EDITOR
Pierfrancesco Morganti
Dermatology Unit, University of Campania "Luigi Vanvitelli", Naples, Italy;
China Medical University, Shenyang, China;
Director of the R&D Nanoscience Centre MAVI, MAVI Sud Srl, Aprilia (Lt), Italy.

Editorial Office
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Lignin: Isolation, Structure and Valorisation

Heiko Lange and Claudia Crestini

Abstract: Lignin is the second most abundant polymer in forest biomass after cellulose. Compared to the ubiquitous use of cellulose, lignin is currently simply being wasted. In fact, as a waste product in the cellulose production, it serves mainly for the generation of energy. The reason for this lies in the challenging structural features and the patchy understanding of the correlation between structural features and polymer characteristics displayed by various lignins. This chapter will introduce the general characteristics and peculiarities of lignin as biopolymer and will present techniques for investigating structural issues. Approaches toward a valorization of lignin will be highlighted, showing the possibilities for using lignin in chemistry and material sciences.

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1. Introduction

The efficient use of all available biomass components is of utmost interest with respect to a sustainable use of renewable resources. Lignin, however, albeit being the second most-abundant component in forest biomass, is still not exploited to its full potential. Lignin is a rather complex natural polymer. Its straightforward use is severely impeded by several difficulties that reach back to the molecular level, and lignin is thus unfortunately still seen as a natural polymer with unpredictable properties.

Especially those areas of research that could eventually truly benefit from using and incorporating lignin as a functional groups-carrying polymeric compound seem to be paralyzed by the old paradigm that "lignin is not good for anything but burning it".

Prejudices and the admittedly tedious initial effort necessary to get an idea of the nature of the lignin at hand contribute to maintaining lignin in a niche. Methods to characterize a given lignin sample do exist, but these methods, although quite matured as such by now, still need further refinement, cross-correlation, and independent validation, in order to further improve and overall streamline the processes of acquiring structural data of lignin. Sound structural data are absolutely necessary for being able to analyse structural features with respect to reactivity of a given lignin, and for understanding which chemistries are suitable for derivatization, functionalization, and depolymerization of lignin samples isolated from different renewable sources.

The isolation of lignin, its structural characterization, and its utilization have been subject to research activities around the globe for decades. Quite some knowledge has been accumulated regarding lignin and its characteristics, and this knowledge is constantly refined with respect to technological advances in adjacent fields such as biorefinery, biotechnology, spectrometry, spectroscopy, etc. Achievements are regularly summarized in reviews and monographs [1–5]. Nevertheless, more fundamental studies on lignin are needed to arrive at a complete understanding; this is true for both lignin *in planta* and isolated lignins.

Lignin, derived from 'lignum', Latin for wood, was introduced as early as 1819 by de Candolle, but not until Payen scientifically described a wood component in 1838, which, from our current perspective must have been a lignin carbohydrate complex (LCC), the term lignin was connected closely to an isolable substance. Schulze, in 1865, chemically defined lignin as a polymer that was different from the cellulose components [5].

Lignin accounts for 15–35% of the dry mass of wood, depending on the type of wood; this makes this natural polyphenol being the second most abundant component in forest biomass after cellulose in absolute numbers [6].

The rather hydrophobic polymer, located mainly in the plant cell walls, is chemically and physically interacting with hemicelluloses and cellulose, respectively [7]: it is located in form of a mixture together with hemicelluloses, eventually forming the aforementioned LCCs, between the cellulose fibrils [1,8].

The tight interplay between these three major plant biopolymers renders the plant cell walls less permeable, leads to increased mechanical strength and rigidity, and thus serves to give not only stability to the plants, but also a better resistance to microbial attacks.

The different functions of lignin in the plant cause its distribution to vary significantly within the different parts of the plant, i.e., between stem, branching points, branches and leafs, and between the different walls of the plant cells themselves [9]. Middle lamella and primary cell walls show higher lignin concentrations than the secondary cell wall. 75–85% of all lignin in the plant, however, is located in the secondary cell walls, since these are significantly more voluminous.

The total amount of lignin present in the plant varies from *ca.* 20% in hardwoods, *ca.* 28% in softwoods and herbaceous angiosperms, and *ca.* 15% in monocots [2,7].

