 Elsevier.

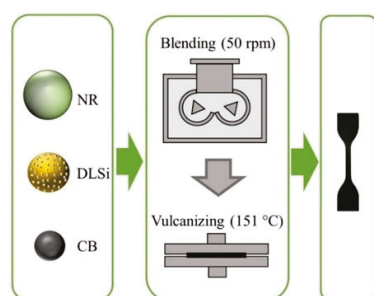


Figure 74. Preparation method of NR-DLSi composites by inserting modified lignin into natural rubber [158]. Used with permission, copyright 2023 Elsevier.

Campos et al. investigated the use of kraft lignin as a reinforcing filler in carboxylated nitrile rubber (XNBR) without any chemical or physical modification [159]. Using a mixture design of experiments (MDOE), they examined the influence of carboxyl group content (1–7%) and lignin loading (0–40 phr) on rubber–lignin interactions. The addition of zinc oxide facilitated ionic bonding with XNBR’s carboxyl groups, improving compatibility with lignin compared to nonpolar rubbers. The results showed a higher carboxyl content (7%),

improved lignin dispersion, and enhanced rubber–lignin interactions. The storage modulus (E') increased with lignin incorporation, demonstrating its reinforcing effect, particularly in XNBR with 7% carboxyl content. The glass transition temperature (T_g) also shifted to higher temperatures with increasing carboxyl and lignin content. Shore A hardness, tear strength, and tensile properties improved significantly, with the X7L40 composite (7% carboxyl, 40 phr lignin) exhibiting the highest enhancements. Notably, stress at 100% strain in XNBR7 (7% carboxyl) rose from 1.7 to 6.3 MPa with lignin addition, whereas in XNBR1 (1% carboxyl), it increased from 1.2 to 1.9 MPa. These results highlight kraft lignin's potential as a reinforcing filler in nitrile rubber, enhancing mechanical properties and overall performance. The foregoing examples are summarized in Table 4.

Table 4. Summary of the preparation, applications, and properties of other lignin-based polymers.

Lignin Type/ Source	Lignin Treatment/ Modification Method	Polymer Matrix	Application	Optimal Lignin Details	Reported Key Properties	Ref.
Dealkaline lignin	Microencapsulated in epoxidized soybean oil	Poly(butylene adipate- <i>co</i> -terephthalate) (PBAT), Tannic acid	Films	1 wt.%	Tensile strength: 7.27 MPa Elongation at break: 439.87%	[120]
Alkali lignin	Enzymatic modification/ decolorization	Keratin	Films	50 wt.% of DML	Tensile strength: 14.8 MPa Elongation at break: 23.7%	[121]
Alkali lignin	Grafting <i>n</i> -octadecyl isocyanate	Castor oil and 1,12-diaminododecane	Films	20 wt.%	Tensile strength: 35 MPa Elongation at break: 80% Toughness: >600 J/m ³	[123]
Dealkaline lignin	Cyanoethylation	Poly(vinyl alcohol) (PVA)	Films	1 wt.%	Tensile strength: 31.1 MPa Elongation at break: 218%	[124]
Sodium lignin sulfonate	Electrostatic adsorption	Chitosan	Films	1 wt.%	Endotoxin adsorption efficiency: 87.6%	[125]
Lignin from Radiata Pine	Treated with ternary DES to form lignin-containing cellulose nanofibrils (LCNF)	Cellulase	Films	LCNF treated at 130 °C	Tensile strength: 52.0 MPa Elongation at break: 8.4% Tensile modulus: 4.2 GPa	[126]
Lignin from Moso bamboo	Pre-hydrolysis and treated with DES	Starch	Films	2 wt.%	Tensile strength: 48.9 MPa Elastic modulus: 2288.8 MPa	[127]
Kraft lignin from <i>Eucalyptus</i> wood	Fractionation with ethyl acetate	Starch	Films	4 wt.% soluble fraction of lignin	Tensile strength: >14 MPa Elastic modulus: 69.24 MPa	[128]
Black liquor lignin from rice straw	Alkylation	Chitosan	Films	8 wt.%	DPHH scavenging activity: 93.25%	[129]
Kraft lignin	Phenolation	Cellulose and PVA	Films	2 h treated lignin	Tensile strength: >40 MPa Toughness: 55 MJ/m ³	[130]
Dealkaline lignin	Amination via Mannich reaction	PVA	Films	30 wt.%	Tensile strength: 3.6 MPa Elongation at break: 107.8%	[131]

Table 4. Cont.

Lignin Type/ Source	Lignin Treatment/ Modification Method	Polymer Matrix	Application	Optimal Lignin Details	Reported Key Properties	Ref.
Lignin from Masson pine	DES pretreatment (choline chloride and lactic acid)	Cellulose	Films	Treated at 140 °C for 4 h	Tensile Stress: >50 MPa 100% absorption of UVB	[132]
Organosolv lignin from potato crop residues	Solvent extraction	Starch	Films	1 wt.%	Tensile strength: >2 MPa Young's modulus: 69 MPa	[133]
Organosolv lignin	Incorporation of silver-embedded clay	PVA	Films	5 wt.%	Tensile strength: 28.56 MPa Elongation at break: 59%	[134]
Softwood kraft lignin	NA	Poly(caprola- ctone) (PCL)	3D-printed dressing material	10 wt.%	Bacterial adhesion reduction: 96.6%	[135]
Enzymatic hydrolysis lignin	Functionalization with $Ti_3C_2T_x$	NA	Coating	33.3 wt.%	Oil–water separation efficiency: >99.5%	[136]
Enzymatic hydrolysis lignin	Hydrothermal treatment with Fe_3O_4	Polydimethylsil- oxane (PDMS)	Coating	25 wt.%	Oil/organic wastes adsorption capacity: 17.17–24.78 g/g	[137]
Industrial lignin	Enzymatic and alkaline treatment	NA	Adhesives and sunscreens	5 wt.%	DPHH scavenging activity: 76.12% UVA protection	[142]
Alkali lignin	DES pretreatment	Furfural	Adhesives/ coatings	33.3 wt.%	Bonding strength: 4.54 MPa	[143]
Organosolv lignin	Esterification	Lauryl methacrylate and isobornyl methacrylate	Elastomers and adhesives	16.5 wt.%	Fracture strength: 6.58 MPa Elongation at break: 337%	[144]
Enzymatic lignin	Purification	<i>n</i> -butyl acrylate and acrylic acid	Elastomers and adhesives	11.8 wt.%	Tensile strength: 14.0 MPa Elongation at break: 510%	[145]
Kraft lignin	NA	Sodium alginate and tricalcium phosphate	3D-printed scaffolds	33.3 wt.%	Compressive strength: >20 MPa Modulus: 90 MPa	[148]
Alkali lignin	NA	Poly(L-lactide- ϵ - caprolactone) (PLGA)	Scaffolds	10 wt.%	Tensile strength: 64.46 MPa Elongation at break: 28.29%	[149]
Herbal lignin	NA	Bitumen (asphalt)	Pavement binders	15 wt.% after aging at 58 °C	Complex shear modulus: 7432 Pa Rutting factor: >2.2 kPa	[150]
Kraft lignin from softwood	NA	Bitumen	Pavement binders	10 wt.% after aging at 58 °C	Complex shear modulus increased by 51% Rutting factor: 3.22 kPa	[151]
Kraft lignin	Amination	Natural/ butadiene rubber	Elastomers/tires	2 phr	Tensile stress: 20 MPa Improved antioxidant and ozone aging resistance	[153]

Table 4. Cont.

Lignin Type/ Source	Lignin Treatment/ Modification Method	Polymer Matrix	Application	Optimal Lignin Details	Reported Key Properties	Ref.
Lignin derivatives	Esterification	Methacrylate	Coating	NA	Broad T_g range: 80–160 °C	[154]
Commercial grade kraft lignin	Depolymerization using ultrasound irradiation	Natural rubber and polyacrylamide	Triboelectric nanogenerators	40 wt.%	Power density: 1411 mW/m ²	[156]
Commercial lignin	NA	Styrene- butadiene rubber	Tire treads	10 wt.%	Tensile strength: 14.7 MPa Elongation at break: 604%	[157]
Soda lignin	Amination	Natural rubber	Fillers in tires	~32 wt.%	Tensile strength: 23.7 MPa Elongation at break: 565%	[158]
Hardwood kraft lignin	NA	Carboxylated nitrile rubber	Fillers in rubbers	40 phr	Shore A hardness: 77 HA Tear strength: 64 kN/m Tensile strength: 16.5 MPa Elongation at break: 434%	[159]

6. Conclusions

Lignin has emerged as a structurally diverse and functionally rich biopolymer that offers significant promise as a sustainable and performance-enhancing component in a wide range of polymeric systems. This review has surveyed the state-of-the-art strategies for lignin incorporation into thermosets, polyurethanes, PLA composites, and film and coating applications, with attention to both chemical and physical mechanisms of integration. In doing so, it becomes apparent that the specific chemical form of lignin, ranging from raw kraft lignin to fractionated and chemically modified derivatives, critically determines the processability, reactivity, and final properties of lignin-containing polymers.

Recent developments have demonstrated reactive modifications to enhance lignin's compatibility and performance in epoxy and polyurethane systems by increasing available functional groups and reducing molecular heterogeneity. Likewise, physical strategies including nanoparticle dispersion or compatibilizer-assisted blending show great utility for film and composite applications, particularly where barrier, thermal, or UV properties are of concern. These approaches reflect a growing understanding of the importance of controlling lignin's dispersion, interfacial adhesion, and chemical compatibility within diverse polymer matrices. However, despite these advances, a number of core challenges persist. The following key insights and future directions summarize the current state and critical advancements needed to realize lignin's full potential:

Versatility and challenges of lignin integration:

Lignin's structural heterogeneity, particularly its wide molecular weight distribution and variable functional composition, continues to hinder reproducibility and scalability in polymer applications. Moreover, inconsistencies in lignin quality across extraction methods (e.g., kraft, organosolv, soda, hydrolysis) introduce variability in downstream performance, limiting industrial uptake.

Impact of lignin form and modification strategy:

The chemical form of lignin (raw vs. modified) significantly influences its reactivity and compatibility with polymer matrices. Reactive modifications such as phenolation, oxyalkylation, and glycidylation have proven effective in enhancing functional group availability and reducing heterogeneity for improved performance in epoxy and polyurethane systems.

Chemical vs. physical integration challenges:

There is a lack of clear distinction between covalent bonding and physical blending in many

studies, with insufficient analytical validation of the proposed integration mechanisms. This ambiguity complicates efforts to develop reliable structure–property relationships and challenges the classification of lignin-based systems as true copolymers, hybrid networks, or filled composites. Future studies should prioritize quantitative methods, such as 2D NMR, MALDI-TOF, FTIR, kinetic analysis, and chromatographic fractionation, to identify and distinguish covalent grafting from surface interactions or entanglement. In the context of PLA composites, this distinction is especially relevant, given that both esterification and filler-type approaches are widely reported but not always experimentally validated.

Importance of life-cycle assessments (LCAs) and techno-economic analyses (TEAs) implementation:

Beyond performance improvements, comprehensive life-cycle assessment (LCA) and techno-economic analysis (TEA) are essential for the real-world adoption of lignin-based materials [160,161]. TEA can help to optimize the production costs by evaluating the feasibility and scalability of lignin valorization routes including feedstock sourcing, process energy input, and purification requirements. Concurrently, LCA provides a quantitative evaluation of environmental impacts, including greenhouse gas emissions, water use, and toxicity. Incorporating TEA and LCA early in the development process will support scalable and sustainable innovations, ultimately accelerating the transition of lignin composites from laboratory research to commercial applications. However, few studies have systematically compared modified lignin-based products with conventional petrochemical analogs using these frameworks. Life-cycle assessments and techno-economic analyses are critical yet underused tools that can guide realistic implementation of lignin-based polymers. These tools support environmentally and economically sound material design and should be integrated into future development workflows.

Sustainability and scalability considerations:

The use of greener solvents and catalysts during lignin modification, the recovery and reuse of unreacted lignin fractions, and the minimization of purification steps are all areas where process intensification can dramatically improve material sustainability profiles. Scalable modification strategies, particularly those avoiding halogenation, heavy metal catalysis, or multistep derivatization, will be key to industrial uptake.

Emerging functional applications:

Beyond conventional thermosets and elastomers, lignin offers unique opportunities for next-generation applications. Its intrinsic UV-absorbing, flame-retardant, and antioxidant properties make it attractive for biomedical, packaging, and aerospace uses. The development of dynamic, reprocessable, and self-healing networks incorporating lignin, such as Diels–Alder-crosslinked PUs or vitrimeric lignin epoxies, represents a promising direction for coupling recyclability with mechanical robustness. Similarly, the intersection of lignin with nanotechnology, such as lignin-based nanoparticles or nanofibers, offers a route to high-performance hybrid materials with hierarchical structures and multifunctionality.

Computational tools and high-throughput methods:

Computational modeling and machine learning approaches remain largely untapped in the lignin polymer space. These tools can assist in decoding complex relationships between lignin structure and polymer performance, optimize blend formulations, and predict processing behavior under varied conditions. Coupled with automated synthesis and high-throughput screening, such strategies can accelerate the design of lignin-based materials tailored for target applications.

Ultimately, the future of lignin in polymer science will be shaped by the field's ability to reconcile its natural complexity with the precision demanded by engineered materials. This will require not only interdisciplinary collaborations among chemists, material scientists, and chemical engineers, but also engagement with stakeholders in forestry, agriculture, and

biorefining to secure consistent, high-quality lignin streams. By embracing lignin’s potential rather than avoiding its variability, the field stands poised to unlock new generations of sustainable, high-performance polymers that redefine the materials landscape for a bio-based economy.

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Abbreviations

The following abbreviations are used in this manuscript:

PUs	Polyurethanes
PLA	Polylactic acid
UV	Ultraviolet
HDIT	Hexamethylene diisocyanate trimer
PDMS	Polydimethylsiloxane
wt.%	Weight percentage
HIT	Hardness
EIT	Elastic modulus
PUFs	Polyurethane foams
PC	Propylene carbonate
MDI	Diphenylmethane-4,4'-diisocyanate
TGA	Thermogravimetric analysis
CUB	Catalytic upstream biorefining
RPFs	Rigid polyurethane foams
SEM	Scanning electron microscopy
DA	Diels–Alder
PCL	Polycaprolactone
T_g	Glass transition temperature
E'	Storage modulus
TDI	Toluene diisocyanate
HL	Hydrolysis lignin
PhL	Phenolated lignin
pMDI	Poly[(phenyl isocyanate)-co-formaldehyde]
LP	Lignin-based polyols
PEG	Polyethylene glycol
PDA	Polydopamine
PA	Phytic acid
NIPUs	Non-isocyanate polyurethanes
CCs	Cyclic carbonates
5CCs	Five-membered cyclic carbonates
CO ₂	Carbon dioxide

RCF	Reductive catalytic fractionation
DETA	Diethylenetriamine
EDA	Ethylenediamine
FT-IR	Fourier Transform Infrared spectroscopy
GPC	Gel permeation chromatography
6CCs	Six-membered cyclic carbonates
EEHL	Epoxidized enzymatic hydrolysis lignin
LNIPU	Lignin/non-isocyanate polyurethanes
WPU	Waterborne polyurethane
APTES	3-aminopropyltriethoxy silane
LNPs	Lignin nanoparticles
<i>E. coli</i>	<i>Escherichia coli</i>
i/h ratio	The ratio of isocyanate to hydroxy functionalities
IPDI	Isophorone diisocyanate
DGEBA	Diglycidyl ether of bisphenol A
BPA	Bisphenol A
CA	Citric acid
ESO	Epoxidized soybean oil
GL	Glycidylated lignin
$T_{5\%}$	Degradation temperatures (5% weight loss)
EL	Enzymatic lignin
ECH	Epichlorohydrin
LER	Lignin epoxy resin
BADGE	Bisphenol A diglycidyl ether
LPAA	Lignin-based phenolic amine
KL	Kraft lignin
NLH	Nano-lignin
GKL	Glycidylated kraft lignin
BMKL	Ball-milled kraft lignin
3D	Three dimensional
PHMS	Polyhydromethylsiloxane
PF	Phenol-formaldehyde
LPF	Lignin-based phenol-formaldehyde
DES	Deep eutectic solvent
PFC	Phenolic resin carbon
PHP	Phosphoric acid and hydrogen peroxide
AL	Alkali lignin
p/a ratio	The ratio of phenol to aldehyde
LMA	Lauryl methacrylate
CROP	Cationic ring-opening polymerization
EL	Ethyl acetate-extracted lignin
THF	Tetrahydrofuran
GLY	Glycidol
BF ₃	Boron trifluoride
$T_{\max}$	Maximum weight loss temperatures
W/O	Water-in-oil
PLLA	Poly (L-lactic acid)
DCM	Dichloromethane
EGDE	Ethylene glycol diglycidyl ether
PEGDE	Poly (ethylene glycol) diglycidyl ether
ENR	Epoxidized natural rubber
EHA	2-ethylhexyl acrylate
ROP	Ring-opening polymerization

TPU	Thermoplastic polyurethane
ALK	Modified alkali lignin
PBAT	Poly (butylene adipate-co-terephthalate)
Ta	Tannic acid
PVA	Poly (vinyl alcohol)
CEL	Cyanoethylated lignin
CNWs	Cellulose nano-whiskers
AgNP	Silver nanoparticle
LS	Lignin sulfonate
TDES	Ternary deep eutectic solvent
LCNF	Lignin-containing cellulose nanofibrils
TPS	Thermoplastic starch
<i>w/v</i>	Weight per volume
DPPH	2,2-diphenyl-1-picrylhydrozyl
<i>C. albicans</i>	<i>Candida albicans</i>
CNF	Cellulose nanofibril
aL	Aminated lignin
Fe(NO ₃) ₃	Ferric nitrate
CL	Lignin-containing cellulose
AlCl ₃	Aluminum chloride
ZnCl ₂	Zinc chloride
HNTs	Halloysite nanotubes
CUR	Curcumin
PCL	Poly(caprolactone)
SSE	Semi-solid extrusion
DPA	<i>D</i> -panthenol
EHL	Enzymatic hydrolysis lignin
MCE	Mixed cellulose ester
CNC	Nanocellulose crystals
TiO ₂	Titanium dioxide
H ₂ O ₂	Hydrogen peroxide
PTIO	2-phenyl-4,4,5,5-tetramethylimidazoline-3-oxide-1-oxyl
K ₂ CO ₃	Potassium carbonate
DLF	Lignin–furfural adhesive
IBOMA	Isobornyl methacrylate
ARGET ATRP	Activators regenerated by electron transfer for atom transfer radical polymerization
RAFT	Reversible addition-fragmentation chain transfer polymerization
BA	<i>n</i> -butyl acrylate
AA	Acrylic acid
SA	Sodium alginate
TCP	Tricalcium phosphate
PLGA	Poly(L-lactide-ε-caprolactone)
DSR	Dynamic shear rheology
CAI	Complex modulus aging index
MM	Mechanical Mixer
HSM	High-Shear Mixer
6PPD	<i>N</i> -(1,3-dimethylbutyl)- <i>N'</i> -phenyl- <i>p</i> -phenylenediamine
DETA	Diethylenetriamine
PArIMAs	Aromatic polymethacrylates
TENGs	Triboelectric nanogenerators
NR	Natural rubber
SSBR	Solution-grade styrene-butadiene rubber
SCA	Silane coupling agent
RI	Reinforcement index

DL	Diethylamine-grafted lignin
CB	Carbon black
DMA	Dynamic mechanical analysis
XNBR	Carboxylated nitrile rubber
MDOE	Mixture design of experiments
NA	Not available

References

- Rath, S.; Pradhan, D.; Du, H.; Mohapatra, S.; Thatoi, H. Transforming lignin into value-added products: Perspectives on lignin chemistry, lignin-based biocomposites, and pathways for augmenting ligninolytic enzyme production. *Adv. Compos. Hybrid Mater.* **2024**, *7*, 27. [CrossRef]
- Li, H.; Liang, Y.; Li, P.; He, C. Conversion of biomass lignin to high-value polyurethane: A review. *J. Bioresour. Bioprod.* **2020**, *5*, 163–179. [CrossRef]
- Shafiq, A.; Ahmad Bhatti, I.; Amjed, N.; Zeshan, M.; Zaheer, A.; Kamal, A.; Naz, S.; Rasheed, T. Lignin derived polyurethanes: Current advances and future prospects in synthesis and applications. *Eur. Polym. J.* **2024**, *209*, 112899. [CrossRef]
- Hasan, M.S.; Sardar, M.R.I.; Shafin, A.A.; Rahman, M.S.; Mahmud, M.; Hossen, M.M. A Brief Review on Applications of Lignin. *J. Chem. Rev.* **2023**, *5*, 56–82. [CrossRef]
- Karunaratna, M.S.; Smith, R.C. Valorization of Lignin as a Sustainable Component of Structural Materials and Composites: Advances from 2011 to 2019. *Sustainability* **2020**, *12*, 734. [CrossRef]
- Fabbri, F.; Bischof, S.; Mayr, S.; Gritsch, S.; Jimenez Bartolome, M.; Schwaiger, N.; Guebitz, G.M.; Weiss, R. The Biomodified Lignin Platform: A Review. *Polymers* **2023**, *15*, 1694. [CrossRef]
- Vasile, C.; Baican, M. Lignins as Promising Renewable Biopolymers and Bioactive Compounds for High-Performance Materials. *Polymers* **2023**, *15*, 3177. [CrossRef]
- Sauceda-Oloño, P.Y.; Kapuge Dona, N.L.; Smith, R.C. The quest for environmentally benign plastics: Advances in greener and more sustainable flame retardant formulations. *Green Chem. Lett. Rev.* **2024**, *17*, 2402403. [CrossRef]
- Sugiarto, S.; Leow, Y.; Tan, C.L.; Wang, G.; Kai, D. How far is Lignin from being a biomedical material? *Bioact. Mater.* **2022**, *8*, 71–94. [CrossRef]
- Abdullah, T.; İlyasoğlu, G.; Memić, A. Designing Lignin-Based Biomaterials as Carriers of Bioactive Molecules. *Pharmaceutics* **2023**, *15*, 1114. [CrossRef]
- Afewerki, S.; Edlund, U. Combined Catalysis: A Powerful Strategy for Engineering Multifunctional Sustainable Lignin-Based Materials. *ACS Nano* **2023**, *17*, 7093–7108. [CrossRef] [PubMed]
- Karagoz, P.; Khiawjan, S.; Marques, M.P.C.; Santzouk, S.; Bugg, T.D.H.; Lye, G.J. Pharmaceutical applications of lignin-derived chemicals and lignin-based materials: Linking lignin source and processing with clinical indication. *Biomass Convers. Biorefin.* **2023**, *14*, 26553–26574. [CrossRef] [PubMed]
- Lv, S.; Liang, S.; Zuo, J.; Zhang, S.; Wang, J.; Wei, D. Lignin-based anti-UV functional materials: Recent advances in preparation and application. *Iran. Polym. J.* **2023**, *32*, 1477–1497. [CrossRef]
- Mukheja, Y.; Kaur, J.; Pathania, K.; Sah, S.P.; Salunke, D.B.; Sangamwar, A.T.; Pawar, S.V. Recent advances in pharmaceutical and biotechnological applications of lignin-based materials. *Int. J. Biol. Macromol.* **2023**, *241*, 124601. [CrossRef]
- Mukherjee, S.; Mukhopadhyay, S. Recent progress in sulfur-containing technical lignin-based polymer composites. *Express Polym. Lett.* **2023**, *17*, 120–151. [CrossRef]
- Taher, M.A.; Wang, X.; Faridul Hasan, K.M.; Miah, M.R.; Zhu, J.; Chen, J. Lignin Modification for Enhanced Performance of Polymer Composites. *ACS Appl. Bio Mater.* **2023**, *6*, 5169–5192. [CrossRef]
- Kaushal, N.; Singh, A.K. Advancement in utilization of bio-based materials including cellulose, lignin, chitosan for bio-inspired surface coatings with special wetting behavior: A review on fabrication and applications. *Int. J. Biol. Macromol.* **2023**, *246*, 125709. [CrossRef]
- Abraham, B.; Syamnath, V.L.; Arun, K.B.; Fathima Zahra, P.M.; Anjusha, P.; Kothakotta, A.; Chen, Y.-H.; Ponnusamy, V.K.; Nisha, P. Lignin-based nanomaterials for food and pharmaceutical applications: Recent trends and future outlook. *Sci. Total Environ.* **2023**, *881*, 163316. [CrossRef]
- Khadem, E.; Ghafarzadeh, M.; Kharaziha, M.; Sun, F.; Zhang, X. Lignin derivatives-based hydrogels for biomedical applications. *Int. J. Biol. Macromol.* **2024**, *261*, 129877. [CrossRef]
- Zheng, Y.; Moreno, A.; Zhang, Y.; Sipponen, M.H.; Dai, L. Harnessing chemical functionality of lignin towards stimuli-responsive materials. *Trends Chem.* **2024**, *6*, 62–78. [CrossRef]
- Mo, J.; Wang, H.; Yan, M.; Huang, J.; Li, R.; Sun, D.; Lei, J.; Qiu, X.; Liu, W. Construction of interfacial dynamic bonds for high performance lignin/polymer biocomposites. *Front. Chem. Sci. Eng.* **2023**, *17*, 1372–1388. [CrossRef]