2. Biosynthesis, Isolation and Structural Features of Lignin

Unlike the structurally well understood cellulose and hemicelluloses, lignin does not display a regular repetition motif, as one might expect for a natural polymer closely interacting with other regularly structured polymers. Furthermore, its structure does not only change between different plant types, but also within the same plant depending on where it fulfils its function; structural changes as well as abundance of lignin thus govern and/or are governed by the need of the plant for stability, protection, etc.

The difficulty in understanding the complex structure of lignin does not only stem from the fact that the isolation processes themselves can give rise to structural modification of lignins, as discussed below, but also from the fact that the biosynthetic pathway is not yet fully understood. It is yet to be elucidated whether there are control mechanisms that determine the structure of lignin in plants, or whether it is allowed to form according to the conditions set by a microenvironment surrounding the site at which the chain propagation takes place. As a result of this presumably uncontrolled biosynthetic pathway that is roughly depicted in Figure 1, lignin displays random sequences of different interunit bonding motifs (Figure 2) [10–12].

Although lignin contains different asymmetric carbon atoms that are formed during its biosynthesis, a preferred absolute configuration, as one might expect from the close interplay with the stereogenic polymeric sugars, does not exist—another fact that suggests that the in vivo lignin formation is probably not controlled in detail. It has been shown, however, that the ratio of the different aromatic units does correlate with the ratio between *threo* and *erythro*-configured aliphatic parts in lignin [13]. Current efforts utilize genetically modified model plants such as, e.g., *Arabidopsis thalianato*, to improve understanding of the formation of lignin, its role in and for the plants, and its interaction with the other components in the plant cell walls [14–17].

The monomers consumed in the radical polymerisations are the three monolignols *p*-coumaryl alcohol (H-unit), coniferyl alcohol (G-unit) and sinapyl alcohol (S-unit), which are synthesised by the plants (Figure 1) [10,11,18]. Depending on the natural source from which the lignin is isolated, and also depending on the method with which the lignin has been extracted from the wood and separated from the cellulose and hemicellulose wood components, the abundances of the different monomer types, as well as the nature and the distribution of the interunit bonding motifs via which they are linked differ.

Three different general types of lignin are generally distinguishable on the basis of fundamental structural aspects: (i) softwood lignin, which is mainly comprised of G-type monomers and a small percentage of H-type monomers; (ii) hardwood lignin, which contains mainly S- and G-units; and (iii) grass lignin, which contains all three monomer types.