22. Zubair, M.; Rauf, Z.; Fatima, S.; Ullah, A. Lignin-derived bionanocomposites as functional food packaging materials. *Sustain. Food Technol.* **2024**, *2*, 945–966. [CrossRef]
23. Fadlallah, S.; Sinha Roy, P.; Garnier, G.; Saito, K.; Allais, F. Are lignin-derived monomers and polymers truly sustainable? An in-depth green metrics calculations approach. *Green Chem.* **2021**, *23*, 1495–1535. [CrossRef]
24. Staccioli, L.; Rodrigues dos Santos, A.M.; Gallego, J.; Kalliola, A.; Fearon, O.; Ortiz, P.; Pitacco, W.; Carvalho, A. A life cycle assessment model to evaluate the environmental sustainability of lignin-based polyols. *Sustain. Prod. Consum.* **2024**, *52*, 624–639. [CrossRef]
25. Jiang, L.; Wang, C.-G.; Chee, P.L.; Qu, C.; Fok, A.Z.; Yong, F.H.; Ong, Z.L.; Kai, D. Strategies for lignin depolymerization and reconstruction towards functional polymers. *Sustain. Energy Fuels* **2023**, *7*, 2953–2973. [CrossRef]
26. Libretti, C.; Santos Correa, L.; Meier, M.A.R. From waste to resource: Advancements in sustainable lignin modification. *Green Chem.* **2024**, *26*, 4358–4386. [CrossRef]
27. Wörner, M.; Werner, L.; Hornung, U.; Islongo Canabarro, N.; Baudouin, D.; Dahmen, N. The Impact of Sulfur-Containing Inorganic Compounds during the Depolymerization of Lignin by Hydrothermal Liquefaction of Black Liquor. *Energy Fuels* **2024**, *38*, 6036–6047. [CrossRef]
28. Li, X.; Meng, Y.; Cheng, Z.; Li, B. Research Progress and Prospect of Stimuli-Responsive Lignin Functional Materials. *Polymers* **2023**, *15*, 3372. [CrossRef]
29. Mondal, S.; Jatrana, A.; Maan, S.; Sharma, P. Lignin modification and valorization in medicine, cosmetics, environmental remediation and agriculture: A review. *Environ. Chem. Lett.* **2023**, *21*, 2171–2197. [CrossRef]
30. Diaz-Baca, J.A.; Fatehi, P. Production and characterization of starch-lignin based materials: A review. *Biotechnol. Adv.* **2024**, *70*, 108281. [CrossRef]
31. Miller, E.; Wolff, M.; Tindall, G.; Bethel, K.; Lynn, B.; Keppler, K.; Thies, M.C.; Davis, E.M. Fabrication and Characterization of Lignin-Based, Thermoresponsive Soft Composites Containing Fractionated and Cleaned Lignin. *ACS Appl. Polym. Mater.* **2023**, *5*, 342–354. [CrossRef]
32. Cen, Q.; Chen, S.; Yang, D.; Zheng, D.; Qiu, X. Full Bio-Based Aerogel Incorporating Lignin for Excellent Flame Retardancy, Mechanical Resistance, and Thermal Insulation. *ACS Sustain. Chem. Eng.* **2023**, *11*, 4473–4484. [CrossRef]
33. Hany, L.J.S.; Rahman, M.M.; Popy, R.S.; Ferdous, T.; Sarker, A.K.; Jahan, M.S. High strength hydrogel using phenolated lignin. *Eur. J. Wood Wood Prod.* **2024**, *82*, 1357–1367. [CrossRef]
34. Hachimi Alaoui, C.; Réthoré, G.; Weiss, P.; Fatimi, A. Sustainable Biomass Lignin-Based Hydrogels: A Review on Properties, Formulation, and Biomedical Applications. *Int. J. Mol. Sci.* **2023**, *24*, 13493. [CrossRef]
35. Luo, C.; He, Z.; Hu, X.; Zhang, Q.; Liang, Y.; Tang, H.; Wang, W.; Xue, D. Preparation of superabsorbent lignin-based composite inhibitor and research on its prevention and control characteristics of coal spontaneous combustion. *Combust. Sci. Technol.* **2024**, *196*, 608–628. [CrossRef]
36. Wang, Q.; Gao, J.; Liu, S.; Wang, Y.; Wu, L. Lignin nanoparticle reinforced multifunctional polyvinyl alcohol/polyurethane composite hydrogel with excellent mechanical, UV-blocking, rheological and thermal properties. *Int. J. Biol. Macromol.* **2023**, *232*, 123338. [CrossRef]
37. Li, S.; Luo, X.; Xiao, H.; Li, D.; Chen, Y. Nitrogen and Sulfur Codoped Hierarchical Porous Carbon Derived from Lignin for High-Performance Zinc Ion Capacitors. *ACS Appl. Energy Mater.* **2023**, *6*, 6700–6711. [CrossRef]
38. Liu, F.; Feng, P.; Yuan, M.; Zhai, G.; Innocent, M.T.; Xiang, H.; Wu, Q.; Lu, Y.; Zhu, M. Continuous Preparation of a Flexible Carbon Nanotube Film from Lignin as a Sulfur Host Material for Lithium–Sulfur Batteries. *ACS Sustain. Chem. Eng.* **2023**, *11*, 16544–16553. [CrossRef]
39. Tian, Y.; Yang, Z.; Wang, H.; Xiong, W.; Lin, X.; Wang, S.; Kong, F.; Li, P.; Xi, Y.; Zhang, F.; et al. Nitrogen-doped 3D carbon hybrids based on modified lignin as sulfur host for high-performance lithium-sulfur batteries. *J. Power Sources* **2024**, *621*, 235322. [CrossRef]
40. dos Reis, G.S.; Petnikota, S.; de Oliveira, H.P.; de Brum, I.A.S.; Thyrel, M.; Dotto, G.L.; Lima, E.C.; Naushad, M.; Hu, T.; Lassi, U.; et al. Statistics design for the synthesis optimization of lignin-sulfonate sulfur-doped mesoporous carbon materials: Promising candidates as adsorbents and supercapacitors materials. *Sci. Rep.* **2024**, *14*, 23354. [CrossRef]
41. Du, B.; Shi, X.; Zhu, H.; Xu, J.; Bai, Y.; Wang, Q.; Wang, X.; Zhou, J. Preparation and characterization of bifunctional wolfsbane-like magnetic Fe₃O₄ nanoparticles-decorated lignin-based carbon nanofibers composites for electromagnetic wave absorption and electrochemical energy storage. *Int. J. Biol. Macromol.* **2023**, *246*, 125574. [CrossRef] [PubMed]
42. Jin, X.; Li, X.; Liu, X.; Du, L.; Su, L.; Ma, Y.; Ren, S. Simple lignin-based, light-driven shape memory polymers with excellent mechanical properties and wide range of glass transition temperatures. *Int. J. Biol. Macromol.* **2023**, *228*, 528–536. [CrossRef] [PubMed]
43. Ma, X.; Wang, X.; Zhao, H.; Xu, X.; Cui, M.; Stott, N.E.; Chen, P.; Zhu, J.; Yan, N.; Chen, J. High-Performance, Light-Stimulation Healable, and Closed-Loop Recyclable Lignin-Based Covalent Adaptable Networks. *Small* **2023**, *19*, 2303215. [CrossRef] [PubMed]

44. Zheng, Q.; Chai, L.; Du, B.; Li, W.; Fu, L.-H.; Chen, X. A pH-Sensitive Lignin-Based Material for Sustained Release of 8-Hydroxyquinoline. *Polymers* **2023**, *15*, 1867. [CrossRef]
45. Zou, Z.; Ismail, B.B.; Zhang, X.; Yang, Z.; Liu, D.; Guo, M. Improving barrier and antibacterial properties of chitosan composite films by incorporating lignin nanoparticles and acylated soy protein isolate nanogel. *Food Hydrocoll.* **2023**, *134*, 108091. [CrossRef]
46. Daassi, R.; Durand, K.; Rodrigue, D.; Stevanovic, T. Optimization of the Electrospray Process to Produce Lignin Nanoparticles for PLA-Based Food Packaging. *Polymers* **2023**, *15*, 2973. [CrossRef]
47. Lage-Rivera, S.; Ares-Pernas, A.; Becerra Permy, J.C.; Gosset, A.; Abad, M.-J. Enhancement of 3D Printability by FDM and Electrical Conductivity of PLA/MWCNT Filaments Using Lignin as Bio-Dispersant. *Polymers* **2023**, *15*, 999. [CrossRef]
48. El-Ganainy, S.M.; Mosa, M.A.; Ismail, A.M.; Khalil, A.E. Lignin-Loaded Carbon Nanoparticles as a Promising Control Agent against *Fusarium verticillioides* in Maize: Physiological and Biochemical Analyses. *Polymers* **2023**, *15*, 1193. [CrossRef]
49. Haider, M.K.; Kharaghani, D.; Sun, L.; Ullah, S.; Sarwar, M.N.; Ullah, A.; Khatri, M.; Yoshiko, Y.; Gopiraman, M.; Kim, I.S. Synthesized bioactive lignin nanoparticles/polycaprolactone nanofibers: A novel nanobiocomposite for bone tissue engineering. *Biomater. Adv.* **2023**, *144*, 213203. [CrossRef]
50. Karunarathna, M.S.; Maladeniya, C.P.; Lauer, M.K.; Tennyson, A.G.; Smith, R.C. Durable composites by vulcanization of oleyl-esterified lignin. *RSC Adv.* **2023**, *13*, 3234–3240. [CrossRef]
51. Tisdale, K.A.; Dona, N.L.K.; Maladeniya, C.P.; Smith, R.C. Green and Atom Economical Route to High Compressive Strength Lignin Oil-Sulfur Composites. *J. Polym. Environ.* **2024**, *32*, 4842–4854. [CrossRef]
52. Tisdale, K.A.; Kapuge Dona, N.L.; Smith, R.C. The Influence of the Comonomer Ratio and Reaction Temperature on the Mechanical, Thermal, and Morphological Properties of Lignin Oil–Sulfur Composites. *Molecules* **2024**, *29*, 4209. [CrossRef] [PubMed]
53. Maladeniya, C.P.; Kapuge Dona, N.L.; Smith, A.D.; Smith, R.C. Thermal and Mechanical Properties of Guaiacol–Fatty Acid–Sulfur Composites. *Macromol* **2023**, *3*, 681–692. [CrossRef]
54. Kapuge Dona, N.L.; Saucedo-Oloño, P.Y.; Smith, R.C. Thermal and Mechanical Properties of Lignin Derivative–Sulfur Composites. *J. Polym. Sci.* **2024**, *63*, 789–799. [CrossRef]
55. Iswanto, A.H.; Lubis, M.A.R.; Sutiawan, J.; Al-Edrus, S.S.O.; Lee, S.H.; Antov, P.; Kristak, L.; Reh, R.; Mardawati, E.; Santoso, A.; et al. Latest Advancements in the Development of High-Performance Lignin- and Tannin-Based Non-Isocyanate Polyurethane Adhesive for Wood Composites. *Polymer* **2023**, *15*, 3864. [CrossRef]
56. Araujo, T.R.; Bresolin, D.; de Oliveira, D.; Sayer, C.; de Araujo, P.H.H.; de Oliveira, J.V. Conventional lignin functionalization for polyurethane applications and a future vision in the use of enzymes as an alternative method. *Eur. Polym. J.* **2023**, *188*, 111934. [CrossRef]
57. Desai, Y.; Jariwala, S.; Gupta, R.K. Bio-Based Polyurethanes and Their Applications. In *Polyurethanes: Preparation, Properties, and Applications Volume 2: Advanced Applications*; ACS Symposium Series; American Chemical Society: San Francisco, CA, USA, 2023; Volume 1453, pp. 1–14.
58. Chandna, S.; Olivares M, C.A.; Baranovskii, E.; Engelmann, G.; Boeker, A.; Tzschucke, C.C.; Haag, R. Lignin Upconversion by Functionalization and Network Formation. *Angew. Chem. Int. Ed.* **2024**, *63*, e202313945. [CrossRef]
59. Shao, L.; Liu, N.; Wang, Z.; Zhan, P.; Zhang, L.; Wu, Z. Functional Lignin-based Polymers: Isolation, Synthetic Methods and High-valued Applications. *ChemistrySelect* **2023**, *8*, e202301633. [CrossRef]
60. Hu, J.; Huang, M.; Zhou, X.; Luo, R.; Li, L.; Li, X. Research Status of Lignin-Based Polyurethane and Its Application in Flexible Electronics. *Polymers* **2024**, *16*, 2340. [CrossRef]
61. Chen, J.; Yang, H.; Qian, Y.; Ouyang, X.; Yang, D.; Pang, Y.; Lei, L.; Qiu, X. Translucent Lignin-Based Omniphobic Polyurethane Coating with Antismudge and UV-Blocking Dual Functionalities. *ACS Sustain. Chem. Eng.* **2023**, *11*, 2613–2622. [CrossRef]
62. Zhang, M.; Jeong, S.; Cho, W.; Ryu, J.; Zhang, B.; Crovella, P.; Ragauskas, A.J.; Wie, J.J.; Yoo, C.G. Green co-solvent-assisted one-pot synthesis of high-performance flexible lignin polyurethane foam. *Chem. Eng. J.* **2024**, *499*, 156142. [CrossRef]
63. Li, C.; Jin, H.; Hou, M.; Guo, X.; Xiao, T.; Cao, X.; Jia, W.; Fatehi, P.; Shi, H. Fractionated lignin as a polyol in polyurethane fabrication. *Int. J. Biol. Macromol.* **2024**, *256*, 128290. [CrossRef] [PubMed]
64. Liu, R.; Yang, Y.; Wu, Y.; Zhu, M.; Su, C.; Wang, Y.; Yao, Z.; Zhao, J.; Cao, H.; Cai, D. Rigid polyurethane foams refined by the lignin oligomers from catalytic upstream biorefining process. *Sustain. Mater. Technol.* **2023**, *35*, e00577. [CrossRef]
65. Du, J.; Wang, H.; Huang, Z.; Liu, X.; Yin, X.; Wu, J.; Lin, W.; Lin, X.; Yi, G. Construction and mechanism study of lignin-based polyurethane with high strength and high self-healing properties. *Int. J. Biol. Macromol.* **2023**, *248*, 125925. [CrossRef]
66. Falireas, P.G.; Gracia-Vitoria, J.; Hensen, A.; Vanbroekhoven, K.; Vendamme, R. Incorporating Phenolated Lignin into Polyurethane Materials: Impact on Mechanical, Thermal, and Adhesion Performance. *Ind. Eng. Chem. Res.* **2024**, *63*, 3921–3935. [CrossRef]
67. Liang, J.; Zhao, Z.; Zhou, T.; Fu, Y.; Tian, G.; Wang, Z. Fabrication of Bio-Based Flexible Polyurethane Foam with Biodegradation by Etherification of Kraft Lignin. *ACS Appl. Polym. Mater.* **2024**, *6*, 4441–4448. [CrossRef]

68. Chen, J.; Wu, J.; Zhong, Y.; Ma, X.; Lv, W.; Zhao, H.; Zhu, J.; Yan, N. Multifunctional superhydrophilic/underwater superoleophobic lignin-based polyurethane foam for highly efficient oil-water separation and water purification. *Sep. Purif. Technol.* **2023**, *311*, 123284. [CrossRef]
69. Yang, Y.; Cao, H.; Liu, R.; Wang, Y.; Zhu, M.; Su, C.; Lv, X.; Zhao, J.; Qin, P.; Cai, D. Fabrication of ultraviolet resistant and anti-bacterial non-isocyanate polyurethanes using the oligomers from the reductive catalytic fractionated lignin oil. *Ind. Crops Prod.* **2023**, *193*, 116213. [CrossRef]
70. Najafi, S.K.; Najafi, F.; Pizzi, A.; Khorshidi, F.H.; Behrooz, R. Effect of Amine Type on Lignin Modification to Evaluate Its Reactivity in Polyol Construction for Non-Isocyanate Polyurethanes (NIPU). *J. Renew. Mater.* **2023**, *11*, 2171. [CrossRef]
71. Zhang, T.; Xue, B.; Yan, Q.; Yuan, Y.; Tan, J.; Guan, Y.; Wen, J.; Li, X.; Zhao, W. New kinds of lignin/non-isocyanate polyurethane hybrid polymers: Facile synthesis, smart properties and adhesive applications. *Ind. Crops Prod.* **2023**, *199*, 116706. [CrossRef]
72. Ng, Q.Y.; Low, J.H.; Pang, M.M.; Idumah, C.I. Properties Enhancement of Waterborne Polyurethane Bio-composite Films with 3-aminopropyltriethoxy Silane Functionalized Lignin. *J. Polym. Environ.* **2023**, *31*, 688–697. [CrossRef]
73. Fan, D.; Huang, Y.; Niu, Y.; Lv, Y.; Li, G. Sustainable waterborne polyurethane/lignin nanoparticles composites: Durability meets degradability. *Polymer* **2024**, *305*, 127179. [CrossRef]
74. Kim, B.M.; Choi, J.S.; Jang, S.; Park, H.; Lee, S.Y.; Jung, J.; Park, J. Sustainable Strategies for Synthesizing Lignin-Incorporated Bio-Based Waterborne Polyurethane with Tunable Characteristics. *Polymers* **2023**, *15*, 3987. [CrossRef] [PubMed]
75. Aminzai, M.T.; Azizi, N.; Nural, Y.; Yabalak, E. A Review on Recent Advances in Polymer-Assisted Green and Sustainable Technology for Remediation of Pharmaceuticals from Water and Wastewater. *Water Air Soil Pollut.* **2023**, *234*, 681. [CrossRef]
76. He, Z.; Wang, M.; Ma, S. Porous lignin-based composites for oil/water separation: A review. *Int. J. Biol. Macromol.* **2024**, *260*, 129569. [CrossRef]
77. Yang, Y.; Wang, Y.; Zhu, M.; Zhao, J.; Cai, D.; Cao, H. Valorization of lignin for renewable non-isocyanate polyurethanes: A state-of-the-art review. *Mater. Today Sustain.* **2023**, *22*, 100367. [CrossRef]
78. Li, D.; Xue, B.; Zhao, Q.; Wang, W.; Li, X.; Wen, J.; Wang, Z.; Zhao, W. Green synthesis of lignin-based non-isocyanate polyurethanes as reusable, self-healable and removable adhesives. *Eur. Polym. J.* **2024**, *221*, 113553. [CrossRef]
79. Lin, L.; Tu, Y.; Li, Z.; Wu, H.; Mao, H.; Wang, C. Synthesis and application of multifunctional lignin-modified cationic waterborne polyurethane in textiles. *Int. J. Biol. Macromol.* **2024**, *262*, 130063. [CrossRef]
80. Lu, X.; Gu, X. A review on lignin-based epoxy resins: Lignin effects on their synthesis and properties. *Int. J. Biol. Macromol.* **2023**, *229*, 778–790. [CrossRef]
81. Arya, A.; Ahamad, A.; Kumar, P.; Chandra, A. A review on lignin based nanocomposites: Fabrication, characterization and application. *Mater. Today Proc.* **2023**, *78*, A10. [CrossRef]
82. Zhou, S.; Huang, K.; Xu, X.; Wang, B.; Zhang, W.; Su, Y.; Hu, K.; Zhang, C.; Zhu, J.; Weng, G.; et al. Rigid-and-Flexible, Degradable, Fully Biobased Thermosets from Lignin and Soybean Oil: Synthesis and Properties. *ACS Sustain. Chem. Eng.* **2023**, *11*, 3466–3473. [CrossRef]
83. Song, P.; Du, L.; Pang, J.; Jiang, G.; Shen, J.; Ma, Y.; Ren, S.; Li, S. Preparation and properties of lignin-based vitrimer system containing dynamic covalent bonds for reusable and recyclable epoxy asphalt. *Ind. Crops Prod.* **2023**, *197*, 116498. [CrossRef]
84. Zou, S.-L.; Xiao, L.-P.; Li, X.-Y.; Yin, W.-Z.; Sun, R.-C. Lignin-based composites with enhanced mechanical properties by acetone fractionation and epoxidation modification. *iScience* **2023**, *26*, 106187. [CrossRef] [PubMed]
85. Li, X.; Li, X.; Ye, J.; Shen, L.; Tan, W.; Luo, X. Preparation and characterization of black liquor lignin-based epoxy composite film. *Mater. Lett.* **2023**, *337*, 133998. [CrossRef]
86. Pappa, C.P.; Cailotto, S.; Gigli, M.; Crestini, C.; Triantafyllidis, K.S. Kraft (Nano)Lignin as Reactive Additive in Epoxy Polymer Bio-Composites. *Polymers* **2024**, *16*, 553. [CrossRef]
87. Xue, M.; Hu, S.; Zhang, S.; Ou, J.; Chen, H.; Liao, Y.; Yue, F. Flexible and Heat-Resisting Lignin-Based Epoxy Resins by Hardwood Kraft Low-Molecular-Weight Lignin as a Sustainable Substitute for Bisphenol A. *ACS Sustain. Chem. Eng.* **2023**, *11*, 16774–16784. [CrossRef]
88. Bagheri, S.; Nejad, M. Fully biobased composite made with epoxidized-lignin, reinforced with bamboo fibers. *Polym. Compos.* **2023**, *44*, 3926–3938. [CrossRef]
89. Gavrilović-Grmuša, I.; Rančić, M.; Tešić, T.; Stupar, S.; Milošević, M.; Gržetić, J. Bio-Epoxy Resins Based on Lignin and Tannic Acids as Wood Adhesives—Characterization and Bonding Properties. *Polymers* **2024**, *16*, 2602. [CrossRef]
90. Li, G.; Li, C.; Li, P.; Sun, R.; Cao, L.; Zhu, P. Lignin-based silicone-modified epoxy resin with enhanced strength and toughness. *Int. J. Adhes. Adhes.* **2024**, *128*, 103564. [CrossRef]
91. Rodrigues, J.S.; de Freitas, A.d.S.M.; Maciel, C.C.; Mendes, S.F.; Diment, D.; Balakshin, M.; Botaro, V.R. Selection of kraft lignin fractions as a partial substitute for phenol in synthesis of phenolic resins: Structure-property correlation. *Ind. Crops Prod.* **2023**, *191*, 115948. [CrossRef]
92. Li, P.; Yang, C.; Yi, D.; Li, S.; Wang, M.; Wang, H.; Jin, Y.; Wu, W. Preparation of spherical porous carbon from lignin-derived phenolic resin and its application in supercapacitor electrodes. *Int. J. Biol. Macromol.* **2023**, *252*, 126271. [CrossRef] [PubMed]

93. Galdino, D.S.; Kondo, M.Y.; De Araujo, V.A.; Ferrufino, G.L.; Faustino, E.; Santos, H.F.; Christoforo, A.L.; Luna, C.M.; Campos, C.I. Thermal and Gluing Properties of Phenol-Based Resin with Lignin for Potential Application in Structural Composites. *Polymers* **2023**, *15*, 357. [CrossRef] [PubMed]
94. Bansode, A.; Portilla Villarreal, L.A.; Wang, Y.; Asafu-Adjaye, O.; Via, B.K.; Farag, R.; Vega Erramuspe, I.B.; Auad, M.L. Kraft Lignin Periodate Oxidation for Biobased Wood Panel Resins. *ACS Appl. Polym. Mater.* **2023**, *5*, 4118–4126. [CrossRef]
95. Zhao, L.; Li, W.; Cheng, Y.; Zhao, J.; Tian, D.; Huang, M.; Shen, F. Preparation and evaluation of lignin-phenol-formaldehyde resin as wood adhesive using unmodified lignin. *Ind. Crops Prod.* **2024**, *211*, 118168. [CrossRef]
96. Gaudenzi, E.; Cardone, F.; Lu, X.; Canestrari, F. The use of lignin for sustainable asphalt pavements: A literature review. *Constr. Build. Mater.* **2023**, *362*, 129773. [CrossRef]
97. Peng, Z.; Jiang, X.; Si, C.; Joao Cárdenas-Oscanoa, A.; Huang, C. Advances of Modified Lignin as Substitute to Develop Lignin-Based Phenol-Formaldehyde Resin Adhesives. *ChemSusChem* **2023**, *16*, e202300174. [CrossRef]
98. Fazeli, M.; Mukherjee, S.; Baniyadi, H.; Abidinejad, R.; Mujtaba, M.; Lipponen, J.; Seppala, J.; Rojas, O.J. Lignin beyond the status quo: Recent and emerging composite applications. *Green Chem.* **2024**, *26*, 593–630. [CrossRef]
99. Ye, H.; You, T.; Nawaz, H.; Xu, F. A comprehensive review on polylactic acid/lignin composites—Structure, synthesis, performance, compatibilization, and applications. *Int. J. Biol. Macromol.* **2024**, *280*, 135886. [CrossRef]
100. Shi, K.; Liu, G.; Sun, H.; Weng, Y. Polylactic Acid/Lignin Composites: A Review. *Polymers* **2023**, *15*, 2807. [CrossRef]
101. Rajeshkumar, G.; Arvindh Seshadri, S.; Devnani, G.L.; Sanjay, M.R.; Siengchin, S.; Prakash Maran, J.; Al-Dhabi, N.A.; Karuppiyah, P.; Mariadhas, V.A.; Sivarajasekar, N.; et al. Environment friendly, renewable and sustainable poly lactic acid (PLA) based natural fiber reinforced composites—A comprehensive review. *J. Clean. Prod.* **2021**, *310*, 127483. [CrossRef]
102. Shi, K.; Liu, G.; Sun, H.; Yang, B.; Weng, Y. Grafting Polymerization of Long-Chain Hydrophobic Acrylic Monomer onto Lignin and Its Application in Poly(Lactic Acid)-Based Wholly Green UV Barrier Composite Films. *ACS Omega* **2023**, *8*, 26926–26937. [CrossRef] [PubMed]
103. Kim, D.; Kim, J.-C.; Kim, J.; Cho, Y.-M.; Yoon, C.-H.; Shin, J.-H.; Kwak, H.W.; Choi, I.-G. Enhancement of elongation at break and UV-protective properties of poly(lactic acid) film with cationic ring opening polymerized (CROP)-lignin. *Int. J. Biol. Macromol.* **2023**, *253*, 127293. [CrossRef] [PubMed]
104. Yu, S.; Lu, Y.; Guo, S.; Guo, T.; Takagi, A.; Kamkar, M.; Rojas, O.J. Lignin-Polylactide Reverse Emulsions for Water and UV-Resistant Composite Films. *ACS Sustain. Chem. Eng.* **2023**, *11*, 12503–12513. [CrossRef]
105. Banpean, A.; Hararak, B.; Winotapun, C.; Wijaranakul, P.; Kitchaicharoenporn, S.; Naimlang, S. Lignin nanoparticles as sustainable biobased nucleating agents of poly(L-lactic acid): Crystallization behavior and effect of particle sizes. *J. Mater. Sci.* **2023**, *58*, 6823–6838. [CrossRef]
106. Shakoar Shar, A.; Wang, N.; Chen, T.; Zhao, X.; Weng, Y. Development of PLA/Lignin Bio-Composites Compatibilized by Ethylene Glycol Diglycidyl Ether and Poly (ethylene glycol) Diglycidyl Ether. *Polymers* **2023**, *15*, 4049. [CrossRef]
107. Ou, W.-X.; Weng, Y.; Zeng, J.-B.; Li, Y.-D. Fully biobased poly(lactic acid)/lignin composites compatibilized by epoxidized natural rubber. *Int. J. Biol. Macromol.* **2023**, *236*, 123960. [CrossRef]
108. Ding, R.; Duan, Z.; Sun, Y.; Yuan, Q.; Tien, T.T.; Zúniga, M.G.; Oh, E.; Nam, J.-D.; Suhr, J. Enhancement of 3D printability and mechanical properties of polylactic acid/lignin biocomposites via interface engineering. *Ind. Crops Prod.* **2023**, *194*, 116286. [CrossRef]
109. Makri, S.P.; Xanthopoulou, E.; Valera, M.A.; Mangas, A.; Marra, G.; Ruiz, V.; Koltsakidis, S.; Tzetzis, D.; Zoikis Karathanasis, A.; Deligkiozi, I.; et al. Poly(Lactic Acid) Composites with Lignin and Nanolignin Synthesized by In Situ Reactive Processing. *Polymers* **2023**, *15*, 2386. [CrossRef]
110. Murillo-Morales, G.; Sethupathy, S.; Zhang, M.; Xu, L.; Ghaznavi, A.; Xu, J.; Yang, B.; Sun, J.; Zhu, D. Characterization and 3D printing of a biodegradable polylactic acid/thermoplastic polyurethane blend with laccase-modified lignin as a nucleating agent. *Int. J. Biol. Macromol.* **2023**, *236*, 123881. [CrossRef]
111. Ju, Z.; Brosse, N.; Hoppe, S.; Wang, Z.; Ziegler-Devin, I.; Zhang, H.; Shu, B. Thermal and mechanical properties of polyethylene glycol (PEG)-modified lignin/polylactic acid (PLA) biocomposites. *Int. J. Biol. Macromol.* **2024**, *262*, 129997. [CrossRef]
112. Beniwal, P.; Toor, A.P. Functionalisation of lignin with urethane linkages and their strengthening effect on PLA composites. *Int. J. Biol. Macromol.* **2024**, *258*, 129005. [CrossRef] [PubMed]
113. Li, B.; Zhu, X.; Xu, C.; Zhang, X.; Fan, Y.; Yu, J. Sustainable strategy for lignin etherification and its promotion of anti-UV aging PLA biocomposite. *Chem. Eng. J.* **2024**, *500*, 156746. [CrossRef]
114. Cao, L.; Chen, P.; Qi, B.; Qi, Z.; Chang, L.; Shan, J.; Sun, C.; Tan, H.; Zhang, Y. A new strategy to prepare bio-based polylactic acid modifier for multi-functionality: Dopamine-grafted modified lignin. *Mater. Today Commun.* **2024**, *41*, 110857. [CrossRef]
115. Zhang, B.; Qiang, G.; Barta, K.; Sun, Z. Bio-based polymers from lignin. *Innov. Mater.* **2024**, *2*, 100062. [CrossRef]
116. Boarino, A.; Klok, H.-A. Opportunities and Challenges for Lignin Valorization in Food Packaging, Antimicrobial, and Agricultural Applications. *Biomacromolecules* **2023**, *24*, 1065–1077. [CrossRef]

117. Ruwoldt, J.; Blindheim, F.H.; Chinga-Carrasco, G. Functional surfaces, films, and coatings with lignin—A critical review. *RSC Adv.* **2023**, *13*, 12529–12553. [CrossRef]
118. Santos, C.C.O.; Ferreira, F.V.; Pinheiro, I.F.; Lona, L.M.F. Lignin valorization through polymer grafting by ring-opening polymerization and its application in health, packaging, and coating. *J. Environ. Chem. Eng.* **2023**, *11*, 109691. [CrossRef]
119. Wang, D.; Gu, Y.; Feng, S.; Yang, W.; Dai, H.; Xiao, H.; Han, J. Lignin-containing biodegradable UV-blocking films: A review. *Green Chem.* **2023**, *25*, 9020–9044. [CrossRef]
120. Olonisakin, K.; Wen, A.; He, S.; Lin, H.; Tao, W.; Chen, S.; Lin, W.; Li, R.; Zhang, X.-x.; Yang, W. The Development of Biodegradable PBAT-Lignin-Tannic Acid Composite Film: Properties, Biodegradability, and Potential Barrier Application in Food Packaging. *Food Bioprocess Technol.* **2023**, *16*, 1525–1540. [CrossRef]
121. Waqas Ali Shah, S.; Ma, K.; Ullah, R.; Ali, E.A.; Qayum, A.; Zahoor; Uddin, N.; Zhu, D. Laccase and dye-decolorizing peroxidase-modified lignin incorporated with keratin-based biodegradable film: An elucidation of structural characterization, antibacterial and antioxidant properties. *Food Chem. X* **2023**, *20*, 101035. [CrossRef]
122. Dong, T.; Chen, W.; Cai, C.; Bai, F.; Zhou, Z.; Wang, J.; Li, X. Water-stable, strong, biodegradable lignocellulose straws replacement for plastic straws. *Chem. Eng. J.* **2023**, *451*, 138970. [CrossRef]
123. Baniasadi, H.; Lipponen, S.; Asplund, M.; Seppälä, J. High-concentration lignin biocomposites with low-melting point biopolyamide. *Chem. Eng. J.* **2023**, *451*, 138564. [CrossRef]
124. Tian, D.; Zhang, J.; Hu, J.; Huang, M.; Zhao, L.; Lei, Y.; Zou, J.; Zhang, S.; Shen, F. A new water-soluble lignin incorporation enhanced the barrier performance of liquid mulching film. *Chem. Eng. J.* **2023**, *452*, 139383. [CrossRef]
125. Xu, T.; Gao, H.; Zhou, J.; He, M.; Ji, X.; Dai, H.; Rojas, O.J. Design of AgNPs doped chitosan/sodium lignin sulfonate/polypyrrole films with antibacterial and endotoxin adsorption functions. *Int. J. Biol. Macromol.* **2023**, *229*, 321–328. [CrossRef]
126. Xie, J.; Xu, J.; Zhang, Z.; Wang, B.; Zhu, S.; Li, J.; Chen, K. New ternary deep eutectic solvents with cycle performance for efficient pretreated radiata pine forming to lignin containing cellulose nanofibrils. *Chem. Eng. J.* **2023**, *451*, 138591. [CrossRef]
127. Sun, X.; Li, Q.; Wu, H.; Zhou, Z.; Feng, S.; Deng, P.; Zou, H.; Tian, D.; Lu, C. Sustainable Starch/Lignin Nanoparticle Composites Biofilms for Food Packaging Applications. *Polymers* **2023**, *15*, 1959. [CrossRef]
128. Rodrigues, J.S.; de Freitas, A.d.S.M.; Lopes, H.S.M.; Pires, A.A.F.; Lemes, A.P.; Ferreira, M.; Botaro, V.R. Improvement of UV stability of thermoplastic starch matrix by addition of selected lignin fraction—Photooxidative degradation. *Int. J. Biol. Macromol.* **2023**, *230*, 123142. [CrossRef]
129. Pham, C.D.; Truong, T.M.; Ly, T.B.; Le, P.K. Application of Lignin from Cellulose Isolation Process in the Fabrication of Chitosan/Lignin Film for UV-Light Blocking and Anti-oxidation. *Waste Biomass Valorization* **2023**, *15*, 1881–1894. [CrossRef]
130. Ou, J.; Hu, S.; Yao, L.; Chen, Y.; Qi, H.; Yue, F. Simultaneous strengthening and toughening lignin/cellulose nanofibril composite films: Effects from flexible hydrogen bonds. *Chem. Eng. J.* **2023**, *453*, 139770. [CrossRef]
131. Gao, S.; Zhu, C.; Ma, L.; Liu, C.; Zhang, H.; Zhang, S. Preparation of an Aminated Lignin/Fe(III)/Polyvinyl Alcohol Film: A Packaging Material with UV Resistance and Slow-Release Function. *Foods* **2023**, *12*, 2794. [CrossRef]
132. Jiang, H.; Chu, Q.; Ma, J.; Wu, S.; Shao, L.; Zhou, X. Dissolution of lignocellulose with high lignin content in AlCl₃/ZnCl₂ aqueous system and properties of the regenerated cellulose film. *Int. J. Biol. Macromol.* **2023**, *234*, 123590. [CrossRef] [PubMed]
133. Zolfaghari, S.; Soltaninejad, A.; Okoro, O.V.; Shavandi, A.; Denayer, J.F.M.; Sadeghi, M.; Karimi, K. Starch biocomposites preparation by incorporating organosolv lignins from potato crop residues. *Int. J. Biol. Macromol.* **2024**, *259*, 129140. [CrossRef] [PubMed]
134. Warale, D.; Shabeena, M.; Prabhu, A.; Kouser, S.; Manasa, D.J.; Nagaraja, G.K. Sustainable organosolv-lignin coated nanosilver-halloysites reinforced poly (vinyl alcohol) nanocomposites for wound healing application. *Int. J. Biol. Macromol.* **2024**, *257*, 128628. [CrossRef] [PubMed]
135. Domínguez-Robles, J.; Cuartas-Gómez, E.; Dynes, S.; Utomo, E.; Anjani, Q.K.; Detamornrat, U.; Donnelly, R.F.; Moreno-Castellanos, N.; Larrañeta, E. Poly(caprolactone)/lignin-based 3D-printed dressings loaded with a novel combination of bioactive agents for wound-healing applications. *Sustain. Mater. Technol.* **2023**, *35*, e00581. [CrossRef]
136. Wang, Q.-M.; Liu, Z.-H.; Lü, Q.-F. Lignin modified Ti₃C₂T_x assisted construction of functionalized interface for separation of oil/water mixture and dye wastewater. *Colloids Surf. A Physicochem. Eng. Asp.* **2023**, *656*, 130371. [CrossRef]
137. Huang, J.; Li, M.; Ren, C.; Huang, W.; Miao, Y.; Wu, Q.; Wang, S. Construction of HLNPs/Fe₃O₄ based superhydrophobic coating with excellent abrasion resistance, UV resistance, flame retardation and oil absorbency. *J. Environ. Chem. Eng.* **2023**, *11*, 109046. [CrossRef]
138. Zhang, Y.-Q.; An, Q.-D.; Xiao, Z.-Y.; Zhu, K.-R.; Dong, X.-L.; Zhai, S.-R. PDMS/magnetic lignin sponge for oil/water separation. *Int. J. Biol. Macromol.* **2023**, *253*, 127368. [CrossRef]
139. Zhang, J.; Jiang, F.; Lu, Y.; Wei, S.; Xu, H.; Zhang, J.; Ge, Y.; Li, Z. Lignin microparticles-reinforced cellulose filter paper for simultaneous removal of emulsified oils and dyes. *Int. J. Biol. Macromol.* **2023**, *230*, 123120. [CrossRef]
140. Guo, Z.; Wang, M.; Qiao, L.; Wang, J.; He, Z. Photothermal, Magnetic, and Superhydrophobic PU Sponge Decorated with a Fe₃O₄/MXene/Lignin Composite for Efficient Oil/Water Separation. *Langmuir* **2023**, *39*, 16935–16953. [CrossRef]

141. Nisar, S.; Raza, Z.A. Corn straw lignin—A sustainable bioinspired finish for superhydrophobic and UV-protective cellulose fabric. *Int. J. Biol. Macromol.* **2024**, *257*, 128393. [CrossRef]
142. Yu, H.; Wang, B.; Wang, Y.; Xu, E.; Wang, R.; Wu, S.; Wu, W.; Ji, B.; Feng, X.; Xu, H.; et al. Lignin Nanoparticles with High Phenolic Content as Efficient Antioxidant and Sun-Blocker for Food and Cosmetics. *ACS Sustain. Chem. Eng.* **2023**, *11*, 4082–4092. [CrossRef]
143. Zhan, B.; Zhang, L.; Deng, Y.; Yan, L. A multifunctional lignin-based composite ultra-adhesive for wood processing. *Green Chem.* **2023**, *25*, 10061–10071. [CrossRef]
144. Wu, M.; Wu, M.; Zhang, M.; Jiang, F.; Zhou, L. Preparation of all biomass lignin-based thermoplastic elastomers by ARGET ATRP. *Ind. Crops Prod.* **2023**, *193*, 116236. [CrossRef]
145. Ou, Y.; Xing, Y.; Yang, Z.; Huang, J.; He, J.; Jiang, F.; Zhang, Y. Strong and ultrafast stimulus-healable lignin-based composite elastomers with excellent adhesion properties. *Int. J. Biol. Macromol.* **2024**, *256*, 128507. [CrossRef]
146. Jiang, B.; Jiao, H.; Guo, X.; Chen, G.; Guo, J.; Wu, W.; Jin, Y.; Cao, G.; Liang, Z. Chemistry, Processing, Structures, Properties, and Applications of Lignin-Based Materials for Additive Manufacturing. *Adv. Sci.* **2023**, *10*, 2206055. [CrossRef]
147. Shah, S.W.A.; Xu, Q.; Ullah, M.W.; Zahoor; Sethupathy, S.; Morales, G.M.; Sun, J.; Zhu, D. Lignin-based additive materials: A review of current status, challenges, and future perspectives. *Addit. Manuf.* **2023**, *74*, 103711. [CrossRef]
148. Silva-Barroso, A.S.; Cabral, C.S.D.; Ferreira, P.; Moreira, A.F.; Correia, I.J. Lignin-enriched tricalcium phosphate/sodium alginate 3D scaffolds for application in bone tissue regeneration. *Int. J. Biol. Macromol.* **2023**, *239*, 124258. [CrossRef]
149. Norouzi, M.; Rafienia, M.; Hosseini, S. Characterization and biological evaluation of new PLGA/fibrin/lignin biocomposite electrospun scaffolds. *Phys. Scr.* **2023**, *98*, 095506. [CrossRef]
150. Su, Y.; Tang, S.; Cai, M.; Nie, Y.; Hu, B.; Wu, S.; Cheng, C. Thermal oxidative aging mechanism of lignin modified bitumen. *Constr. Build. Mater.* **2023**, *363*, 129863. [CrossRef]
151. Rezazad Gohari, A.; Lamothe, S.; Bilodeau, J.-P.; Mansourian, A.; Carter, A. Laboratory Study on Influence of Blending Conditions on Chemo-Thermal Characteristics of Lignin-Modified Bitumen. *Appl. Sci.* **2023**, *13*, 7766. [CrossRef]
152. Wu, Z.; Lin, X.; Teng, J.; Li, M.; Song, J.; Huang, C.; Wang, R.; Ying, H.; Zhang, L.; Zhu, C. Recent Advances of Lignin Functionalization for High-Performance and Advanced Functional Rubber Composites. *Biomacromolecules* **2023**, *24*, 4553–4567. [CrossRef] [PubMed]
153. Chung, J.-Y.; Hwang, U.; Kim, J.; Kim, N.-Y.; Nam, J.; Jung, J.; Kim, S.-H.; Cho, J.K.; Lee, B.; Park, I.-K.; et al. Amine-Functionalized Lignin as an Eco-Friendly Antioxidant for Rubber Compounds. *ACS Sustain. Chem. Eng.* **2023**, *11*, 2303–2313. [CrossRef]
154. Laanesoo, S.; Bonjour, O.; Sedrik, R.; Tamsalu, I.; Jannasch, P.; Vares, L. Combining isosorbide and lignin-related benzoic acids for high-Tg polymethacrylates. *Eur. Polym. J.* **2024**, *202*, 112595. [CrossRef]
155. Feng, Y.; Yu, J.; Shao, C.; Sun, R. Advances toward sustainable lignin-based gel for energy storage and smart sensing. *Pap. Biomater.* **2023**, *8*, 20–36. [CrossRef]
156. Nanthagal, K.; Khoonsap, S.; Harnchana, V.; Suphasorn, P.; Chanlek, N.; Sinthiptharakoon, K.; Lapawae, K.; Amnuaypanich, S. Unprecedented Triboelectric Effect of Lignin on Enhancing the Electrical Outputs of Natural-Rubber-Based Triboelectric Nanogenerators (TENGs). *ACS Sustain. Chem. Eng.* **2023**, *11*, 1311–1323. [CrossRef]
157. Chowdhury, S.G.; Ghosh, S.; Pal, A.; Pal, K.; Chanda, J.; Samui, B.K.; Bhattacharyya, S.K.; Mukhopadhyay, R.; Bandyopadhyay, A. Evaluation of lignin as potential green filler in an optimally designed solution grade styrene-butadiene rubber (SSBR) based tyre tread compound. *Plast. Rubber Compos.* **2023**, *52*, 63–74. [CrossRef]
158. Qiu, J.; Yuan, S.; Xiao, H.; Liu, J.; Shen, T.; Tan, Z.; Zhuang, W.; Ying, H.; Li, M.; Zhu, C. Study on lignin amination for lignin/SiO₂ nano-hybrids towards sustainable natural rubber composites. *Int. J. Biol. Macromol.* **2023**, *233*, 123547. [CrossRef]
159. Campos, G.N.; da Rocha, E.B.D.; Furtado, C.R.G.; de Figueiredo, M.A.G.; de Sousa, A.M.F. Using carboxyl groups to improve the compatibility of XNBR/lignin composites. *Polym. Compos.* **2023**, *45*, 4124–4137. [CrossRef]
160. Moretti, C.; Corona, B.; Hoefnagels, R.; Vural-Gürsel, I.; Gosselink, R.; Junginger, M. Review of life cycle assessments of lignin and derived products: Lessons learned. *Sci. Total Environ.* **2021**, *770*, 144656. [CrossRef]
161. Scown, C.D.; Baral, N.R.; Yang, M.; Vora, N.; Huntington, T. Technoeconomic analysis for biofuels and bioproducts. *Curr. Opin. Biotechnol.* **2021**, *67*, 58–64. [CrossRef]

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Transparent Wood Fabrication and Applications: A Review

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Abstract: Wood cellulose is an abundant bio-based resource with diverse applications in construction, cosmetics, packaging, and the pulp and paper industries. Transparent wood (TW) is a novel, high-quality wood material with several advantages over traditional transparent materials (e.g., glass and plastic). These benefits include renewability, UV shielding, lightweight properties, low thermal expansion, reduced glare, and improved mechanical strength. TW has significant potential for various applications, including transparent roofs, windows, home lighting structures, electronic devices, home decoration, solar cells, packaging, smart packaging materials, and other high-value-added products. The mechanical properties of TW, such as tensile strength and optical transmittance, are typically up to 500 MPa (Young's modulus of 50 GPa) and 10–90%, respectively. Fabrication methods, wood types, and processing conditions significantly influence the mechanical and optical properties of TW. In addition, recent research has highlighted the feasibility of TW and large-scale production, making it an emerging research topic for future exploration. This review attempted to provide recent and updated manufacturing methods of TW as well as current and future applications. In particular, the effects of structural modification through various chemical pretreatment methods and impregnation methods using various polymers on the properties of TW biocomposites were also reviewed.

Keywords: bio-based materials; polymer resin; cellulose nanofiber; lignocellulose; delignification

1. Introduction

Wood has long been a fundamental material widely used in various industries, such as pulp and paper, construction, flooring, furniture, and fuel. As the most abundant organic raw material on the planet, wood has played an essential role in human life for thousands of years. Recently, the commercialization of high-value-added wood for various uses other than traditional applications has attracted increasing research attention. In particular, wood has a low carbon footprint, is environmentally friendly and is renewable.

Among its various emerging applications, transparent wood (TW) has gained significant attention for its potential use in high-value-added products, such as smart windows, rooftops, decorative materials, solar cells, insulation, lighting management, and UV shielding [1–10]. Fink first introduced TW prepared by impregnating (infiltrating) different types of polymers into wood structures [11]. Previous studies [9–14] have highlighted the numerous advantages and potential future applications of TW. TW intended for commercial and

industrial use must be environmentally friendly and safe for the human body, ensuring the absence of toxic or harmful chemicals.

Transparent wood fabrication begins with the chemical pretreatment of lignocellulosic biomass, involving either of the following processes: (1) complete lignin removal [11] or (2) chemical modification of lignin with partial delignification while retaining a controlled amount of lignin [8,15–18]. To produce a high-quality transparent wood material, the delignified wood is impregnated with a polymer with a refractive index (RI) closely matching that of the wood matrix [11]. Subsequently, the RI difference between the cell wall and lumen is reduced, resulting in less light scattering and higher transparency. In recent studies, various polymers, including epoxy, prepolymerized methyl methacrylate (PMMA), polyvinyl pyrrolidone (PVP), polyethylene glycol/methyl methacrylate (PEG/MMA), polyvinyl alcohol (PVA), polyurethane (PU), epoxy vitrimers, TEMPO-treated cellulose nanofiber (CNF), and chitosan (CTS) polymer, have been used to make TW [14,15,17,19–22]. However, in recent years, various environmental issues have placed many restrictions on using conventional petrochemical-based resin materials (e.g., epoxy or PMMA). Consequently, bio-based polymers, such as limonene acrylate monomer, CNF, and CTS [14,23], have been increasingly used to produce eco-friendly TW. In addition, self-densified and compacted TW has demonstrated enhanced mechanical properties [24,25].