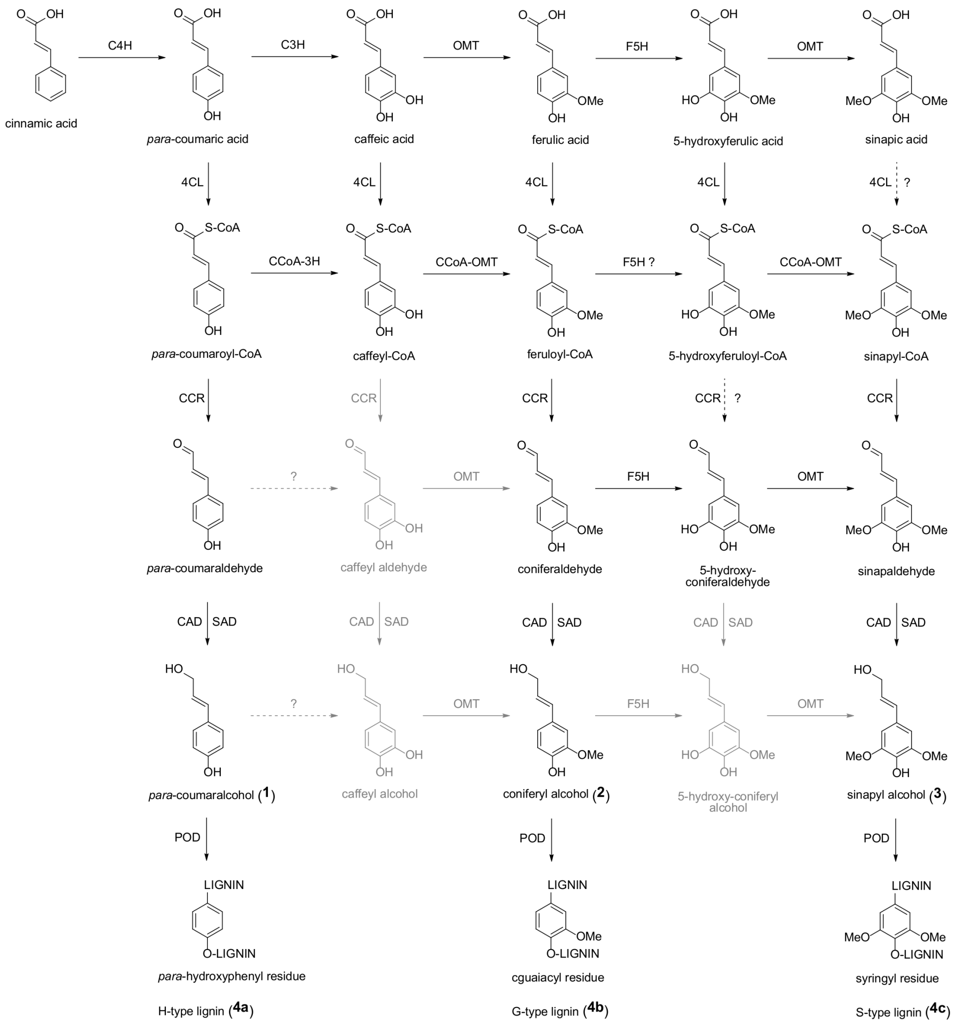


Figure 1. Main elements of the biosynthesis of lignin as described in Ref. [11]. (C4H—cinnamate-4-hydroxylase; C3H—cinnamate-3-hydroxylase; OMT—O-methyltransferase; F5H—ferulate-5-hydroxylase; 4CL—CoA-ligase; CCR—cinnamoyl co-enzyme A reductase; CCoA-3H—coumaroyl-co-enzyme A 3 hydroxylase; CCoA-OMT—coumaroyl-co-enzyme O-methyl transferase; CAD—cinnamyl alcohol dehydrogenase; SAD—short- chain alcohol dehydrogenase; POD—peroxidase.

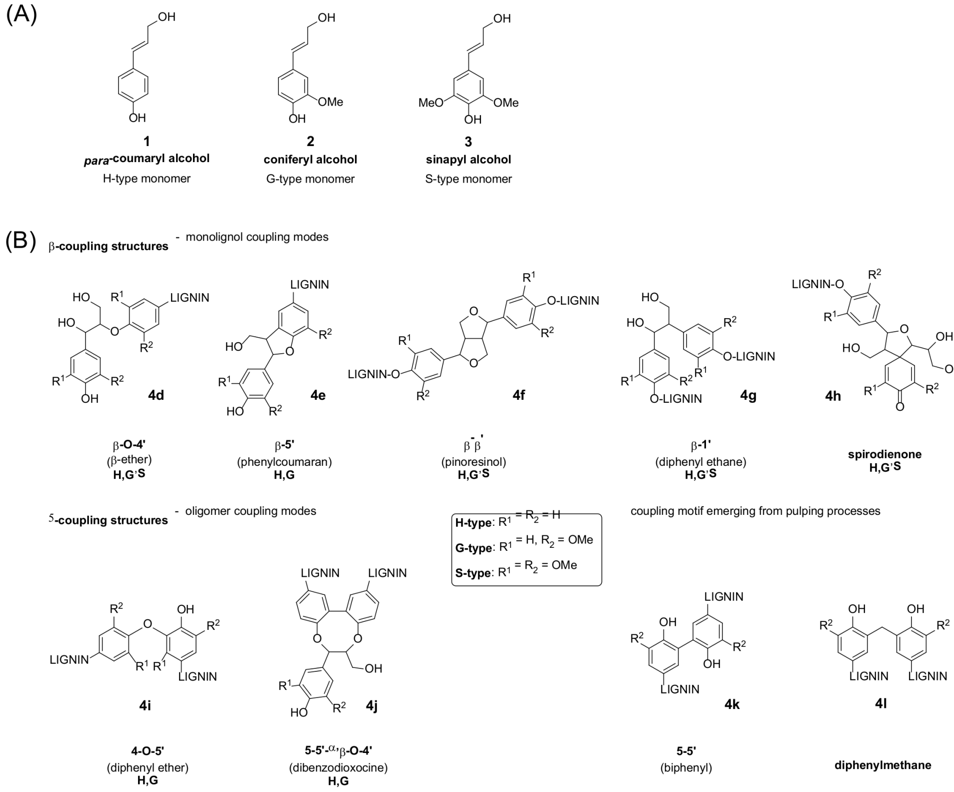
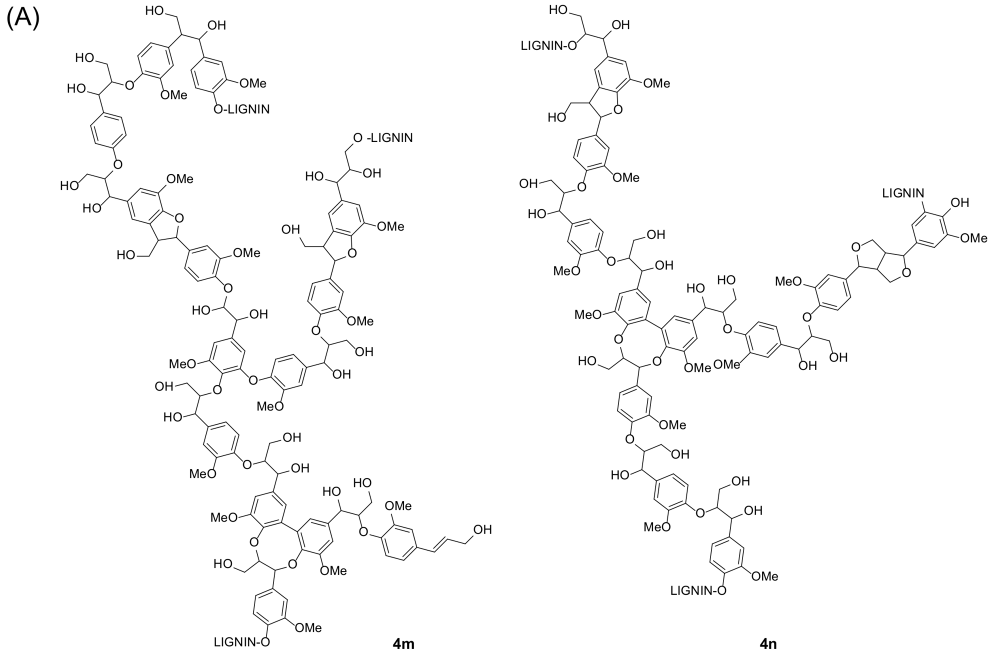