Light transmittance is the most critical property of TW, typically influenced by wood thickness, delignification process, light transmittance of the polymer, and difference in light transmittance between the polymer and wood. Previous studies have reported transmittance values of TW varying from 10% to 90% [1,2,5,10,17,21]. Several factors, including wood species and type, delignification process, bleaching conditions, thickness, tensile strength, expansion rate, light transmittance, and polymer properties, influence the performance of TW [2,5,17]. Owing to the limitations of conventional glass, such as brittleness and low thermal insulation, developing a method for producing commercially viable TW with enhanced tensile strength and less brittleness is crucial. Studies have demonstrated that the mechanical properties of TW could be substantially improved. For example, reinforcing with epoxy resin significantly improved its longitudinal tensile strength from 42.7 MPa to 45.4 MPa and from 4.5 MPa to 23.4 MPa in the radial direction [21]. The improvement in the tensile strength of TW was reported to be approximately 106% and 520% in the longitudinal and radial directions, respectively, corresponding to 10 MPa to 200 MPa or higher [1,5,13]. Furthermore, multilayered TW has been developed to achieve enhanced mechanical properties in all directions [9,26,27]. By contrast, some studies have reported a reduction in mechanical strength, with reductions from approximately 220 MPa to approximately 150 MPa [2,19] or from 60 MPa to 30 MPa [28,29].

For the industrial application of TW, understanding the influence of raw materials and fabrication processes on its mechanical properties is crucial. This review explores the various types of TW, their production methods, and the impact of polymer impregnation and manufacturing processes on the mechanical and optical properties of TW. Furthermore, it explores advancements in nanostructured materials, high thermal insulation, and thermal conductivity. This review examines various factors affecting the transmittance of TW. Furthermore, it explores factors affecting transparency, such as wood types, polymers used, material thickness, and manufacturing processes.

2. Review of the Fabrication Process of TW

Currently, softwood and hardwood species are used in composites TW with various polymers, including PMMA, PVA, PVA-lignin nanoparticles, PVA-chitin nanofibers, chitin nanocrystals, chitin-chitosan [8], CNF, CTS, PU, and PEG/MMA. Various bleaching agents (catalysts) are used, such as NaClO_2 , H_2O_2 , NaOH , acetic acid, sodium acetate, ethylenedi-

aminetetraacetic acid (EDTA), and other combinations are used to delignify wood-based raw materials. Figure 1 shows the general process for producing TW.

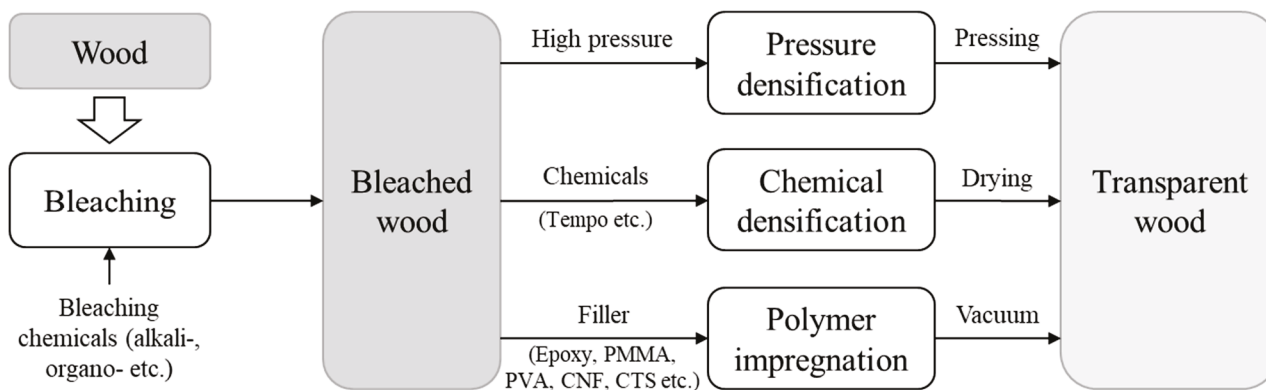


Figure 1. General fabrication methods of TW.

2.1. Bleaching and Delignification

Delignification is a crucial initial step in producing TW, although complete lignin removal is not always necessary [15,16,18,30]. Several studies [15–17] have indicated that even partial lignin removal from wood sources can result in TW. Various bleaching and delignification methods are used in the early stages of TW production. Wood delignification involves using alkalis, organic acids, NaClO_2 , H_2O_2 , and enzymes. Bleaching conditions influence the transmittance and mechanical properties of TW. Wood type, wood thickness, reaction time, and chemical dosage also affect the bleaching process. A common delignification method involves oxidative bleaching. This process involves treating dried wood by immersing it in a sodium chlorite (NaClO_2) bleaching solution with acetate buffer, maintained at a pH of 4.5 and temperature of 70–80 °C. As mentioned in the Introduction, bleaching using NaClO_2 is the preferred method for producing TW. Other studies used a combination of H_2O_2 in the delignification process [21,28,31]. Wood can be bleached using different methods, bleaching agents, temperatures, and durations. Bleaching removes lignin content, alters its color, and transforms brown wood into a white one. Lignin modification retains lignin while decolorizing the wood. The bleaching process breaks down the bonds within the complex lignin structure. The most easily cleaved lignin bonds include α -O-5, β -O-5, and γ -O-5. The mechanisms involved in the bleaching process have been widely studied in the pulp and paper industry. Details on wood and pulp bleaching are discussed in several studies [16,32–34]. Highly delignified and bleached wood turns white and exhibits a highly porous structure, facilitating efficient polymer infiltration. However, lignin modification primarily whitens or decolorizes wood. This approach has gained significant interest in the large-scale and efficient production of TW, as demonstrated by [35]. Their study highlighted the potential of lignin modification in producing TW and its viability for large-scale production. Xia et al. applied NaOH and H_2O_2 to wood samples, followed by UV illumination, until the samples turned completely white [35]. Subsequently, various TW products were prepared through vacuum infiltration using epoxy (300/21 epoxy resin, Aeromarine Products Inc., San Diego, CA, USA). TW prepared through lignin modification has the potential for various applications and large-scale production. Figure 2 illustrates the lignin modification and delignification processes, along with their mechanisms.

A washing step is performed after bleaching to remove unreacted chemicals, extracts, lignin, and impurities. During this process, the pH is neutralized, and a light vacuum may be applied to the pretreated wood to remove residual chemicals and volatile components. Subsequently, the treated wood undergoes a solvent-exchange process, where it is immersed in an ethanol and acetone solution. Depending on the purity, the amount of solvent

required for the exchange process typically ranges from two to three times the volume of wood. After undergoing these processes, the wood material is ready for TW production. However, solvent exchange is not always necessary. TW can be produced by directly infiltrating the polymer into the dried, bleached wood without a solvent-exchange process. Table 1 lists the different delignification and bleaching agents used for TW fabrication.

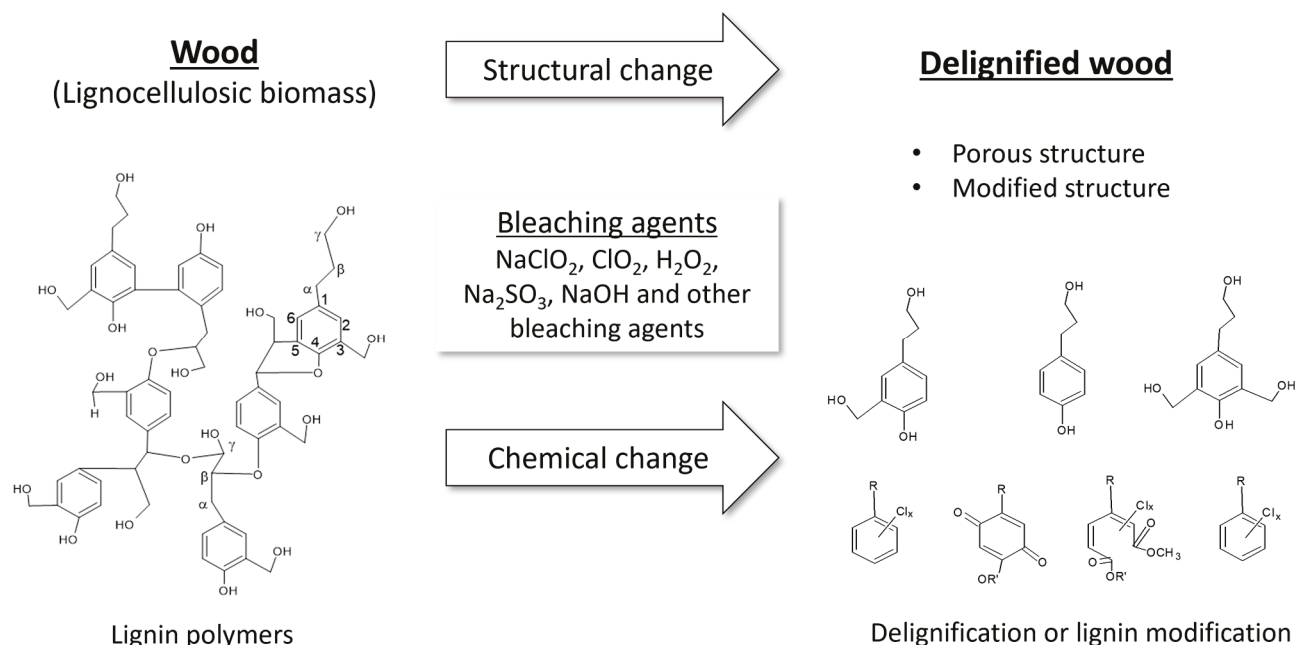


Figure 2. Delignification, lignin modification, and the development of porous wood structure through bleaching [16,32–34].

Table 1. Bleaching methods using various woods and bleaching agents for TW production.

Wood Species; Size (W:L:T)	Bleaching Agents and Delignification	References
Poplar veneer; 80:80:3 mm ³	KOH (>98%) and DI water followed by NaClO (>98%) for 8 h at 120–130 °C. The amount of lignin content before and after bleaching is not indicated.	[36]
Basswood	Boiling with NaOH (2.5 mol L ^{−1}) and Na ₂ SO ₃ (0.4 mol L ^{−1}) for 12 h. Second Step: H ₂ O ₂ , 2.5 mol L ^{−1} for 12 h. In the first stage, lignin content = ~12–14%; second stage, the lignin content ≤ 3.0%.	[21]
Basswood	Soaking in NaOH (2.5 mol/L) and Na ₂ SO ₃ (0.4 mol/L). Boiling for 12 h. Bleaching with H ₂ O ₂ , (2.5 mol/L). This resulted in 33%, 50%, and nearly 100% lignin removal.	[37]
Poplar (<i>Populus</i> sp.) and Balsa wood. Width (80–300 mm), Length from 25 to 300 mm and thickness from 1–10 mm	NaOH (10 wt%) and Na ₂ SO ₃ (5 wt%) boiling for 2–4 h, followed by boiling in DI water. Further whitening using H ₂ O ₂ (30 wt%) in boiling. The lignin content is ~2.8%.	[38]
Balsa (<i>Ochroma pyramidale</i>), alder (<i>Alnus glutinosa</i>), birch (<i>Betula pendula</i>), and beech (<i>Fagus sylvestris</i>); 0.7–3 mm thickness	Peracetic acid (PAA) and CH ₃ COOOH. Treated at 80 °C using aqueous PAA solution (4 wt%) at a pH of 4. 8 (adjusted with NaOH), followed by washing with DI water and acetone. Lignin removal from 18.2% to 27.9% of untreated to 0.9 to 2.0% of treated biomass.	[23]
Basswood (<i>Tilia</i>); 20:20:0.42 mm ³	2.0 wt% NaClO ₂ and 0.1 wt% acetic acid glacial, bleaching time for 30, 60, 90, 120, and 150 min. This resulted in lignin removal of 33, 38, 47, 51, and 64%, with treatment time of 30, 60, 90, 120, and 150 min, respectively.	[17]

Table 1. Cont.

Wood Species; Size (W:L:T)	Bleaching Agents and Delignification	References
Basswood (<i>Tilia</i>); 20:20:0.42 mm ³	6.0 wt% H ₂ O ₂ , 1.0 wt% trisodium citrate 95% dihydrate, 1.0 wt% of NaOH, and 92 wt%. Bleaching time of 30, 60, 90, 120, and 150 min, and lignin content varied from 24.3, 19.5, 18.4, 16.6, 15.2 and 14.9 wt%.	[30]
Beechwood	NaClO ₂ (5.0 wt%) in acetate buffer solution at 95 °C for 12 h. The lignin content was not indicated.	[2]
Birch (<i>Betula alnoides</i> , <i>Betula</i>) and New Zealand pine (<i>Pinus radiata</i> D. Don); 20:20:0.5 mm ³	NaClO ₂ (0.4–1.0%) at 70–90 °C for 45–135 min. The pH 4.6 was adjusted by adding CH ₃ COOH.	[18]

2.2. Impregnation of Bleached Wood with Various Polymers

2.2.1. Previous Approaches of Impregnation Process

Previous studies have identified resin (filler) penetration as the preferred and most effective method for producing TW using materials obtained after the initial delignification process [2,38,39]. Delignified and bleached wood materials typically have empty spaces from which components such as lignin and hemicellulose are removed, resulting in a porous structure. To achieve transparency, these pores are filled with a material with a refractive index close to or equal to that of wood. In some cases, the selected polymers also provide UV-shielding properties. According to Fink [11], wood generally has a refractive index of approximately 1.53. Commonly used polymers have refractive indices ranging from 1.50 to 1.53, making polymer resin suitable for TW fabrication. The delignified porous wood material is filled with polymer through impregnation or penetration. Various types of polymers, such as PMMA, PVP, PU, epoxy resin, quantum dots, PVA-lignin, PVA-lignin nanoparticles, PVA-chitin nanocrystals, chitin crystal, PEG/MMA, ATO/MMA, CNF, and CTS, have been used for this purpose [1,2,6–8,11,15,19–21]. Matching the refractive index (RI) of polymer for TW production is one of the important factors. However, it is difficult to match the various RIs depending on the polymer structure to produce TW. Polymers used in TW, including PMMA, epoxy resin, and PVA, may contain SiO₂, TiO₂, and lignin to modulate the RI of composite materials. These nanoparticles may play a role in controlling the RIs of polymers and wood. However, further study is needed to evaluate the properties of TW composite materials when infiltrated into TW.

Polymer impregnation typically takes 30 min to several hours and involves repeated cycles of impregnation, release, and penetration. However, the low specific gravity of wood during impregnation makes effective polymer infiltration into the porous wood challenging. Alternative methods, such as compression or self-densification of oxidized wood using TEMPO and other solvents, have been explored to improve polymer penetration. For instance, Zu et al. [24] produced TW using a high-pressure compression method on bleached wood.

2.2.2. Pressurization of Delignified Wood

Li et al. produced TW by densifying wood after TEMPO oxidation [25]. Samanta et al. have prepared TW biocomposites for smart window applications [40]. These TW composites were prepared using thiol and ene monomers containing chromic components and a mixture of thermo- and photo-responsive chromophores. To produce TW through pressing, the lignin content of the wood materials must first be removed. Different bleaching chemical agents are used for delignification, such as NaClO₂, NaClO, and H₂O₂. After lignin is removed from the natural wood, the resulting bleached wood is subjected to pressurize between microporous filtering films for 1 h to 3 h and subsequently dried.

2.2.3. Polymer Infiltration

Wood materials can be processed with or without delignification and bleaching to produce TW. When bleaching or delignification is performed, several chemical agents (NaOH, Na₂SO₃, NaClO₂, NaClO, and H₂O₂) are used to remove a portion or the entire lignin content from the wood substrate, resulting in different types of TW [2,17,18,23]. Depending on the lignin content, TW can be categorized into lignin-retaining, partially bleached, and fully bleached TW. To prepare TW, pretreated wood (processed through solvent exchange using ethanol or acetone) or wood bleached using bleaching agents (subjected to solvent exchange using ethanol or acetone) is infiltrated with various polymers, such as epoxy resin, PMMA, quantum dots, PVA, PLA, chitosan, and cellulose nanofibers through a vacuum process [1,2,16,21,31]. This renders the wood transparent, with varying levels of transmittance and haziness.

The infiltrated components used for fabricating TW for smart windows consisted of pentaerythritol tetrakis (3-mercaptopropionate) (PETMP), 1,3,5-triazine-2,4,6(1*H*,3*H*,5*H*)-trione (TATATO), and the UV initiator 1-hydroxy-cyclohexyl phenyl ketone (0.5%). In addition, Hai et al. have developed bio-based TW for packaging and wood straws by infiltrating wood with dissolved chitosan and TEMPO-nanocellulose [14]. A study by Qiu et al. explored the transition from high-haze wood to TW using phase-change materials [41]. They used various copolymers, including styrene (St), butyl acrylate (BA), and 1-octadecene (ODE), for the infiltration. At low temperatures (e.g., 5 °C), the wood exhibited high haze and low transparency. However, increasing the temperature to 25 °C or 50 °C for 2 min rendered the wood transparent. To prepare thermo-responsive flexible TW, the authors infiltrated wood with a combination of (St/BA/ODE) St and BA (1:1 ratio) containing 0.3% divinylbenzene (DVB, 55%) as the cross-linker and 0.2% 2,2'-azobis-(2-methylpropionitrile) (AIBN) as the initiator [41]. The mixture was precopolymerized at 85 °C for 35 min. By adding 5% 1-octadecene (ODE 90%) to the St/BA mixture, a thermo-responsive flexible opaque and TW composited was prepared. Table 2 presents the various methods used for TW fabrication and their applications.

Table 2. TW fabrication methods and applications.

Wood Species	Fabrication Method and Filler (Polymer)	Applications	Ref.
Basswood	15 wt% Polyvinylpyrrolidone (PVP) ethanol. Degassed under 200 Pa for ~10 min	Highly efficient broadband light management in solar cells	[37]
Poplar (<i>Populus</i> sp.) and Balsa wood	Impregnation using vacuum infiltration process with prepolymerized MMA solution (PMMA)	TW for Energy-saving building	[38]
Silver birch wood (<i>Betula pendula</i>)	Impregnation using vacuum infiltration with PEG/MMA (70/30 wt%) solution	Thermal energy storage and reversible optical transmittance	[19]
Balsa wood	Impregnation using vacuum infiltration with polyvinyl alcohol (PVA) solution	Thermally insulated TW for energy-efficient windows	[13]
Douglas fir, Bass, Balsa, and Pinewood	Infiltration into the delignified wood scaffold using epoxy resin, followed by solidification for 24 h	Aesthetic TW for energy-efficient buildings	[10]
Basswood	Impregnation with 2 epoxy resins (E-128 resin and D-630) at a mass ratio of 3:1	Thick translucent wood walls and indoor light performance	[5]
Balsa wood	Infiltration using poly-methyl methacrylate (PMMA) and laser deposition for conductive film layer using indium tin oxide (ITO).	TW substrate for perovskite solar cells	[4]

Table 2. Cont.

Wood Species	Fabrication Method and Filler (Polymer)	Applications	Ref.
Beechwood	Impregnation using vacuum infiltration with poly methyl methacrylate (PMMA) for 1 h with three repetitions, followed by heat treatment in a box furnace at 85 °C for 12 h	Next-generation smart building materials	[2]
Beechwood	Impregnation using vacuum infiltration with Cs ₂ WO ₃ /prepolymerized methyl methacrylate (MMA) mixed solution, followed by infiltration for 30 min with three repetitions	TW containing Cs ₂ WO ₃ nanoparticles for heat-shielding-window	[39]
Fir wood	Impregnation using vacuum infiltration with TEMPO-treated nanocellulose and chitosan solution	Food packaging materials, medical packaging materials, and straw	[14]
Balsa wood	Impregnation using vacuum infiltration with PVA, PVA-lignin nanoparticle	UV-shielding windows application	[8]

2.2.4. Self-Densified TW

Self-densified TW is a recent method that involves bleaching using sodium chlorite (NaClO₂) and an acetate buffer (pH 4.6) at 80 °C for 12 h. The natural wood is transformed into white wood, washed, and immersed in a 0.1 M sodium phosphate (PBS) solvent. Subsequently, the wood is treated using the TEMPO oxidation method. Following this, the wood is dried at ambient temperature to produce self-densified TW [25].

3. Physical Properties of TW

3.1. Optical Transmittance and Haze

Transmittance is the most crucial property of TW. A higher transmittance allows more light to pass through, improving visibility. Light transmittance in TW is primarily influenced by wood thickness, bleaching conditions, type of impregnated polymers, resins, and wood type.

High light transmittance and sufficient wood thickness are crucial for the successful industrial or commercial application of TW. However, while thicker wood is suitable for construction, it tends to have lower transmittance. In general, a thicker TW exhibits higher blurriness and lower light transmittance, making it challenging to achieve clear visibility.

TW with high transmittance or translucency is preferred for applications such as solar cells, light management, and structural elements. However, products requiring high transparency demand minimal haze. Therefore, applications in buildings and residential areas require a balance between transmittance and visibility. Yaddanapudi et al. reported that TW made from beech and PMMA exhibited transmittance ranging from 10 to 70%, depending on the wood thickness (0.1 mm to 0.7 mm) [2]. Balsa wood has been widely used for manufacturing TW, with transmittance ranging from 10 to 90%, depending on the thickness [1,6,10,15]. Several studies have reported methods for preparing TWs of various thicknesses. For example, Fu et al. reported TWs with a thickness of 3.5 mm and a transmittance of about 70–90% depending on the infiltrated polymer component [6], and Mi et al. reported various types of TWs for aesthetic wood applications with a thickness of 2 mm and a transmittance of about 80% [10]. Hai et al. recently reported various types of TWs with very thin layers for various applications with a transmittance of about 70–80% [14]. In addition, Hai et al. combined PVA and lignin nanoparticles to produce TWs with a thickness of 1–2 mm for UV-blocking window applications [8,9]. High turbidity and high transmittance are prioritized based on the TW application. For example, high-

transmittance and high-haze products are suitable for applications such as solar cells, outdoor displays, and home lighting management.

In a recent study, Jia et al. [29] developed TW with a transmittance of approximately 90% and haze of 10%, demonstrating clear visibility and high potential as a building material.

3.2. UV-Shielding Properties

Another notable advantage of TW is its UV-shielding properties. Numerous studies on TW and glass have shown that, unlike glass with little to no UV-shielding properties, TW exhibits excellent UV-shielding capabilities. TW with different types of filler materials holds significant potential for UV-shielding applications. UV rays from the sun are known to have various negative effects on humans, such as skin burns and cancer. Therefore, TW with high UV-shielding properties offers a significant advantage for commercial success [7,8,10,13]. TW produced by infiltrating PMMA/antimony-doped tin oxide (ATO), PMMA, NCF, and CTS solutions has shown significant UV-shielding capability, with transmittance demonstrating high UV protection properties (~80%) in the 200–400 nm range. Furthermore, recent developments have explored partial delignification and reinforcing lignin nanoparticles to enhance UV shielding [8,14]. In one study, bleached wood was infiltrated with a composite of PVA and lignin nanoparticles to create UV-shielding TW. In addition, a simpler approach involving delignification using NaOH without bleaching was demonstrated to produce TW with UV-shielding properties [8,14]. Therefore, UV-shielding properties offer significant advantages for window applications.

3.3. Density, Modulus, Strength, and Toughness

Wood is a natural material with several unique properties, such as lightweight and high tensile strength, Young's modulus, and toughness. These properties vary depending on the wood species, growth conditions, and whether it is earlywood or latewood. In general, the density of wood is approximately 0.65 g/cm³. Studies have reported that the density of latewood and earlywood in Douglas fir ranges from 300 kg/m³ to 800 kg/m³ [10,14]. Another previous study reported Balsa wood with a density ranging from 112 kgm^{−3} to 170–200 kgm^{−3} [25,40]. TW is a suitable alternative for building and construction applications owing to its superior properties compared with other materials, such as natural wood, steel, and glass. Therefore, mechanical properties, such as tensile strength, Young's modulus, toughness, and density, should be considered. The literature reviews have confirmed that the mechanical properties of TW are superior to those of natural wood [5,10,14,38]. Wang et al. reported that natural wood had a tensile strength of approximately 42.8 MPa and a modulus of 5.7 GPa [38]. By contrast, TW exhibited an average tensile strength and modulus of 52.3 MPa and 2.4 GPa, respectively, with elongation at break increasing from 1% to approximately 5% compared with untreated wood. Mi et al. reported that TW exhibited significantly enhanced tensile strength and toughness compared with natural wood [10]. The tensile strengths of natural wood in the radial and longitudinal directions were less than 10 MPa and approximately 40 MPa, respectively, whereas those of TW exceeded 20 MPa and 95 MPa, respectively. In addition, Mi et al. showed that the toughness of TW increased fivefold in the radial and longitudinal directions [10].