Figure 2. Characteristic interunit bonding motifs and functional groups found in different lignins.

Oxidative coupling of the monomers occurs, however, preferentially at only a limited number of positions, namely O-4, C-1, C-3, C-5 and C-β (IUPAC numbering), thus eventually reflecting some selectivity of enzymes putatively involved in the biosynthesis. Interesting recent findings suggest that the formation of lignin polymers does not end with the death of the plant cell, but that the major part of the lignin in a plant cell is produced after its death, as long as monomers are available [19]. This adds another source of uncertainty to the structural puzzle. Besides, based on the theoretically possible coupling modes, it is currently still controversially discussed in the community whether lignin *in planta* is a branched, and thus three-dimensional polymer, or whether it consists of linear chains that are rather oligomeric than polymeric (Figure 3) [20–22]. Newer studies have established that at least milled wood lignins and some organosolv lignins comprise linear oligomeric chains [22,23]. Newest studies revised the structure of kraft lignins, revealing the fact that it is generally composed of two main, structurally different parts: one consisting of truly polymeric chains, slightly brached, and one of small chains, brached and presumably exhibiting only a low amount of aliphatic moieties [24].

Different techniques and processes have been established for the isolation of lignin; the most important ones are those yielding: (i) milled wood lignin (MWL) (obtained via Björkman's procedure) (Figure 3B) [25]; (ii) acidolysis lignin [26,27]; (iii) enzymatic mild acidolysis lignin [28]; (iv) organosolv lignin (obtained in the Allcel process) [29,30]; (v) kraft lignin (KL) (Figure 4A) [31,32]; (vi) lignosulfonate (obtained in the Howard process) (Figure 4B) [33]; (vii) pyrolysis lignin [34–37]; and (viii) steam explosion lignin [38–40] (Table 1). Since any isolation process can affect the natural structure of lignin, i.e., isolation methods may introduce new functional groups, and/or cause partial degradation, and the isolated lignins are thus not necessarily representing the natural lignin polymers.



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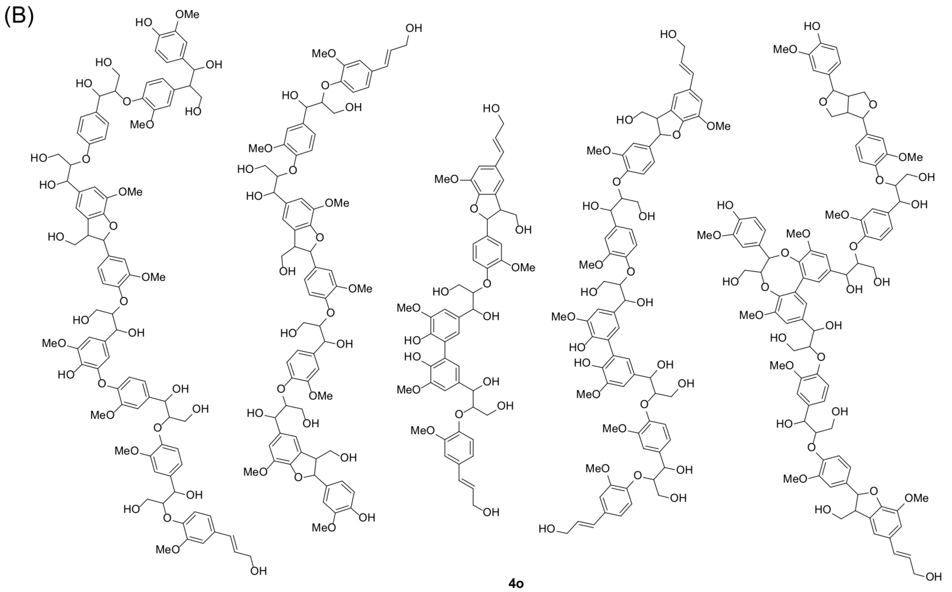

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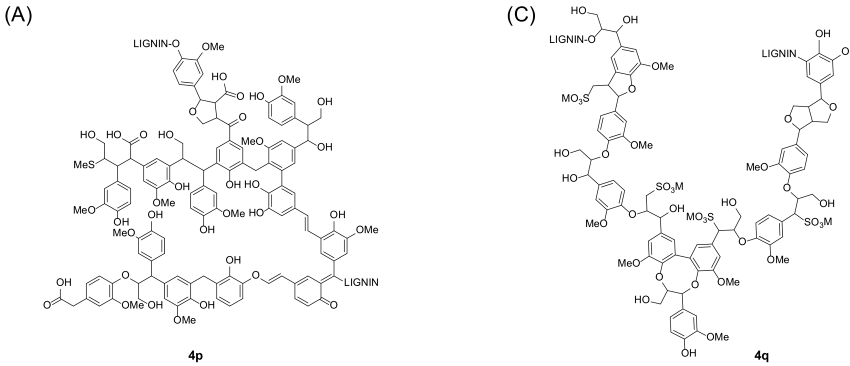
Figure 3. Lignin structures showing characteristic interunit bonding motifs and functional groups for different types of lignin: (**A**) branched polymeric lignin (outdated view); (**B**) linear chains of oligomeric milled wood lignin (actual view).

More recent efforts aimed at circumventing this issue by (i) generating a 'stand-alone' 'synthetic lignin' in form of dehydrogenation polymers (DHP) by reacting monolignols in the presence of oxidative enzymes [41–43], or (ii) by evaluating and refining current standard analyses techniques as discussed below for the investigation of lignin inside undamaged woody tissues [44].



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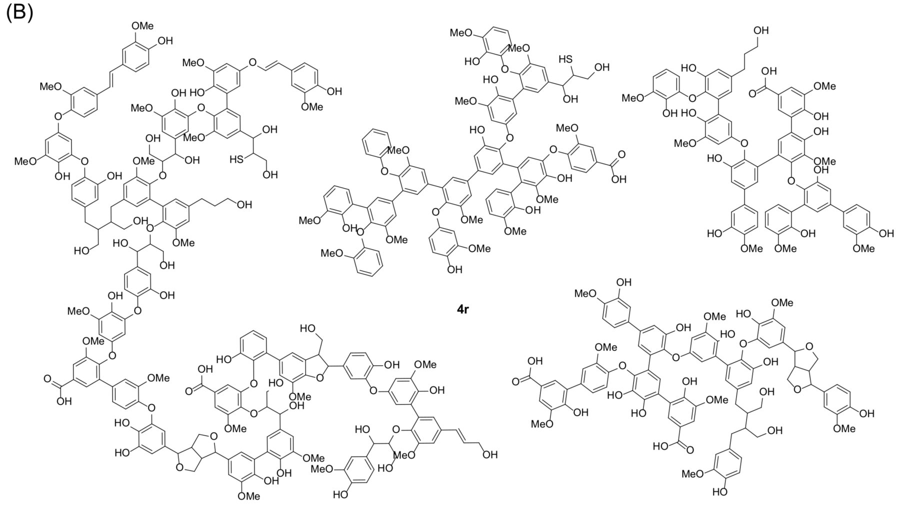

Figure 4. Lignin structures showing characteristic interunit bonding motifs and functional groups for different types of lignin (continued): (**A**) kraft lignin (outdated view); (**B**) kraft lignin (proposed revised structure composed of polymeric and oligomeric units); (**C**) lignosulfonate.