3.4. Thermal Conductivity

Heat transfer is a key factor in construction and building applications. Modern architecture increasingly features high-rise or large-scale buildings with open designs and extensive glass windows to allow natural light into the buildings. Glass has traditionally been the preferred material for windows. However, glass windows have several disadvantages, such as high thermal conductivity, heavyweight, and brittleness. TW is

an emerging material with significant advantages over glass, such as lightweight properties, ductility, UV shielding, and low thermal conductivity. Wood is known to be a low-thermal-conductivity material, making TW a promising alternative for reducing thermal conductivity. Several studies have explored the thermal conductivity and UV-shielding properties of TW [4,5,10,13]. These studies have reported that TW exhibited 3–4 times lesser thermal conductivity ($0.32\text{--}0.15\text{ W m}^{-1}\text{ K}^{-1}$) than glass ($1.0\text{ W m}^{-1}\text{ K}^{-1}$). Thus, large-scale production of TW is expected to play a significant role in future construction applications.

4. Factors Affecting Optical and Mechanical Properties of TW

The mechanical characteristics of TW are one of its critical properties, and they vary based on their intended use. As previously discussed, TW is used in home structures, smart houses, packaging, and glass substitutes. The mechanical properties of TW used for these applications are primarily influenced by the wood type, processing conditions, and type of impregnating polymer used. To function as a substitute for fragile glass, TW must have the mechanical strength required for its use as a building material. Zu et al. demonstrated that simple bleached and compressed TW exhibited superior mechanical properties compared with wood impregnated with PMMA, epoxy resin, PVA, and NCF [24]. However, TW produced through compression alone had the disadvantage of limited thickness increase. In a recent study, Li et al. reported that TW produced through TEMPO oxidation and high-pressure compression exhibited high tensile strength and Young's modulus, reaching approximately 500 MPa and 50 GPa, respectively, despite being a relatively thin material [25].

TW impregnated with PVA and PMMA exhibited extremely poor mechanical properties. This can be attributed to various factors, such as wood type, bleaching conditions, and other processing factors. According to Jungstedt et al. [42], birch exhibited an initial longitudinal tensile strength of approximately 167 MPa, while materials made by infiltrating PMMA under different conditions achieved tensile strengths of 193 and 263 MPa. Yu et al. [39] reported that natural wood had a strength of 55.1 MPa, increasing to 60.1 MPa following PMMA impregnation. Qui et al. [41] noted that native Balsa wood had a strength of approximately 20 MPa, whereas polymer-impregnated materials exhibited a tensile strength of approximately 10–20 MPa, depending on the resin type and penetration conditions. Research indicates that the type of wood used in manufacturing TW significantly influences its mechanical properties and tensile strength development.

Table 3 lists the mechanical properties of various wood types and TW in the longitudinal ($//$) and radial ($\perp$) directions. In addition, it summarized the effects of bleaching agents, bleaching conditions, wood thickness, and wood type on transmittance.

Table 3. Various mechanical properties of TW from different wood sources.

Name of Wood	Wood Sizes (mm)	Bleaching Agents	Polymer	Tensile Strength (MPa)	Young's Modulus (GPa)	Transmittance (%)	Ref.
Beechwood	T: 0.1–0.7	NaClO_2	PMMA	NW: 220 DL: 75 TW: 150	NW: 1.52 DL: 2.5 TW: 2.1	>10–70	[2]
Douglas fir (<i>Pseudotsuga menziesii</i>)	320×170 T: 0.6–2	NaClO_2	Epoxy resin	NW $\perp$: 6.24 NW $//$: N/A TW $\perp$: 21.6 TW $//$: 92	N/A	>80	[10]
Balsa wood (<i>Ochroma pyramidale</i>)	20×20 T: 0.7–3.7	NaClO_2	PMMA	NW: N/A DL: 10 TW: 95	NW: N/A DL: 0.22 T: 2.05	40–90	[1]
Pine, birch, and ash wood veneer	100×100 T: 1.5	H_2O_2 , NaClO_2	PMMA	100.7	N/A	80	[16]
Basswood	50×50 T: 3	NaOH ; Na_2SiO_3 , H_2O_2	Epoxy resin	NW $\perp$: <5 NW $//$: <45 TW $\perp$: <23.4 TW $//$: 45.4	NW $\perp$: N/A NW $//$: N/A T $\perp$: 1.22 T $//$: 2.37	80–90	[21]

Table 3. Cont.

Name of Wood	Wood Sizes (mm)	Bleaching Agents	Polymer	Tensile Strength (MPa)	Young's Modulus (GPa)	Transmittance (%)	Ref.
Balsa wood	20 × 40 T: 0.8	NaClO	Polyvinyl alcohol (PVA)	NW _⊥ : 1.15 NW _{//} : 18.8 TW _⊥ : 67 TW _{//} : 143 NW: 61.5 LMW: 33.3 T-PG0: 39.9 T-PG50: 22.6 T-PG100: 13.3	N/A	90	[10]
Poplar wood (<i>Populus deltoides</i>)	20 × 20 T: 1	NaOH, Na ₂ EDTA, MgSO ₄ , and H ₂ O ₂	PVA and PG	NW _⊥ : 8.5 NW _{//} : 55 TW _⊥ : 33.3 TW _{//} : 44.4 NW: 78.3 DL: 21.9 T: 93.0 ATO _{0.3-0.7} T: 96.4–113.1	NW: 1.98 LMW: 1.49 T-PG0: 1.51 T-PG50: 0.80 T-PG100: 0.26	65–80	[28]
Basswood	T: ~0.7	NaClO, NaClO ₂	Epoxy resin	NW _⊥ : 1.02 NW _{//} : 12.01 TW _⊥ : 4.12–63.05 TW _{//} : 45.12–75.12 NW: 121.9 DL: 84–8–114.3 TW: 152.4–171.4	N/A	90	[29]
Poplar wood (<i>P. adenopoda Maxim</i>)	25 × 25 and 50 × 50 T: 1	NaClO ₂	ATO/PMMA	NW: 129.6 TW-TES: 70.5	NW: 3.74 DL: 1.71 T: 3.38 ATO _{0.3-0.7} T: 4.27–4.46	45–80	[7]
Silver birch wood (<i>Betula pendula</i>)	20 × 20; 20 × 20 T: 0.5–1.5	NaClO ₂	PEG/PMMA	NW _⊥ : 1.02 NW _{//} : 12.01 TW _⊥ : 4.12–63.05 TW _{//} : 45.12–75.12 NW: 121.9 DL: 84–8–114.3 TW: 152.4–171.4	NW:14.5 T: 14.9	60–80	[19]
Balsa wood (<i>Ochroma pyramidale</i>)	20 × 20 T: 1, 1.5, 2, and 5	NaClO ₂	Epoxy resin	NW _⊥ : 1 NW _{//} : 75 TW _⊥ : 26 TW _{//} : 258 TW _⊥ : 26 TW _{//} : 171	N/A	10–80	[15]
Basswood (<i>Tilia</i>)	20 × 20 T: 0.42	NaClO ₂	PMMA	NW _⊥ : 1 NW _{//} : 75 TW _⊥ : 26 TW _{//} : 258 TW _⊥ : 26 TW _{//} : 171	N/A	10–60	[17]
Fir	100 × 100 100 × 200 T: 0.1	NaClO ₂	Cellulose nanofiber, Chitosan	NW _⊥ : 1 NW _{//} : 75 TW _⊥ : 26 TW _{//} : 258 TW _⊥ : 26 TW _{//} : 171	15 13	75–80	[14]

5. Potential Applications of TW

5.1. TW for Building and House Structure

Previous studies [2,5,11,16] have highlighted the potential applications of TW, including housing structures, smart houses, walls, and rooftops. For example, several studies [1,39] have shown that Cs₂WO₃/PMMA composites exhibited excellent insulation properties, making them ideal for use as window materials. Li et al. developed TW of various thicknesses, ranging from 20 mm to 50 mm [5]. The 20 mm thick TW impregnated with epoxy resin exhibited a transmittance of up to 40%, making it suitable for use in wall structures. In addition, Li et al. reported that TW made by impregnating epoxy resin exhibited significantly enhanced transmittance compared with TW infiltrated with PMMA [5]. Therefore, to develop advanced TW for housing structures, reducing the turbidity of TW and further exploring technologies related to the use of various woods and fabrication processes are crucial.

5.2. Light Management, House Decoration, Solar Cells and Electric Devices

TW has potential applications in buildings, lighting management, and home decorations [3,4,10,13,39]. It can be used in rooftops, lighting management, and decoration. Yu et al. reported that TW resulted in enhanced heat-shielding properties compared to glass [39]. Model houses with Cs₂WO₃/MMA TW windows exhibited better insulation than existing houses using ITO glass windows, resulting in nearly double the indoor temperature. According to Li et al. [1], TW exhibited minimal glare, and Lang et al. [43] noted that TW infiltrated with PMMA and coated with PETDOT:PSS demonstrated significant potential as a glass substitute for windows. In addition, Mi et al. [10] noted that TW could improve lighting and insulation and create pleasant interior lighting. These properties make TW a promising material for smart houses, buildings, and windows.

In addition, TW can be used in other industries, such as electronics, sensors, and solar cells. Recent studies [5,19,21,38] have demonstrated its potential in lighting management,

solar cell materials, thermal energy storage systems, and energy-saving applications. Wang et al. [38] revealed that TW and transparent-wood-based fibers exhibit extremely low thermal conductivity of approximately 0.2 W/mK. By contrast, ITO glass has a thermal conductivity of approximately 1.0 W/mK, approximately five times higher than that of TW. This makes TW a viable energy-saving material for buildings. Montanari et al. [19] produced TW by immersing delignified wood in a PEG/MMA (70/30 *w/w*) polymer solution three times. The resulting TW exhibited excellent thermal energy storage and energy-saving potential. Li et al. [5] suggested combining solar cells and wooden building materials as an energy-saving solution. Other studies [5,21] explored TW for solar cell applications, reporting conversion efficiencies ranging from 14.4% to 16.8%. Another study used TW to fabricate perovskite solar cells, consisting of a TW/ITO layer/TiO₂/perovskite/spiro-OMeTAD/Au layer. The perovskite solar cells exhibited a current density of 21.9 mA·cm⁻², voltage of 1.09 V, and charge rate of 70.2%. These findings indicate that TW is a promising eco-friendly housing material.

5.3. TW as Green Bio-Based Packaging Materials

Bio-based TW, fabricated by impregnating CNF and CTS suspensions into bleached porous wood, has demonstrated excellent antioxidant and UV-shielding properties, improved mechanical properties, and enhanced transmittance. Hai et al., 2021 produced bio-based TW in significantly larger sizes (200 mm × 100 mm × 0.1 mm) compared with those in previously published works and used two types of TW for packaging applications [14]. This highly flexible material can be used for transparent straws, transparent bags, and medical window packaging. However, despite the many advantages of bio-based materials, such as cellulose nanofibers and chitosan solutions, hydrophilicity remains a significant challenge. Further efforts are necessary to address this limitation and improve the thickness of bio-based TW. Figure 3 illustrates the fabrication process and TW applications. In addition, Zhang et al. recently also developed biobased transparent wood for food packaging [44]. The authors demonstrated that food packaging has good UV-shielding functionality, antioxidation, and so on.

5.4. Patents on TW

TW is a promising material for developing green products, potentially replacing traditional window glass, structural components in buildings, and decorations and improving sunlight management. TW development has rapidly progressed in recent years. However, the emerging trend of bio-based materials and their feasibility for real-world applications has led to the filing of several patents worldwide. Advanced TW offers excellent mechanical properties, ease of fabrication, low thermal expansion, and minimal environmental impact. Consequently, research groups, companies, and institutions have increasingly pursued patents related to TW. In 2017, Cellutech filed a patent for a method of preparing TW [46]. Cellutech listed a range of polymers for TW fabrication, such as poly(hexafluoropropylene oxide), hydroxypropyl cellulose, poly(tetrafluoroethylene-co-hexafluoropropylene), poly(pentadecafluoro octyl acrylate), and poly (tetrafluoro-3-(heptafluoropropoxy) propyl acrylate), among others. In 2017, the University of Maryland secured a patent for TW with a high transmittance of 92% [47]. The patent lists various polymers for TW fabrication, including polyester fiberglass, polyurethane polymers, vulcanized rubber, bakelite, duroplast, urea-formaldehyde, melamine resin, diallyl phthalate (DAP), polyimides and bismaleimides, and cyanate esters or poycyanurates, among others [46,47]. In addition, they patented a process and various polymers for creating bio-based TW. With TW being an emerging field with significant potential for industrial applications, research since 2016 has led to several patents from companies, universities, and institutions [46]. As

the development of TW continues, more patents and industrial applications are expected to emerge in the future.

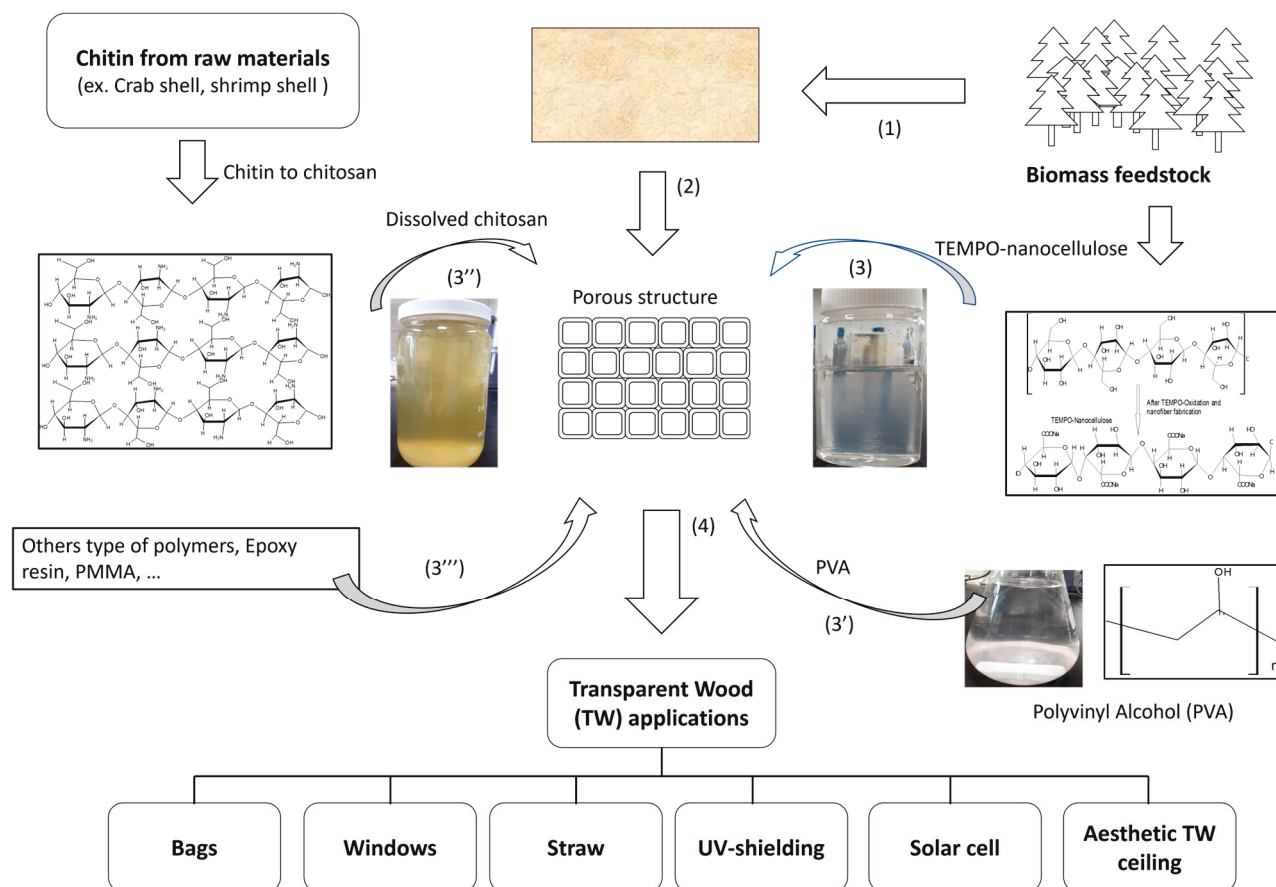


Figure 3. Fabrication process and applications of TW: (1) from wood-to-veneer and TEMPO-treated nanocellulose; (2) bleached wood; (3) TEMPO-treated nanocellulose; (3') PVA; (3'') chitosan; (3''') other types of polymers; (4) TW applications; TW bag, window, straw, solar cells, and aesthetic wood ceiling [14,16,28,35,39,41,43,45].

5.5. Future Trends and Challenges

TW has gained significant research attention in recent years owing to its potential applications and unique properties. However, several challenges remain, such as high hydrophilicity, permeability, production scalability, and chemical consumption. To address these challenges, researchers have proposed various solutions, including hydrophobic coatings with materials such as silicates and quantum dots, improving the thickness of bio-based TW through layer-by-layer deposition.

Currently, three primary approaches are employed for TW production: penetration, compression, and self-densification of TEMPO-oxidized bleached wood. Although conventional chemical polymers, such as PMMA, epoxy resin, and PVA, were initially used for impregnation, recent research has expanded the selection to include various materials. Combinations of different polymers are also being explored, although conventional fillers, such as PMMA and epoxy resin, remain toxic and harmful to human health and the environment. By contrast, bio-based materials, such as PVA, CNF, and CTS, are nontoxic but exhibit poor physical properties, including water absorption and hydrophilicity. Nevertheless, bio-based materials, such as PLA, CNF, and CTS, are preferred owing to their minimal environmental impact and lack of human hazards. In addition, CNF are considered as a potential material to infiltrate porous wood to produce TW, but the high energy cost for production of CNF to be reduced. The high energy consumption and waste generation and

disposal costs due to the use of chemicals in CNF production remain issues that will be solved in the future. However, the high hydrophilicity of these materials necessitates a hydrophobic coating for the resulting TW to prevent moisture absorption and humidity damage caused by weather changes.

The size and thickness limitations of TW pose challenges for industrial and commercial applications. However, Xia et al. [35] successfully fabricated meter-scale TW with a thickness of 1 mm, marking a significant breakthrough in production scalability. Consequently, research focused on commercialization is expected to accelerate in the near future. Overcoming these challenges holds significant potential for future applications of bio-based TW, particularly in improving mechanical properties, thermal expansion, light management, and the customizing of thickness and filler content for improved flexibility and toughness. Ongoing research focuses on optimizing hydrophilicity and hydrophobicity through various physicochemical methods, which can further expand the potential of TW for various applications.

6. Conclusions

The potential applications of TW primarily include materials for residential building structures, smart houses, windows, solar cells, and packaging, with significant expansion anticipated in the future. High-density TW of consistent thickness is suitable for building, window, and home structural applications. However, it can also be converted or fabricated into a thin film, making it suitable for rolling, decorative, and packaging purposes. Currently, the primary methods for manufacturing TW include penetration, high-pressure compression, and self-densification.

The fabrication of TW using eco-friendly materials such as NCF, CTS, and other bio-based materials has great potential. However, various eco-friendly polymer materials and manufacturing methods for TWs with desirable mechanical and functional properties should be developed. In particular, the mechanical properties of TW, including tensile strength, Young's modulus, and transmittance, are greatly influenced by wood species, polymers, and processing methods. As highlighted in this review, eco-friendly TW offers significant market potential and a wide range of applications.

This paper provides a review of the latest and updated manufacturing methods, properties, and applications of bio-based composite material (TW). In particular, the effects of structural modifications using various chemical reagents and combinations using various polymers on the properties of TW biocomposites were also examined.

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References

- Li, T.; Zhu, M.W.; Yang, Z.; Song, J.W.; Dai, J.Q.; Yao, Y.G.; Luo, W.; Pastel, G.; Yang, B.; Hu, L.B. Wood Composite as an Energy Efficient Building Material: Guided Sunlight Transmittance and Effective Thermal Insulation. *Adv. Energy Mater.* **2016**, *6*, 1601122. [CrossRef]
- Yaddanapudi, H.S.; Hickerson, N.; Saini, S.; Tiwari, A. Fabrication and characterization of transparent wood for next generation smart building applications. *Vacuum* **2017**, *146*, 649–654. [CrossRef]
- Li, Y.; Vasileva, E.; Sychugov, I.; Popov, S.; Berglund, L. Optically transparent wood: Recent progress, opportunities, and challenges. *Adv. Opt. Mater.* **2019**, *6*, 1800059. [CrossRef]
- Li, Y.; Cheng, M.; Jungstedt, E.; Xu, B.; Sun, L.; Berglund, L. Optically transparent wood substrate for perovskite solar cells. *ACS Sustain. Chem. Eng.* **2019**, *7*, 6061–6067. [CrossRef]
- Li, H.; Guo, X.; He, Y.; Zheng, R. House model with 2–5 cm thick translucent wood walls and its indoor light performance. *Eur. J. Wood Wood Prod.* **2019**, *77*, 843–851. [CrossRef]
- Fu, Q.L.; Yan, M.; Jungstedt, E.; Yang, X.; Li, Y.Y.; Berglund, L.A. Transparent plywood as a load-bearing and luminescent biocomposite. *Compos. Sci. Technol.* **2018**, *164*, 296–303. [CrossRef]
- Qiu, Z.; Xiao, Z.; Gao, L.; Li, J.; Wang, H.; Wang, Y.; Xie, Y. Transparent wood bearing a shielding effect to infrared heat and ultraviolet via incorporation of modified antimony-doped tin oxide nanoparticles. *Compos. Sci. Technol.* **2019**, *172*, 43–48. [CrossRef]
- Hai, L.V.; Cho, S.W.; Kwon, G.J.; Lee, D.Y.; Ma, S.Y.; Bandi, R.; Kim, J.K.; Han, S.Y.; Dadigala, R.; Lee, S.H. Fabrication of eco-friendly transparent wood for UV-shielding functionality. *Ind. Crop. Prod.* **2023**, *201*, 116918. [CrossRef]
- Hai, L.V.; Hoa, P.D.; Kim, J.; Lee, S.H. All-biobased multilayer transparent wood infiltrated with cellulose and chitosan nanofibers: Improving anisotropic mechanical and ultraviolet shielding properties. *Ind. Crop. Prod.* **2024**, *222*, 119508. [CrossRef]
- Mi, R.Y.; Chen, C.J.; Keplinger, T.; Pei, Y.; He, S.M.; Liu, D.P.; Li, J.G.; Dai, J.Q.; Hitz, E.; Yang, B.; et al. Scalable aesthetic transparent wood for energy efficient buildings. *Nat. Commun.* **2020**, *11*, 3836. [CrossRef]
- Fink, S. Transparent wood—A new approach in the functional study of wood structure. *Holzforschung* **1992**, *46*, 403–408. [CrossRef]
- Katunský, D.; Kanócz, J.; Karl'a, V. Structural elements with transparent wood in architecture. *Int. Rev. Appl. Sci. Eng.* **2018**, *9*, 101–106. [CrossRef]
- Mi, R.; Li, T.; Dalgo, D.; Chen, C.; Kuang, Y.; He, S.; Zhao, X.; Xie, W.; Gan, W.; Zhu, J. A clear, strong, and thermally insulated transparent wood for energy efficient windows. *Adv. Funct. Mater.* **2020**, *30*, 1907511. [CrossRef]
- Hai, L.V.; Muthoka, R.M.; Panicker, P.S.; Agumba, D.O.; Pham, H.D.; Kim, J. All-biobased transparent-wood: A new approach and its environmental-friendly packaging application. *Carbohydr. Polym.* **2021**, *264*, 118012. [CrossRef]
- Qin, J.; Li, X.; Shao, Y.; Shi, K.; Zhao, X.; Feng, T.; Hu, Y. Optimization of delignification process for efficient preparation of transparent wood with high strength and high transmittance. *Vacuum* **2018**, *158*, 158–165. [CrossRef]
- Li, Y.Y.; Fu, Q.L.; Rojas, R.; Yan, M.; Lawoko, M.; Berglund, L. Lignin-Retaining Transparent Wood. *Chemsuschem* **2017**, *10*, 3445–3451. [CrossRef] [PubMed]
- Wu, J.M.; Wu, Y.; Yang, F.; Tang, C.Y.; Huang, Q.T.; Zhang, J.L. Impact of delignification on morphological, optical and mechanical properties of transparent wood. *Compos. Part A Appl. Sci. Manuf.* **2019**, *117*, 324–331. [CrossRef]
- Wu, Y.; Zhou, J.C.; Huang, Q.T.; Yang, F.; Wang, Y.J.; Wang, J. Study on the Properties of Partially Transparent Wood under Different Delignification Processes. *Polym. Basel* **2020**, *12*, 661. [CrossRef]
- Montanari, C.I.; Li, Y.; Chen, H.; Yan, M.; Berglund, L.A. Transparent wood for thermal energy storage and reversible optical transmittance. *ACS Appl. Mater. Interfaces* **2019**, *11*, 20465–20472. [CrossRef] [PubMed]
- Chen, H.; Baitenov, A.; Li, Y.; Vasileva, E.; Popov, S.; Sychugov, I.; Yan, M.; Berglund, L. Thickness dependence of optical transmittance of transparent wood: Chemical modification effects. *ACS Appl. Mater. Interfaces* **2019**, *11*, 35451–35457. [CrossRef]
- Zhu, M.W.; Song, J.W.; Li, T.; Gong, A.; Wang, Y.B.; Dai, J.Q.; Yao, Y.G.; Luo, W.; Henderson, D.; Hu, L.B. Highly Anisotropic, Highly Transparent Wood Composites. *Adv. Mater.* **2016**, *28*, 5181–5187. [CrossRef] [PubMed]
- Wang, K.L.; Dong, Y.M.; Ling, Z.; Liu, X.R.; Shi, S.Q.; Li, J.Z. Transparent wood developed by introducing epoxy vitrimers into a delignified wood template. *Compos. Sci. Technol.* **2021**, *207*, 108690. [CrossRef]
- Montanari, C.; Ogawa, Y.; Olsén, P.; Berglund, L.A. High performance, fully bio-based, and optically transparent wood biocomposites. *Adv. Sci.* **2021**, *8*, 2100559. [CrossRef]
- Zhu, M.W.; Wang, Y.L.; Zhu, S.Z.; Xu, L.S.; Jia, C.; Dai, J.Q.; Song, J.W.; Yao, Y.G.; Wang, Y.B.; Li, Y.F.; et al. Anisotropic, Transparent Films with Aligned Cellulose Nanofibers. *Adv. Mater.* **2017**, *29*, 1606284. [CrossRef]
- Li, K.; Wang, S.N.; Chen, H.; Yang, X.; Berglund, L.A.; Zhou, Q. Self-Densification of Highly Mesoporous Wood Structure into a Strong and Transparent Film. *Adv. Mater.* **2020**, *32*, 2003653. [CrossRef]
- Zhou, J.C.; Wang, Y.J.; Wang, J.; Wu, Y. Multilayer Transparent Wood with Log Color Composed of Different Tree Species. *ACS Omega* **2022**, *7*, 46303–46310. [CrossRef]

27. Bisht, P.; Pandey, K.K. Optical and mechanical properties of multilayered transparent wood. *Mater. Today Commun.* **2024**, *38*, 107871. [CrossRef]
28. Rao, A.N.S.; Nagarajappa, G.B.; Nair, S.; Chathoth, A.M.; Pandey, K.K. Flexible transparent wood prepared from poplar veneer and polyvinyl alcohol. *Compos. Sci. Technol.* **2019**, *182*, 107719. [CrossRef]
29. Jia, C.; Chen, C.J.; Mi, R.Y.; Li, T.; Dai, J.Q.; Yang, Z.; Pei, Y.; He, S.M.; Bian, H.Y.; Jang, S.H.; et al. Clear Wood toward High-Performance Building Materials. *ACS Nano* **2019**, *13*, 9993–10001. [CrossRef]
30. Wu, Y.; Zhou, J.C.; Huang, Q.T.; Yang, F.; Wang, Y.J.; Liang, X.M.; Li, J.Z. Study on the Colorimetry Properties of Transparent Wood Prepared from Six Wood Species. *ACS Omega* **2020**, *5*, 1782–1788. [CrossRef]
31. Wu, Y.; Wu, J.M.; Yang, F.; Tang, C.Y.; Huang, Q.T. Effect of H₂O₂ Bleaching Treatment on the Properties of Finished Transparent Wood. *Polym. Basel* **2019**, *11*, 776. [CrossRef]
32. Brogdon, B.N.; Lucia, L.A. New insights into lignin modification during chlorine dioxide bleaching sequences (IV): The impact of modifications in the (EP) and (EOP) stages on the D stage. *J. Wood Chem. Technol.* **2007**, *25*, 149–170. [CrossRef]
33. Tarvo, V.; Lehtimaa, T.; Kuitunen, S.; Alopaeus, V.; Vuorinen, T.; Aittamaa, J. A model for chlorine dioxide delignification of chemical pulp. *J. Wood Chem. Technol.* **2010**, *30*, 230–268. [CrossRef]
34. Elhelece, W.A.; Abousekkina, M.M. Theoretical interpretation of the changes occur on cellulosic wastes as a result of different chemical treatments. *Eur. Chem. Bull.* **2013**, *2*, 328–334. [CrossRef]
35. Xia, Q.Q.; Chen, C.J.; Li, T.; He, S.M.; Gao, J.L.; Wang, X.Z.; Hu, L.B. Solar-assisted fabrication of large-scale, patternable transparent wood. *Sci. Adv.* **2021**, *7*, eabd7342. [CrossRef]
36. Zou, W.H.; Sun, D.L.; Wang, Z.H.; Li, R.Y.; Yu, W.X.; Zhang, P.F. Eco-friendly transparent poplar-based composites that are stable and flexible at high temperature. *RSC Adv.* **2019**, *9*, 21566–21571. [CrossRef]
37. Zhu, M.W.; Li, T.; Davis, C.S.; Yao, Y.G.; Dai, J.Q.; Wang, Y.B.; AlQatari, F.; Gilman, J.W.; Hu, L.B. Transparent and haze wood composites for highly efficient broadband light management in solar cells. *Nano Energy* **2016**, *26*, 332–339. [CrossRef]
38. Wang, X.; Zhan, T.; Liu, Y.; Shi, J.; Pan, B.; Zhang, Y.; Cai, L.; Shi, S.Q. Large-size transparent wood for energy-saving building applications. *Chemsuschem* **2018**, *11*, 4086–4093. [CrossRef]
39. Yu, Z.Y.; Yao, Y.J.; Yao, J.N.; Zhang, L.M.; Chen, Z.; Gao, Y.F.; Luo, H.J. Transparent wood containing CsWO nanoparticles for heat-shielding window applications. *J. Mater. Chem. A* **2017**, *5*, 6019–6024. [CrossRef]
40. Samanta, A.; Chen, H.; Samanta, P.; Popov, S.; Sychugov, I.; Berglund, L.A. Reversible dual-stimuli-responsive chromic transparent wood biocomposites for smart window applications. *ACS Appl. Mater. Interfaces* **2021**, *13*, 3270–3277. [CrossRef]
41. Qiu, Z.; Wang, S.; Wang, Y.; Li, J.; Xiao, Z.; Wang, H.; Liang, D.; Xie, Y. Transparent wood with thermo-reversible optical properties based on phase-change material. *Compos. Sci. Technol.* **2020**, *200*, 108407. [CrossRef]
42. Jungstedt, E.; Montanari, C.; Östlund, S.; Berglund, L. Mechanical properties of transparent high strength biocomposites from delignified wood veneer. *Compos. Part A Appl. Sci. Manuf.* **2020**, *133*, 105853. [CrossRef]
43. Lang, A.W.; Li, Y.Y.; De Keersmaecker, M.; Shen, D.E.; Österholm, A.M.; Berglund, L.; Reynolds, J.R. Transparent Wood Smart Windows: Polymer Electrochromic Devices Based on Poly(3,4-Ethylenedioxythiophene):Poly(Styrene Sulfonate) Electrodes. *Chemsuschem* **2018**, *11*, 854–863. [CrossRef] [PubMed]
44. Li, Y.Y.; Fu, Q.L.; Yu, S.; Yan, M.; Berglund, L. Optically Transparent Wood from a Nanoporous Cellulosic Template: Combining Functional and Structural Performance. *Biomacromolecules* **2016**, *17*, 1358–1364. [CrossRef]
45. Zhang, K.; Sutton, I.; Smith, M.D.; Harper, D.P.; Wang, S.; Wu, T.; Li, M. Ambient-densified and polymer-free transparent wood film for smart food packaging window. *IScience* **2023**, *26*, 108455. [CrossRef]
46. Hu, L.; Zhu, M.; Li, T.; Gong, A.S.; Jianwei, S. Transparent Wood Composite, Systems and Method of Fabrication. WO2017136714A1, 3 February 2017.
47. Li, Y.; Rojas, R.; Berglund, L. Transparent Wood and a Method for Its Preparation. AU2018245016B2, 29 March 2018.

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Review

Unusual Lignocellulosic Bioresins: Adhesives and Coatings for Metals and Glass

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Abstract: This minireview presents some unusual but encouraging examples of lignocellulosic-based adhesives and coatings used for metals, glass, and some other difficult-to-adhere materials. The reactions and applications presented are as follows. (i) The reactions of tannins and wood lignin with phosphate salts, in particular triethylphosphate, to adhere and join steel and aluminum to Teflon, in particular for non-stick frying pans. These adhesive coatings have been shown to sustain the relevant factory industrial test of 410 °C for 11 min and, moreover, to present a 50% material loss even at 900 °C for 5 min. (ii) Non-isocyanate polyurethanes (NIPU) based on glucose and sucrose as coatings of steel and glass. These were obtained by the carbonation of carbohydrates through reaction with the inexpensive dimethyl carbonate followed by reaction with a diamine; all materials used were bio-sourced. Lastly, (iii) the use of citric acid-based adhesive coupled with any hydroxyl groups carrying material for coating metals is also described. These three approaches give a clear indication of the possibilities and capabilities of biomaterials in this field. All these are presented and discussed.

Keywords: tannin; lignin; citric acid; glucose; non-isocyanate polyurethanes; bio-sourced adhesives; bio-sourced coatings; metals; glass; Teflon

1. Introduction

Mainly general bio-sourced resin reviews exist, although often, they are mainly aimed at clarifying the nature of the polymers formed rather than emphasizing the possibility of these biopolymers for adhesives, coatings, and bonding applications [1]. Bio-sourced resins of all sorts have been developed mainly to bond and coat wood, and in these specific cases, such resins are tested for adhesives and even used industrially [2–8]. Sometimes, among the types of resins developed, some of them show unusually good properties for bonding and coating more difficult substrates than wood, such as metals and even glass. Lignocellulosic-based bio-adhesives to bond metals and glass have already been reported and are mainly based on alginates [9]. There are, however, many other articles dealing with different biomaterials used for bioadhesives for metals, glass, and silicates for a number of applications as well as lignocellulosic materials [10–24] and even some reviews on the same subject [25,26]. The literature used in this study was gathered from the database of Google Scholar using the keywords complete bioresins for metals and glass considering the period between 2014 and 2024. This minireview presents some metal binding adhesive cases based on different bio-sourced lignocellulosic materials.

2. Tannin Triethyl Phosphate for Biobinders for Metal/Teflon Assemblies

Reacting triethylphosphate (TEP) with flavonoid tannins yields a thermally resistant adhesive capable of binding Teflon to metals like steel and aluminum [27].

On the flavonoid unit structure (Figure 1) the reaction with TEP occurs at temperatures in the 160–185 °C range and leads to the formation of several structures, e.g., Figure 2.

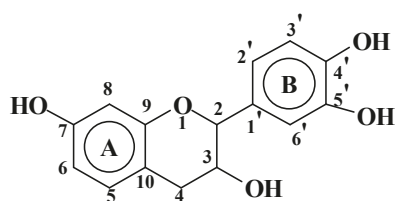


Figure 1. Structure of a flavonoid unit with atom numbering.

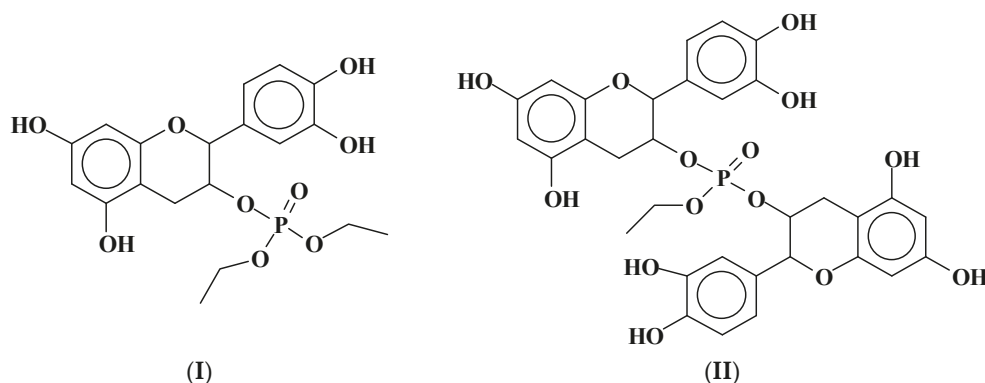


Figure 2. Basic structures (I) and (II) produced by the reaction of triethylphosphate on the C3 site of tannin flavonoid units [27].

The reaction appears to take place first with the flavonoid units' $-C_3 -OH$ groups that have alcoholic behavior. This site appears to be the favorite TEP reaction site on flavonoid tannin units, and it is extensive. It must be pointed out that the reaction seems to have a temperature of activation below which it does not appear to occur. Thus, it occurs readily at 185 °C but does not occur at 100 °C.

A lower level of the TEP reaction also takes place on the phenolic $-OH$ groups on the flavonoid unit's B-ring $C4'$ and $C5'$ sites, as shown by the detected structures in Figure 3.

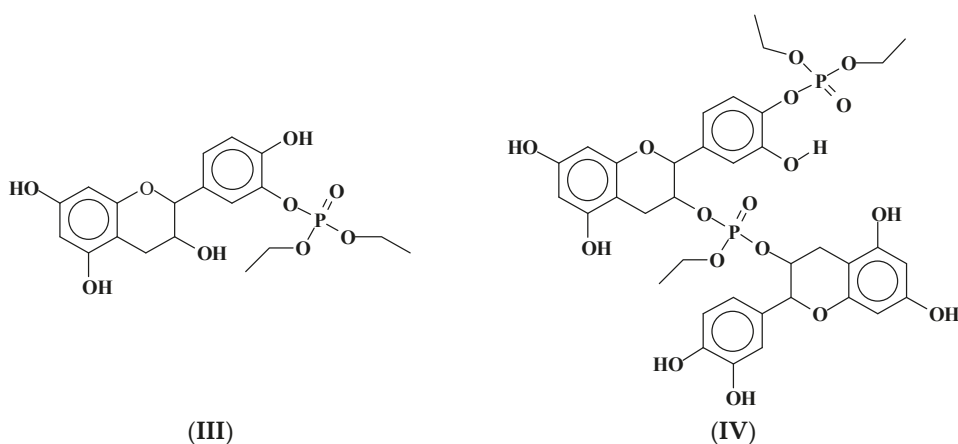


Figure 3. Basic structures (III) and (IV) produced by the reaction of triethylphosphate at the aromatic B-ring $C4'$ and $C5'$ sites of tannin flavonoid units, coupled with the reaction at the C3 site [27].

Mimosa tannin was used in this research work. It is composed of catechin units (approximately 15%), robinetinidin units (approximately 65–70%), and fisetinidin units (approximately 15–20%), which are bonded to C4 to C6 in the tannin oligomers, with the exception of catechin, which is linked to C4 to C8 and which is, in general, placed as the lower terminal unit of the tannin oligomers. Mimosa trimers, for example, present a structure such as that shown in Figure 4.

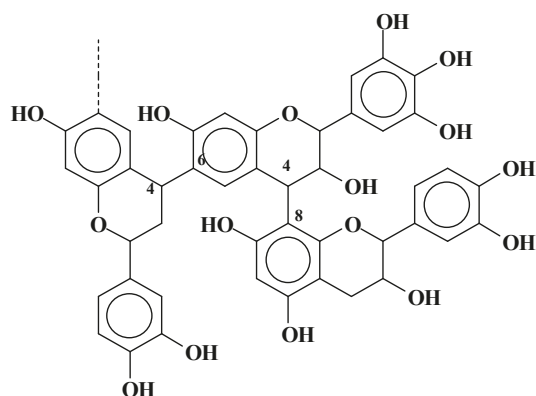


Figure 4. Structure of a trimer of mimosa tannin showing C4–C8 linkage between the terminal and before-terminal flavonoid unit and C4–C6 linkage between the before-terminal flavonoid unit and all subsequent flavonoid units [2].

This is reported to explain that structures I, II, III, and IV are then valid for both catechin as well as for robinetinidin in the tannin of the mimosa tannin; these two units present the same molecular weight.

MALDI–ToF mass spectrometry confirmed the formation of the structures above and below with a note that a TEP reaction was not found to occur on the A-ring –OH groups but only on the B-ring ones, and the latter occurred as a lesser reaction pattern (Figure 5).

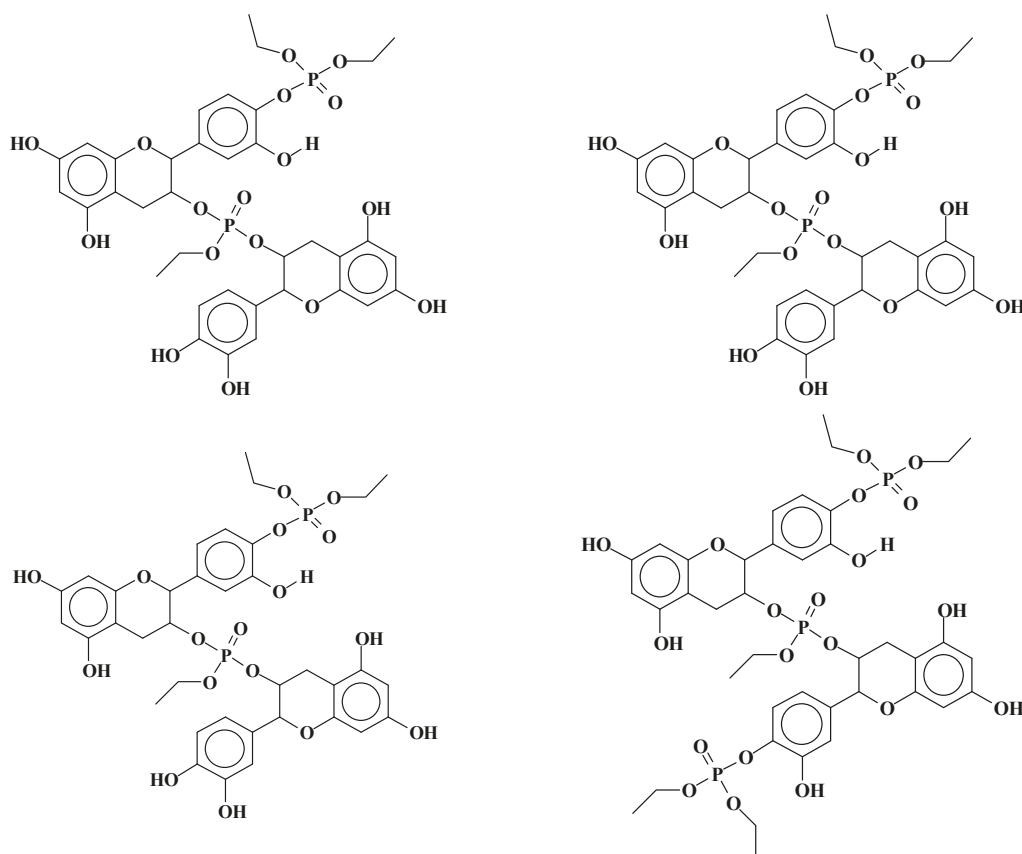


Figure 5. A variety of structures detected in the reaction of flavonoid tannins with triethylphosphate [27].

The research work also reported that the formulation to which ammonia is added seems to favor the reaction of TEP with the tannin flavonoid unit. The amination of the hydroxyl groups of the tannin, both of phenolic and alcoholic nature, is well-known [28].

This appears to be due to the amination of some of the hydroxyl groups of the flavonoid to be transformed in $-NH_2$ groups. The reaction of the aminated C3 of the aminated tannin detected by NMR was also confirmed by the MALDI ToF investigation, with species such as that shown in Figure 6 being detected.

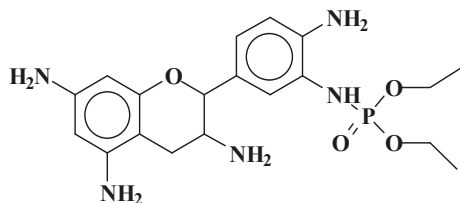


Figure 6. Structure of the reaction of triethylphosphate with an aminated flavonoid tannin unit [27].

Moreover, the possibility of the formation of higher-molecular-weight oligomers was confirmed by MALDI ToF analysis by reacting TEP with resorcinol as a simple model compound of the A-ring of flavonoid units yielding higher-molecular-weight oligomers within the range examined, as shown in Figure 7.

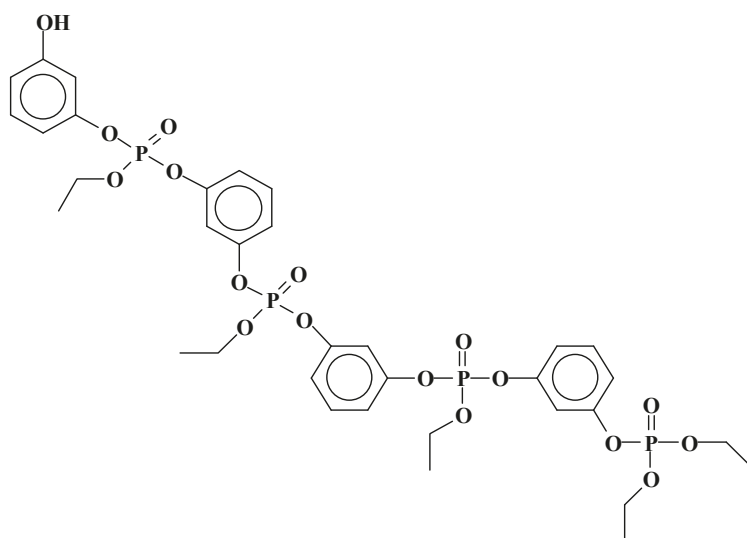


Figure 7. Higher-molecular-weight oligomer species detected by reaction of resorcinol used as a simple model compound of aromatic rings of tannin [27].

Such model compounds' approach indicates that while the C3-OH of flavonoid units is the favorite reaction site for TEP, followed by the phenolic $-OH$ s on the flavonoid units B-ring, the possibility of the reaction of TEP on the flavonoid unit's A-rings also exists, and it is likely to occur. This possibility was confirmed by the finding that in the ^{13}C NMR analysis in this research work, it was found that a consistent number of catechin and/or robinetinidin units presented open heterocyclic rings of the flavonoid units, thus showing that phenolic A-rings effectively have a resorcinol or phloroglucinol nature once reaction with TEP was carried out, as shown in Figure 8.

The research work also tested the progression of the degradation of the tannin alone, tannin+ NH_3 , tannin+TEP, and tannin+ NH_3 +TEP up to 900 °C for a period of 120 min. The materials showed a multi-step degradation process. An initial weight loss step was detected mostly for tannin (~10%) and tannin+ NH_3 (~5%) in the 50–170 °C range due to the evaporation of absorbed water and volatiles (CO, H, and CO₂). Indications of the higher thermal stability of the materials prepared using TEP emerged from this test. A second step of the thermal degradation for the tannin alone occurs at around 320 °C, while for tannin+ NH_3 , tannin+TEP, and tannin+TEP+ NH_3 , this was respectively observed at 370, 350, and 405 °C. For the tannin alone, the temperature at which 50% was decomposed was 520 °C. For tannin+ NH_3 , it was 750 °C, and 50% decomposition of tannin+TEP and

tannin+TEP+NH₃ occurred after only 5 min at 900 °C. The article also compares the residual material after heating at 900 °C. After 57 min at 900 °C, the residual materials under an argon atmosphere for tannin, tannin+NH₃, tannin+TEP, and tannin+TEP+NH₃ were 17%, 37%, 38%, and 44%, respectively. Thus, a high thermal stability has been observed for resins prepared by reacting tannin with TEP. It is important to note that these adhesive coatings have been shown to sustain the relevant factory industrial test of 410 °C for 11 min [29–31].

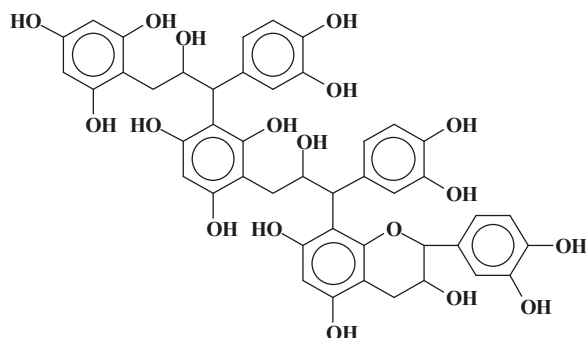


Figure 8. Structure of a flavonoid tannin trimer showing a number of flavonoid units where the heterocyclic ring of the structure has been cleaved and the structure is open [2].

Such a reaction of TEP with a tannin has been patented and industrially applied to bond Teflon on steel and/or aluminum [27,29]. This was carried out to manufacture non-stick frying pans without using the currently used synthetic, oil-derived phenol formaldehyde resin. It must be pointed out that such adhesive passed the relevant test for the application of non-stick frying pans, namely resisting heating at 410 °C for more than 11 min. It then constitutes a major “green” advance for non-stick frying pans, but its use can be extended to other metal bonding applications.

3. Lignin Triethyl Phosphate for Wood Surface Coatings and Bioadhesive for Metals/Teflon Assembly

Considering that the reaction of TEP with tannins is patented, a further development was carried out for the same application but using lignin to react with triethylphosphate, and the results obtained were also very encouraging; this latter work was not patented [32,33]. Thus, new thermosetting resins were obtained by reacting TEP with lignin to develop through a different lignocellulosic material new heat-resistant bio-sourced paints, lacquers, adhesives, coatings, and resins for numerous different applications and different surfaces [32,33]. Moreover, guaiacol (purity > 99%) and glycerol (purity > 99.5%) were also used as simple model compounds of lignin to clarify what occurs. Lignin and TEP appear to polycondense because of the TEP reacting with both the phenolic hydroxyl groups and the side chain aliphatic hydroxyls groups of lignin. Higher temperatures and the addition of ammonia favor the reaction. Model compounds, namely guaiacol and glycerol, were used initially to interpret the reaction of TEP with lignin’s aromatic and aliphatic parts. What was found with the model compounds was then confirmed by reacting a desulfurized softwood kraft lignin (BioChoice kraft lignin) with TEP.

Acetone-insoluble hard, rigid, dark cured resin solids were obtained when reacting TEP and lignin at 180 °C and 220 °C. When TEP was reacted with glycerol at 180 °C, a transparent liquid resin was obtained, while when guaiacol was reacted with TEP without or with NH₃ the resulting product was so burned that it was not possible to recover any resin solids after heat treatment. Finally, a flexible black semisolid was obtained when TEP and lignin were reacted at 90 °C; a resin soluble in acetone but not in water was obtained. The TEP heated at 90 °C with glycerol remained a transparent liquid before and after heating it. The resins prepared were analyzed by MALDI ToF, CP-MAS ¹³C NMR, and FTIR spectrometry. The NMR analysis showed, together with some unreacted lignin units, lignin units where TEP had reacted with the lignin phenolic –OH (Figure 9).

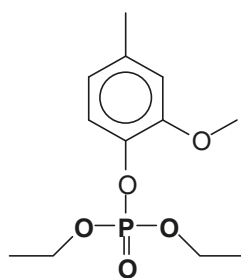


Figure 9. Example of TEP reaction site on lignin structure [32].

Also, the lignin aliphatic $-\text{CH}_2\text{OH}$ groups of the unit's side chain appeared to react with TEP.

A variety of structures formed by the reaction were determined by combining ^{13}C NMR and MALDI ToF analysis, such as those shown in Figure 10.

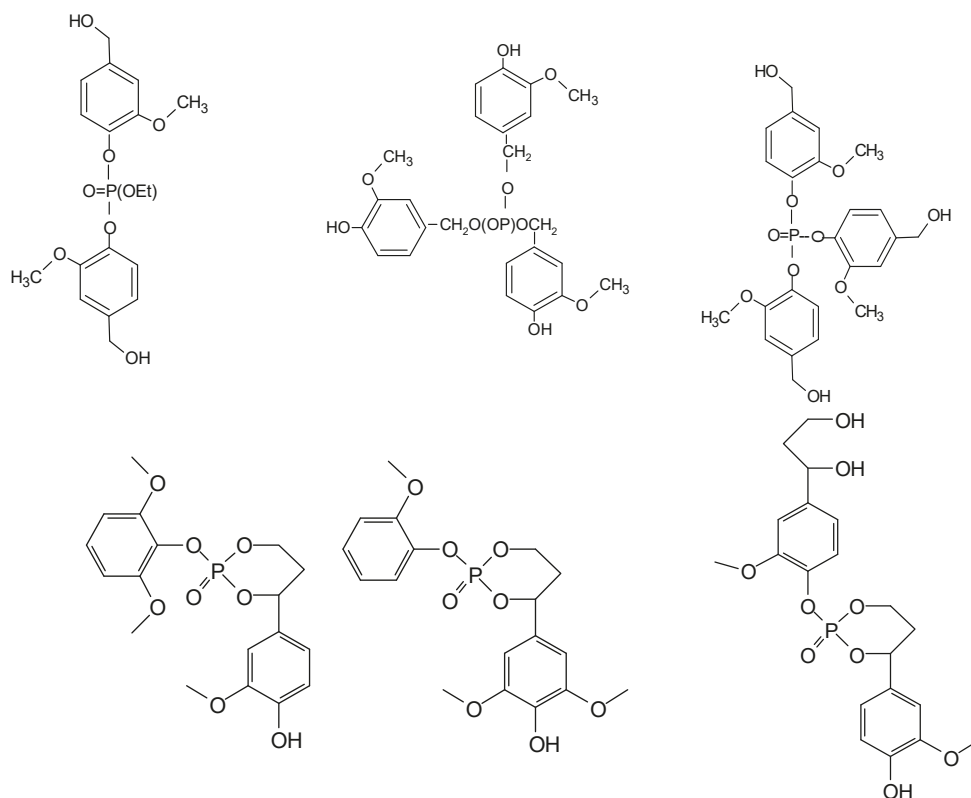


Figure 10. An example of the variety of structures formed by the reaction of triethyl phosphate with lignin units. Note also the cyclic structures formed by intramolecular reaction [32].

Branched and cyclic structures were also identified, the latter generated by intramolecular reactions, as shown in Figure 10.

Amination, in some cases by substitution with ammonia of the lignin phenolic $-\text{OH}$ groups, also appeared to occur. This type of reaction occurs with ease and has been reported for other phenolic compounds [28].

All these reactions yielded characterized structures, as shown in Figure 11.

Finally, several indications of lignin demethylation reactions were noticed in the NMR analysis. Higher-molecular-weight oligomers of lignin units or their sequences reacted through TEP were also detected, such as that shown in Figure 12.

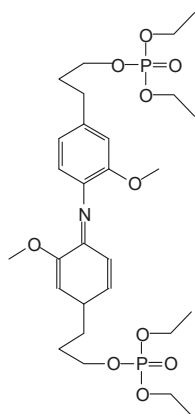


Figure 11. Example of a detected aminated lignin structure obtained by reaction with triethylphosphate [32].

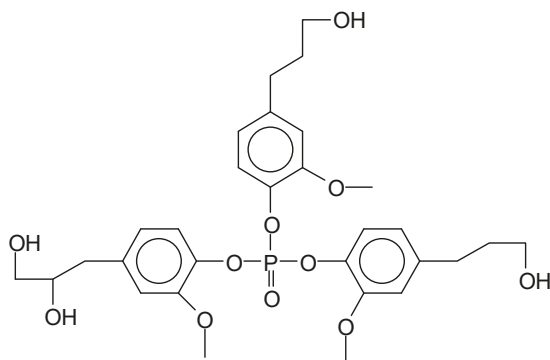


Figure 12. An example of a detected higher-molecular-weight oligomer produced by the reaction of triethylphosphate linking two lignin units [32]. Note: TEP can be linked through the phenolic –OH groups, as in the figure; with the aliphatic lignin –OHs; or with both –OH types.

In Figure 12, the lignin units are linked through TEP. TEP can be reacted with either the phenolic or the aliphatic lignin unit's sites.

The effectiveness of the product yielded by the TEP reaction with lignin for surface coating applications on wood were measured by the dynamic sessile water drop test on a coated wood surface. Figure 13 shows the results obtained, confirming the effectiveness of the reacted product as a wood surface finish.

It is clear from Figure 13 that a much higher initial water contact angle is obtained for the TEP/lignin resin-coated wood surface than for the untreated wood surface. Furthermore, the water contact angle remains almost constant for the coated surface while showing a rapid time-dependent decrease on the untreated surface.

As for tannins, it appears that the TEP/lignin reaction is temperature-dependent, being favored from 180 °C. Hardened and insoluble resins are obtained, also with ammonia added, as additional cross-linking reactions do occur.

However, the reaction of lignin and tannins (and possibly other biopolymers) with TEP was initially developed for the specific use to bind Teflon on steel or aluminum for non-stick frying pans (Figure 14) [27,29].

This biopolyphenol–TEP adhesive has been used as a substitute for synthetic phenol-formaldehyde resins but also has the advantage of not using any formaldehyde. It needed to adhere well to both the non-stick frying pan metal base (steel and/or aluminum) and simultaneously to its Teflon coating. Figure 14 shows a polyphenol–TEP resin adhered to the metal base without any Teflon (left) and the finished pan with the Teflon applied (right), according to a proprietary process. The test for the TEP/lignin resin needed to be capable of withstanding more than 410 °C for 11 min under many repeated temperature applications. The bio-lignin/TEP assembly passed this repeated test well. Contrary to the

tannin/TEP resin, the lignin/TEP resin system is not patented [32]. The reaction appears also for lignin, as for the tannin, to be dependent on the temperature. Thus, it is certainly favored from a 180 °C temperature, yielding insoluble hardened resins, as well as when ammonia is present, due to additional cross-linking reactions.

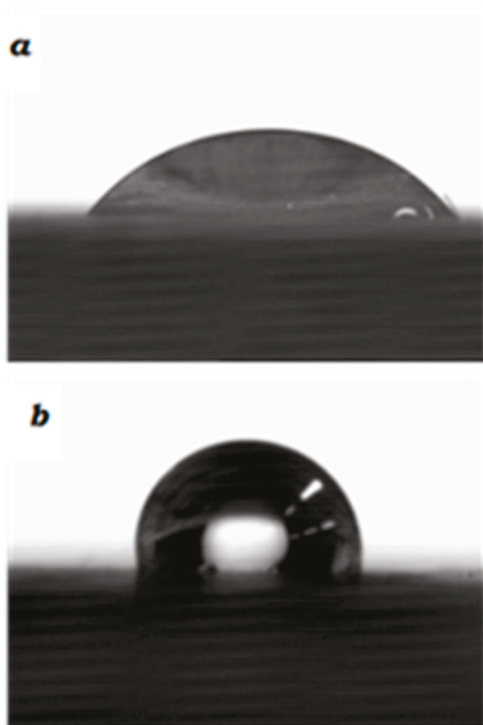
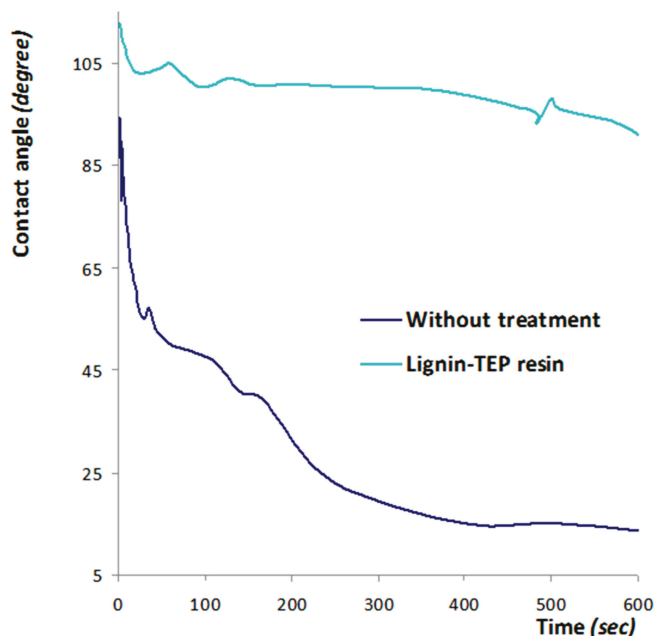


Figure 13. (Top) Water contact angle variation as a function of time of the lignin-TEP-based resin coated beech wood surface and the untreated beech sample control. (Bottom) Water drop shape after 60 s on: (a) untreated beech wood (control) and (b) beech wood surface coated with a lignin-TEP-based resin [32].



Figure 14. Frying pan metal base with the polyphenolic-TEP binder applied to it (**left**) and finished pan with Teflon applied on the binder according to a proprietary process (**right**) [33].

4. Totally Bio-Sourced Non-Isocyanate Polyurethanes (NIPU)

Polyurethanes, incorrectly called biopolyurethanes, can and have already been prepared by the use of bio-sourced polyols issued from renewable materials and used for a number of possible applications [34–41]. However, polymeric isocyanates have always been used to prepare such polyurethanes even if reacted with bio-sourced polyols. However, this approach can hardly be considered to lead to totally bio-sourced polyurethanes, seeing that toxic isocyanates are still used. Research works on alternate reaction routes yielding non-isocyanate-based polyurethanes (NIPUs) have now been known for a few years. These, however, are not bio-sourced and especially not bio-sourced starting from lignocellulosic materials. However, as good as these polyhydroxyurethanes [42–59] may be, they have shown to suffer from a few application drawbacks inherent to the approaches used, and this is on top of the lack of the use of bio-sourced renewable materials. The reaction is based on the reaction of single or double five-membered cycle carbonate groups [42] reacted with diamines [43–59]. The main technological barriers to their use are the need for the synthesis of single or double cyclic carbonates, bio-based or not, and thus an additional reaction, as well as the particular slowness in curing due to the slow reaction of opening of the cycles of the carbonates.

Non-isocyanate polyurethanes (NIPUs) that are totally bio-sourced have been obtained by reacting glucose and/or sucrose in a reaction with dimethyl carbonate, an inexpensive chemical, and hexamethylene diamine, also bio-sourced, or other bio-sourced diamine or polyamines [60]. Dimethyl carbonate hydroxyl group carboxymethylation generally occurs at a temperature around 90 °C by bimolecular nucleophilic substitution, acyl-cleaving, under alkaline catalysis [61]. While this approach was initially successfully used for bio-sourced polyphenolic materials, these latter NIPUs have been tested on wood but have never been tested for coatings of metallic surfaces. The main objective of using glucose and sucrose was to demonstrate that NIPUs could be prepared from carbohydrates, a relatively inexpensive material in abundant supply. The oligomers produced in this manner were identified by MALDI-ToF, CP-MAS ^{13}C NMR, and FTIR spectrometry. On steel, the NIPU resins were cured at 300 °C for 3 min, for both the glucose-based and sucrose-based coatings. The glucose derived NIPUs appeared to harden at a sensibly lower temperature and appeared to be easier to handle and spread than the ones prepared from sucrose due to the apparent glucose-based NIPU resin's lower energy of activation of hardening. Steel coatings was, among others, the tested application of the glucose-based NIPUs [60]. Cross-cut testing was carried out on the coated steel plates, and the results obtained were very encouraging. The coating results were already good at 103 °C for the glucose derived NIPU, while a markedly higher hardening temperature was needed for the sucrose-derived NIPU coating. Some of the higher-molecular-weight oligomeric NIPU species identified, both linear and branched, are shown in Figure 15 for glucose and in Figure 16 for sucrose.

The potential of this type of NIPU resin is shown by its capability of coating a steel surface. Figure 17 shows the hardened coating appearance of a steel plate with a glucose-derived NIPU resin and a sucrose NIPU resin. The clearly good appearance of the glucose derived coating film is apparent, its appearance also indicating this coating's spreading ease. This was shown to be far better than the sucrose-derived NIPU coating [61]. The very bad appearance of the sucrose NIPU coating in Figure 17 indicates the very difficult spreading of such a coating due to the very high viscosity of this NIPU. Of particular interest is the cross-cut test results for the coating adhesion [61], which are shown in Figure 18, indicating that none of the cutting lattice squares were detached and showing that the cut line edges were smooth. Thus, the glucose-derived NIPU coating's adhesion onto the metal plates was judged to be excellent.

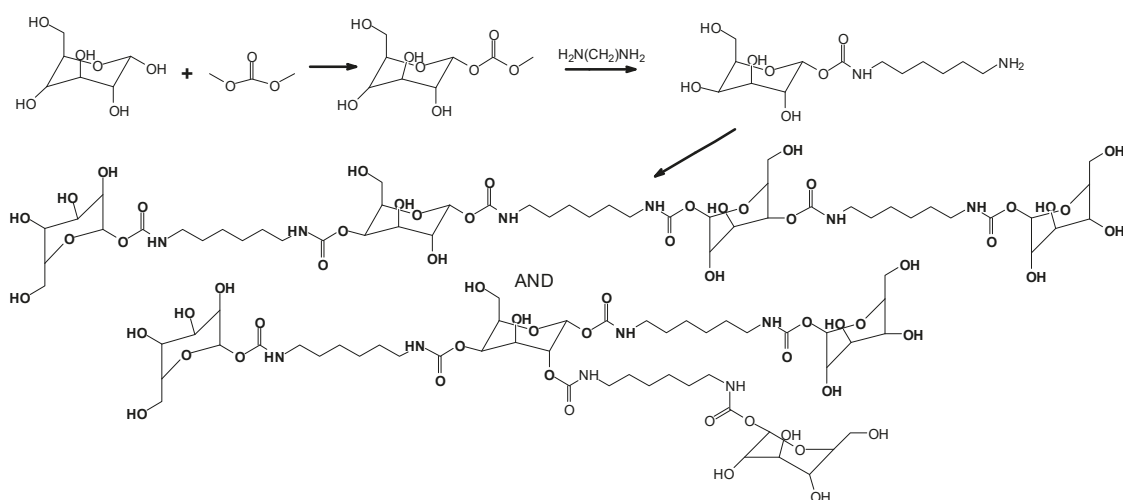


Figure 15. Linear and branched oligomers identified in glucose-based NIPUs [60].

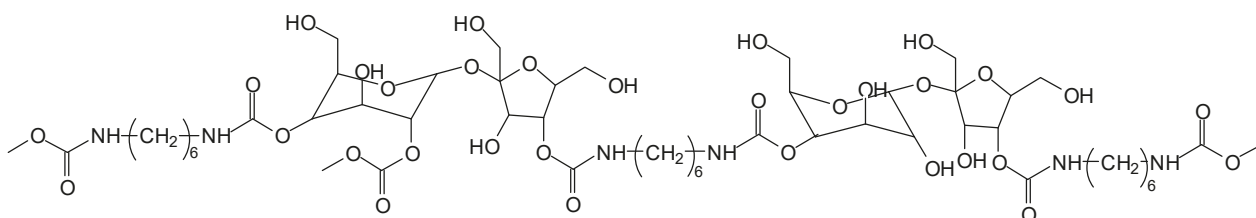


Figure 16. A polymeric species identified in sucrose-based NIPUs [62].

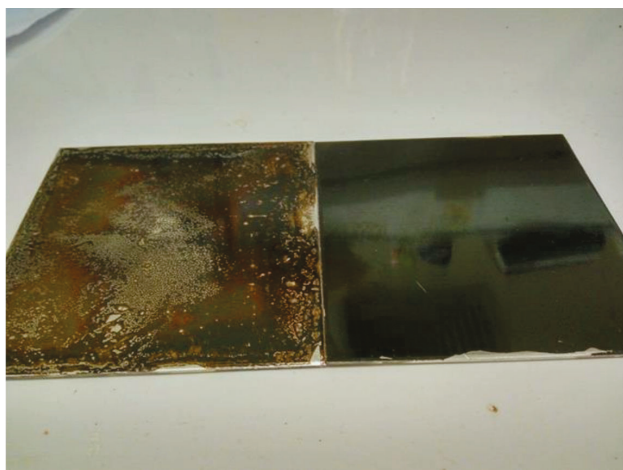


Figure 17. Appearance of glucose and sucrose NIPU coatings on steel. Left: sucrose NIPU coating. Right: glucose NIPU coating [61].

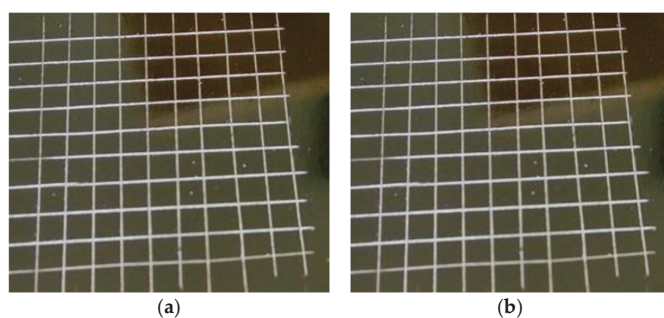


Figure 18. Cross-cut test according to [63] of a glucose-based surface coating on stainless steel cured for 3 min at 300 °C. (a) Before washing in hot water; (b) after washing in hot water [61].

5. PVA–Citric Acid Metal and Glass Bonding

Citric acid has in recent years gained considerable publicity as a wood binder, either alone or in combination with a variety of hydroxyl-carrying biomaterials, such as glucose, sucrose, starch, tannin, lignin, and others [64–77]. All this work has mainly aimed to the application of thermosetting wood adhesives, but a few research works exist in which some bio-adhesive systems have been used for adherence to metals or glass either as a coating or as an adhesive [78–82], in particular for glass [83–86].

A novel polyester-type citric acid/PVA adhesive (CPVA) was developed for plywood adhesive, but that was also able to bond steel and glass. It was accomplished by directly mixing the aqueous solution of PVA with citric acid, which was used to bond various substrates, including wood, but in particular glass, and steel sheets [87]. The adhesion strength of the adhesives was investigated by lap-shear tests in tension, using a universal testing machine under ambient conditions with a stretching speed of 1 mm/min. Taking glass slides as an example, the tested adhesive was compressed between two glass slides of 76 mm × 25 mm with a bonding area of 25 mm × 25 mm [87]. The results show that CPVA adhesives exhibit bonding properties on different substrates. Steel sheets, glass, and ceramics were selected for bonding, and lap-shear tests were performed. The bonding strength applied on glass and steel showed a clear trend, as shown in Table 1. The bonding strength of CPVA on both the glass and steel sheet was greater than 6 MPa, demonstrating a very strong bond being formed. Moreover, the bonding strengths of CPVA adhesives applied on glass and steel are both higher than that obtained with traditional polyurethane with soy protein and PVA/PAA adhesives. It appears that the CPVA adhesive has a greater potential for adhesion on glass and steel. Thus, CPVA could serve as a potential candidate for eco-friendly bonding on traditionally difficult-to-bond substrates.

Table 1. Dependence of the adhesion strength of glass and steel joints as a function of the ratio of citric acid/PVA (CA/PVA).

	CA:PVA Mass Ratio					
	0:1	0.25:1	0.5:1	0.75:1	1:1	1.1:1
Glass sheet (MPa)	5.2	5.75	6.0	6.1	6.4	5.9
Steel sheet (MPa)	4.7	5.5	5.7	5.8	6.3	5.9

The research work even compared the strength of the best PVA-citric acid adhesive with that of competitive commercial adhesives, showing that better results were obtained with this adhesive, as well as comparing the resistance of all of them to a warm water (63 °C) test, as shown in Table 2.

Table 2. Strength results of steel joints bonded with different adhesives after a treatment in 63 °C water for 3 h.

Adhesive Type	MPa
PF	0.77
MUF	1.36
Modified Soy protein	1.07
Modified Starch	0.81
PVA-citric acid	1.52

6. Conclusions

While the great majority of the rapidly increasing number of publications on lignocellulosic based (and other bio-sourced materials) adhesive and coatings are aimed for application to wood products bonding and wood surface finishing, there are relatively few works aimed at using lignocellulosic-derived bio-resins for bonding applications of, and coating on, metals, glass, and other difficult-to-bond substrates. While the natural tendency to use lignocellulosic materials for wood applications, the cases presented here have been shown to render readers aware that there are great opportunities to develop a variety of other higher-tech applications for lignocellulosic materials. Literally, the field is open to considerable and fast development once researchers start to become aware of the many unexplored possibilities that exist. This mini review has presented some unusual cases based on some lignocellulosic materials used for such non-traditional applications, showing encouraging potential for further considerable developments.

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References

1. Caillol, S.; Boutevin, B.; Pascault, J.-P. Biosourced Epoxy monomers and polymers. In *Handbook of Adhesive Technology*, 3rd ed.; Pizzi, A., Mittal, J.K., Eds.; CRC Press: Boca Raton, FL, USA, 2018.
2. Pizzi, A. (Ed.) Tannin-based adhesives. In *Wood Adhesives Chemistry and Technology*; Dekker: New York, NY, USA, 1983; pp. 177–246.
3. Pizzi, A. *Advanced Wood Adhesives Technology*; Dekker: New York, NY, USA, 1994.
4. Pizzi, A. Little Secrets for the Successful Industrial Use of Tannin Adhesives: A Review. *J. Renew. Mater.* **2023**, *11*, 3403–3415. [CrossRef]
5. Pizzi, A. Lignin Adhesives. In *Handbook of Adhesive Technology*, 2nd ed.; Pizzi, A., Mittal, J.K., Eds.; Dekker: New York, NY, USA, 2003.
6. Pizzi, A. Recent development in advanced lignin-based adhesives and binders. In *Advanced Lignin Technologies*; IntechOpen: London, UK, 2024.
7. Dunky, M. Wood Adhesives Based on Natural Resources: A Critical Review. Part I. Protein-Based Adhesives. *Rev. Adhes. Adhes.* **2020**, *8*, 199–332. [CrossRef]
8. Dunky, M. Wood Adhesives Based on Natural Resources: A Critical Review. Part II. Carbohydrate-Based Adhesives. *Rev. Adhes. Adhes.* **2020**, *8*, 333–378. [CrossRef]
9. Maiti, B.; Abramov, A.; Finn, M.G.; Díaz, D.D. Biopolymers as sustainable metal bio-adhesives. *J. Appl. Polym. Sci.* **2021**, *138*, 49783. [CrossRef]
10. Ma, Y.; Kou, Z.; Hu, Y.; Zhou, J.; Bei, Y.; Hu, L.; Huang, Q.; Jia, P.; Zhou, Y. Research advances in bio-based adhesives. *Int. J. Adhes. Adhes.* **2023**, *126*, 103444. [CrossRef]
11. Dang, Q.D.; Moon, J.R.; Jeon, Y.S.; Kim, J.H. Supramolecular adhesive gels based on biocompatible poly(2-ethyl-2-oxazoline) and tannic acid via hydrogen bonding complexation. *J. Appl. Polym. Sci.* **2019**, *137*, 48285. [CrossRef]
12. Zhao, Q.X.; Mu, S.D.; Long, Y.R.; Zhou, J.; Chen, W.Y.; Astruc, D.; Gaidau, C.; Gu, H.B. Tannin-tethered gelatin hydrogels with considerable self-healing and adhesive performances. *Macromol. Mater. Eng.* **2019**, *304*, 1800664. [CrossRef]
13. Zhang, H.; Chen, T.; Li, Y.; Han, Y.; Sun, Y.; Sun, G. Novel lignin-containing high-performance adhesive for extreme environment. *Int. J. Biol. Macromol.* **2020**, *164*, 1832–1839. [CrossRef]

14. Liu, W.; Fang, C.; Chen, F.; Qiu, X. Strong, reusable, and self-healing lignin-containing polyurea adhesives. *ChemSusChem* **2020**, *13*, 4691–4701. [CrossRef]
15. Zhu, Y.; Di, B.; Chen, H.; Wang, X.; Tian, Y. In situ synthesis of novel biomass lignin/silica based epoxy resin adhesive from renewable resources at different pHs. *J. Adhes. Sci. Technol.* **2019**, *33*, 1806–1820. [CrossRef]
16. Chen, H.; Ling, M.; Hencz, L.; Ling, H.Y.; Li, G.; Lin, Z.; Liu, G.; Zhang, S. Exploring chemical, mechanical, and electrical functionalities of binders for advanced energy-storage devices. *Chem. Rev.* **2018**, *118*, 8936–8982. [CrossRef] [PubMed]
17. Ling, M.; Xu, Y.; Zhao, H.; Gu, X.; Qiu, J.; Li, S.; Wu, M.; Song, X.; Yan, C.; Liu, G.; et al. Dual-functional gum arabic binder for silicon anodes in lithium ion batteries. *Nano Energy* **2015**, *12*, 178–185. [CrossRef]
18. Li, Z.; Wan, Z.; Zeng, X.; Zhang, S.; Yan, L.; Ji, J.; Wang, H.; Ma, Q.; Liu, T.; Lin, Z.; et al. A robust network binder via localized linking by small molecules for high-area-capacity silicon anodes in lithium-ion batteries. *Nano Energy* **2020**, *79*, 105430. [CrossRef]
19. Panchireddy, S.; Grignard, B.; Thomassin, J.-M.; Jerome, C.; Detrembleur, C. Bio-based poly(hydroxyurethane) glues for metal substrates. *Polym. Chem.* **2018**, *9*, 2650–2659. [CrossRef]
20. Jenkins, C.L.; Siebert, H.M.; Wilker, J.J. Integrating Mussel Chemistry into a Bio-Based Polymer to Create Degradable Adhesives. *Macromolecules* **2017**, *50*, 561–568. [CrossRef]
21. Schneiderman, D.K.; Vanderlaan, M.E.; Mannion, A.M.; Panthani, T.R.; Batiste, D.C.; Wang, J.Z.; Bates, F.S.; Macosko, C.W.; Hillmyer, M.A. Chemically Recyclable Biobased Polyurethanes. *ACS Macro Lett.* **2016**, *5*, 515–518. [CrossRef]
22. Strube, O.I.; Rüdiger, A.A.; Bremser, W. Buildup of biobased adhesive layers by enzymatically controlled deposition on the example of casein. *Int. J. Adhes. Adhes.* **2015**, *63*, 9–13. [CrossRef]
23. Ruediger, A.A.; Bremser, W.; Strube, O.I. The enzyme-mediated autodeposition of casein: Effect of enzyme immobilization on deposition of protein structures. *J. Coat. Technol. Res.* **2016**, *13*, 597–611. [CrossRef]
24. Kim, S.; Jeong, Y.K.; Wang, Y.; Lee, H.; Choi, J.W. A “Sticky” Mucin-Inspired DNA-Polysaccharide Binder for Silicon and Silicon-Graphite Blended Anodes in Lithium-Ion Batteries. *Adv. Mater.* **2018**, *30*, 1707594. [CrossRef]
25. Hussin, M.H.; Latif, N.H.A.; Hamidon, T.S.; Idris, N.N.; Hashim, R.; Appaturi, J.N.; Brosse, N.; Ziegler-Devin, I.; Chrusiel, L.; Fatriasari, W.; et al. Latest advancements in high-performance bio-based wood adhesives: A critical review. *J. Mater. Res. Technol.* **2022**, *21*, 3909–3946. [CrossRef]
26. Dunky, M. Applications and Industrial Implementations of Naturally-Based Adhesives. In *Biobased Adhesives: Sources, Characteristics, and Applications*; Dunky, M., Mittal, K.L., Eds.; Wiley-Scrivener: New York, NY, USA, 2023.
27. Basso, M.; Pizzi, A.; Maris, J.P.; Delmotte, L.; Colin, B.; Rogaume, Y. MALDI-TOF, 13C NMR and FTIR analysis of the cross-linking reaction of condensed tannins by triethyl phosphate. *Ind. Crops Prod.* **2017**, *95*, 621–631. [CrossRef]
28. Braghiroli, F.; Fierro, V.; Pizzi, A.; Rode, K.; Radke, W.; Delmotte, L.; Parmentier, J.; Celzard, A. Condensation reaction of flavonoid tannins with ammonia. *Ind. Crops Prod.* **2013**, *44*, 330–335. [CrossRef]
29. Polesel-Maris, J.; Joutang, I. Anti-Adhesive Coatings Based on Condensed Tannins. WO Patent 2017/037393A1, 9 March 2017.
30. Norme Francaise XP D21-503. Articles Culinaires Métalliques à Usage Domestique pour Cuisinières et Plaques de Cuisson—Compatibilité avec les Différentes Sources de Chaleur: Exigences et Méthodes d’Essai. 2003. Available online: <https://www.boutique.afnor.org/fr-fr/norme/xp-d21503/articles-culinaires-metalliques-a-usage-domestique-pour-cuisinieres-et-plaq/fa135063/22040> (accessed on 12 November 2024).
31. Normes HACCP (Hazard Analysis Critical Control Point), RÈGLEMENT (CE) No 852/2004 DU PARLEMENT EUROPÉEN ET DU CONSEIL du 29 Avril 2004 Relatif à l’hygiène des Denrées Alimentaire. 2004. Available online: https://www.economie.gouv.fr/files/directions_services/dgcrf/securite/produits_alimentaires/Hygiene_alimentaire/ha-reg-852.pdf (accessed on 12 November 2024).
32. Basso, M.C.; Pizzi, A.; Delmotte, L.; Abdalla, S. MALDI-TOF and 13C NMR Analysis of the Cross-linking Reaction of Lignin by Triethyl Phosphate. *Polymers* **2017**, *9*, 206. [CrossRef] [PubMed]
33. Pizzi, A. Lignin Adhesives. In *Handbook of Adhesive Technology*, 3rd ed.; Pizzi, A., Mittal, J.K., Eds.; Dekker: New York, NY, USA, 2017.
34. Peng, Y.; Zheng, Z.; Sun, P.; Wang, X.; Zhang, T. Synthesis and characterization of polyphenol-based polyurethane. *New J. Chem.* **2012**, *37*, 729–734. [CrossRef]
35. Ge, J.-J.; Sakai, K. Compressive Properties and Biodegradabilities of Polyurethane Foams Derived from Condensed Tannins. *Mokuzai Gakkaishi* **1993**, *39*, 801–806.
36. Ge, J.-J.; Sakai, K. Decomposition of polyurethane foams derived from condensed tannin II: Hydrolysis and aminolysis of polyurethane foams. *J. Wood Sci.* **1998**, *44*, 103–105. [CrossRef]
37. Ge, J.; Shi, X.; Cai, M.; Wu, R.; Wang, M. A novel biodegradable antimicrobial PU foam from wattle tannin. *J. Appl. Polym. Sci.* **2003**, *90*, 2756–2763. [CrossRef]
38. Faruk, O.; Sain, M. Continuous Extrusion Foaming of Lignin Enhanced Thermoplastic Polyurethane (TPU). *J. Biobased Mater. Bioenergy* **2013**, *7*, 309–314. [CrossRef]
39. Hatakeyama, H.; Hatakeyama, T. Advances of Polyurethane Foams Derived from Lignin. *J. Renew. Mater.* **2013**, *1*, 113–123. [CrossRef]
40. Liu, J.; Liu, H.; Deng, L.; Liao, B.; Guo, Q. Improving aging resistance and mechanical properties of waterborne polyurethanes modified by lignin amines. *J. Appl. Polym. Sci.* **2013**, *130*, 1736–1742. [CrossRef]

41. Wang, Z.; Xu, S.; Hu, W.-P.; Xie, Y.-J. Fractionation of the Biopolyols from Lignocellulosic Biomass for the Production of Rigid Foams. *BioEnergy Res.* **2013**, *6*, 896–902. [CrossRef]
42. Whelan, J.M., Jr.; Hill, M.; Cotter, R.J. Multiple Cyclic Carbonate Polymers. U.S. Patent 3,072,613, 8 January 1963.
43. Rokicki, G.; Piotrowska, A. A new route to polyurethanes from ethylene carbonate, diamines and diols. *Polymer* **2002**, *43*, 2927–2935. [CrossRef]
44. Kihara, N.; Endo, T. Synthesis and properties of poly(hydroxyurethane)s. *J. Polym. Sci. Part A Polym. Chem.* **1993**, *31*, 2765–2773. [CrossRef]
45. Kihara, N.; Kushida, Y.; Endo, T. Optically active poly(hydroxyurethane)s derived from cyclic carbonate and L-lysine derivatives. *J. Polym. Sci. Part A Polym. Chem.* **1996**, *34*, 2173–2179. [CrossRef]
46. Tomita, H.; Sanda, F.; Endo, T. Structural analysis of polyhydroxyurethane obtained by polyaddition of bifunctional five-membered cyclic carbonate and diamine based on the model reaction. *J. Polym. Sci. Part A Polym. Chem.* **2001**, *39*, 851–859. [CrossRef]
47. Tomita, H.; Sanda, F.; Endo, T. Polyaddition behavior of bis(five- and six-membered cyclic carbonate)s with diamine. *J. Polym. Sci. Part A Polym. Chem.* **2001**, *39*, 860–867. [CrossRef]
48. Tomita, H.; Sanda, F.; Endo, T. Model reaction for the synthesis of polyhydroxyurethanes from cyclic carbonates with amines: Substituent effect on the reactivity and selectivity of ring-opening direction in the reaction of five-membered cyclic carbonates with amine. *J. Polym. Sci. Part A Polym. Chem.* **2001**, *39*, 3678–3685. [CrossRef]
49. Birukov, O.; Potashnikova, R.; Leykin, A.; Figovsky, O.; Shapovalov, L. Advantages in chemistry and technology of non-isocyanate polyurethane. *J. Sci. Israel-Technol. Adv.* **2009**, *11*, 160–167.
50. Figovsky, O.; Shapovalov, L. Features of reaction amino-cyclocarbonate for production of new type polyurethanes. *Macromol. Symp.* **2002**, *187*, 325–332. [CrossRef]
51. Camara, F.; Benyahya, S.; Besse, V.; Boutevin, G.; Auvergne, R.; Boutevin, B.; Caillol, S. Reactivity of secondary amines for the synthesis of non-isocyanate polyurethanes. *Eur. Polym. J.* **2014**, *55*, 17–26. [CrossRef]
52. Blattmann, H.; Fleischer, M.; Bähr, M.; Mülhaupt, R. Isocyanate- and phosgene-free routes to polyfunctional cyclic carbonates and green polyurethanes by fixation of carbon dioxide. *Macromol. Rapid Commun.* **2014**, *35*, 1238–1254. [CrossRef] [PubMed]
53. Boyer, A.; Cloutet, E.; Tassaing, T.; Gadenne, B.; Alfes, C.; Cramail, H. Solubility in CO₂ and carbonation studies of epoxidized fatty acid diesters: Towards novel precursors for polyurethane synthesis. *Green Chem.* **2010**, *12*, 2205–2213. [CrossRef]
54. Kim, M.; Kim, H.; Ha, C.; Park, D.; Lee, J. Syntheses and thermal properties of poly(hydroxy)urethanes by polyaddition reaction of bis(cyclic carbonate) and diamines. *J. Appl. Polym. Sci.* **2001**, *81*, 2735–2743. [CrossRef]
55. Ochiai, B.; Inoue, S.; Endo, T. Salt effect on polyaddition of bifunctional cyclic carbonate and diamine. *J. Polym. Sci. Part A Polym. Chem.* **2005**, *43*, 6282–6286. [CrossRef]
56. Ubaghs, L.; Fricke, N.; Keul, H.; Höcker, H. Polyurethanes with pendant hydroxyl groups: Synthesis and Characterization. *Macromol. Rapid Commun.* **2004**, *25*, 517–521. [CrossRef]
57. Fleischer, M.; Blattmann, H.; Mülhaupt, R. Glycerol-, pentaerythritol- and trimethylolpropane-based polyurethanes and their cellulose carbonate composites prepared via the non-isocyanate route with catalytic carbon dioxide fixation. *Green Chem.* **2013**, *15*, 934–942. [CrossRef]
58. Besse, V.; Auvergne, R.; Carlotti, S.; Boutevin, G.; Otazaghine, B.; Caillol, S.; Pascault, J.-P.; Boutevin, B. Synthesis of isosorbide based polyurethanes: An isocyanate free method. *React. Funct. Polym.* **2013**, *73*, 588–594. [CrossRef]
59. Cornille, A.; Auvergne, R.; Figovsky, O.; Boutevin, B.; Caillol, S. A perspective approach to sustainable routes for non-isocyanate polyurethanes. *Eur. Polym. J.* **2017**, *87*, 535–552. [CrossRef]
60. Xi, X.; Pizzi, A.; Delmotte, L. Isocyanate-Free Polyurethane Coatings and Adhesives from Mono- and Di-Saccharides. *Polymers* **2018**, *10*, 402. [CrossRef]
61. Tundo, P.; Selva, M. The Chemistry of Dimethyl Carbonate. *Acc. Chem. Res.* **2002**, *35*, 706–716. [CrossRef]
62. Xi, X.; Wu, Z.; Pizzi, A.; Gerardin, C.; Lei, H.; Zhang, B.; Du, G. Non-isocyanate polyurethane adhesive from sucrose used for particleboard. *Wood Sci. Technol.* **2019**, *53*, 393–405. [CrossRef]
63. *Norme Francaise NF EN ISO 2409; Peintures et Vernis—Essai de Quadrillage.* AFNOR: St. Denis, France, 2020.
64. Li, J.; Lei, H.; Xi, X.; Li, C.; Hou, D.; Song, J.; Du, G. A sustainable tannin-citric acid wood adhesive with favorable bonding properties and water resistance. *Ind. Crops Prod.* **2023**, *201*, 116933. [CrossRef]
65. Umemura, K.; Ueda, T.; Munawar, S.S.; Kawai, S. Application of citric acid as natural adhesive for wood. *J. Appl. Polym. Sci.* **2011**, *123*, 1991–1996. [CrossRef]
66. Umemura, K.; Sugihara, O.; Kawai, S. Investigation of a new natural adhesive composed of citric acid and sucrose for particleboard. *J. Wood Sci.* **2013**, *59*, 203–208. [CrossRef]
67. Del Menezzi, C.; Amirou, S.; Pizzi, A.; Xi, X.; Delmotte, L. Reactions with Wood Carbohydrates and Lignin of Citric Acid as a Bond Promoter of Wood Veneer Panels. *Polymers* **2018**, *10*, 833. [CrossRef]
68. Li, C.; Lei, H.; Wu, Z.; Xi, X.; DU, G.; Pizzi, A. Fully Biobased Adhesive from Glucose and Citric Acid for Plywood with High Performance. *ACS Appl. Mater. Interfaces* **2022**, *14*, 23859–23867. [CrossRef]
69. Liao, R.; Xu, J.; Umemura, K. Low Density Sugarcane Bagasse Particleboard Bonded with Citric Acid and Sucrose: Effect of board density and additive content. *BioResources* **2015**, *11*, 2174–2185. [CrossRef]

70. Widyorini, R.; Nugraha, P.A.; Rahman, M.Z.A.; Prayitno, T.A. Bonding ability of a new adhesive composed of citric acid-sucrose for particleboard. *BioResources* **2016**, *11*, 4526–4535. [CrossRef]
71. Lin, C.-F.; Karlsson, O.; Jones, D.; Sandberg, D. Bio-based adhesive derived from citric acid and sorbitol for wood-composite manufacture. *Wood Mater. Sci. Eng.* **2022**, *17*, 397–399. [CrossRef]
72. Kurkowiak, K.; Hentges, D.; Dumarçay, S.; Gérardin, P.; Militz, H. Understanding the Mode of Action of Sorbitol and Citric Acid (SorCA) in Wood. In Proceedings of the Tenth European Conference on Wood Modification, Nancy, France, 25–26 April 2022; pp. 209–216.
73. Kusumah, S.S.; Jayadi; Wibowo, D.T.; A Pramasari, D.; A Widyaningrum, B.; Darmawan, T.; Ismadi; Dwianto, W.; Umemura, K. Investigation of eco-friendly plywood bonded with citric acid—Starch based adhesive. In *IOP Conference Series: Earth and Environmental Science*; IOP Publishing: Bristol, UK, 2020; Volume 460. [CrossRef]
74. Li, C.; Hou, D.; Lei, H.; Xi, X.; Du, G.; Zhang, H.; Cao, M.; Tondi, G. Effective and eco-friendly safe self-antimildew strategy to simultaneously improve the water resistance and bonding strength of starch-based adhesive. *Int. J. Biol. Macromol.* **2023**, *248*, 125889. [CrossRef]
75. Cai, L.; Chen, Y.; Lu, Z.; Wei, M.; Zhao, X.; Xie, Y.; Li, J.; Xiao, S. Citric acid/chitosan adhesive with viscosity-controlled for wood bonding through supramolecular self-assembly. *Carbohydr. Polym.* **2024**, *329*, 121765. [CrossRef] [PubMed]
76. Sakai, S.; Chen, S.; Matsuo-Ueda, M.; Umemura, K. Curing behavior of sucrose with p-toluenesulfonic acid. *Polymers* **2023**, *15*, 4592. [CrossRef] [PubMed]
77. Crisostomo, M.; Del Menezzi, C.; Militz, H.; Kurkowiak, K.; Mayer, A.; Carvalho, L.; Martins, J. Effect of Citric Acid on the Properties of Sapwood of *Pinus sylvestris* Submitted to Thermomechanical Treatment. *Forests* **2023**, *14*, 1839. [CrossRef]
78. Li, X.; Wang, Z.; Li, W.; Sun, J. Superstrong Water-Based Supramolecular Adhesives Derived from Poly(Vinyl Alcohol)/Poly(Acrylic Acid) Complexes. *ACS Mater. Lett.* **2021**, *3*, 875–882. [CrossRef]
79. Machalická, K.; Eliášová, M. Adhesive Joints in Glass Structures: Effects of Various Materials in the Connection, Thickness of the Adhesive Layer, and Ageing. *Int. J. Adhes. Adhes.* **2017**, *72*, 10–22. [CrossRef]
80. Overend, M.; Jin, Q.; Watson, J. The Selection and Performance of Adhesives for a Steel–Glass Connection. *Int. J. Adhes. Adhes.* **2011**, *31*, 587–597. [CrossRef]
81. Qi, G.; Sun, X.S. Peel Adhesion Properties of Modified Soy Protein Adhesive on a Glass Panel. *Ind. Crops Prod.* **2010**, *32*, 208–212. [CrossRef]
82. Kumar, A.; Kumar, K.; Ghosh, P.; Rath, A.; Yadav, K. MWCNTs toward Superior Strength of Epoxy Adhesive Joint on Mild Steel Adherent. *Compos. Part B Eng.* **2018**, *143*, 207–216. [CrossRef]
83. Roberts, A.D.; Finnigan, W.; Kelly, P.P.; Faulkner, M.; Breitling, R.; Takano, E.; Scrutton, N.S.; Blaker, J.J.; Hay, S. Non-covalent protein-based adhesives for transparent substrates—Bovine serum albumin vs. recombinant spider silk. *Mater. Today Bio* **2020**, *7*, 100068. [CrossRef]
84. Zhou, Y.; Fang, Z.; Zeng, G.; Tang, Z.; Zhang, F.; Luo, J.; Li, X.; Li, K.; Li, J. Tough protein based adhesive reinforced by molecular spring strengthening strategy. *Chem. Eng. J.* **2022**, *436*, 135023. [CrossRef]
85. Allen, G.; Griffin, S.; Clifford, B.; Phillips, C.; Van Keulen, G. Development of bio-inspired protein-based materials as novel transparent adhesives for glass laminates. *Access Microbiol.* **2019**, *1*. [CrossRef]
86. Heinritz, C.; Ng, X.J.; Scheibel, T. Bio-inspired Protein-Based and Activatable Adhesion Systems. *Adv. Funct. Mater.* **2024**, *34*, 2303609. [CrossRef]
87. Xu, D.; Li, C.; Zhou, Y.; Wang, Z.; Hou, D.; Lei, H.; Chen, Z.; Du, G. Feasibly developing Polyester-type poly(vinyl alcohol) adhesive in one go with high water resistance and bonding performance. *Langmuir*, 2024; *in press*.

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