Table 1. Overview comparing the (average) characteristics of various lignins obtained through the different characteristic isolation techniques (for combined references on data shown here, refer to Ref. [45]).

Lignin Type	C9 Molecular Formula	Monomer Molecular Weight [u]	Number-Average Molecular Weight (M_n)	Poly-Dispersity
Milled wood lignin [a]	$C_9H_{7.80}O_{2.41}(OCH_3)_{0.95}$	198	2800–14,200	3.7–12.9
Cellulolytic enzyme lignin [b]	$C_9H_{8.02}O_{2.82}(OCH_3)_{0.90}$	187	~1900	5.7–6.7
Enzymatic mild acidolysis lignin (EMAL) [b]	$C_9H_{8.02}O_{2.82}(OCH_3)_{0.90}$	187	~2000	~3
Kraft lignin [c]	$C_9H_{8.5}O_{2.1}S_{0.1}(OCH_3)_{0.8}(CO_2H)_{0.2}$	180	1000–3000	2–4
Lignosulfonate (softwood) [d]	$C_9H_{8.5}O_{2.5}(OCH_3)_{0.85}\ (SO_3H)_{0.4}$	215–254	5000–20,000	4–9
Lignosulfonate (hardwood) [d]	$C_9H_{7.5}O_{2.5}(OCH_3)_{0.39}\ (SO_3H)_{0.6}$	188	5000–20,000	4–9
Organosolv lignin [e]	$C_9H_{8.53}O_{2.45}(OCH_3)_{1.04}$	188	>1000	2.4–6.4
Pyrolysis lignin [f]	$C_9H_{6.3\text{-}7.3}O_{0.6\text{-}1.4}(OCH_3)_{0.3\text{-}0.8}(OH)_{1\text{-}1.2}$	n.d.	300–600	2.0–2.2
Steam explosion lignin [g]	$C_9H_{8.53}O_{2.45}(OCH_3)_{1.04}$	188	1100–2300	1.5–2.8

[a] Norway spruce wood; [b] Isolated from milled Norway spruce wood; [c] Norway spruce wood; [d] Norway spruce wood; [e] Norway spruce wood; [f] Beech wood; [g] Japanese white birch wood and larch wood.

Total amounts of lignin in a sample, however, are still best and commonly determined as Klason lignin by the Klason method [46,47]. If necessary, the amount of Klason lignin can be corrected for the parts of lignin that are soluble under the conditions used in the Klason method, i.e., the so-called acid soluble lignin.

3. Biodegradation

As mentioned above, lignin plays a dual role for the plant: it gives stability to the plant cells and thus to the plant, and it serves to protect the plant and its cells against attacks by microbes or saprophytic organisms. On the molecular bases, the protection is effected by simply preventing the microbes to hydrolytically cleave the cellulosic polymers, by physically shielding the cellulose and presenting a polymer that does not give rise to facile (bio)degradation [48,49].

Most of the phenols are used for the construction of alkyl aryl ethers, and the redox potential needed for effective bond cleavages is too high, as that the plant-based oxidoreductases which are involved in the construction of lignin, could be strong enough to ('accidently') destroy it. Lignin degradation can thus only be effected by organisms outside the plant that host a special group of enzymes, known as lignocellulolytic enzymes [50–52]. Evolution of these organisms that serve to recycle woody biomass and to feed back the carbon sources in the carbon cycle of the planet started presumable in the first half of the Palaeozoic Era, when the first vascular plants grew on dry land mass [53,54]. The group of lignocellulolytic enzymes (cellulases [55], hemicellulases [56] and ligninases [57]), which is found widespread in different fungi, e.g., *Trichoderma reesei* (ascomycetes), *Phanerochaete chrysosporium* (white-rot) and *Fomitopsis palustris* (brown-rot) (both basidiomycetes phyla), comprises both cellulose and lignin degrading enzymes. Organisms such as, e.g., *Ceriporiopsis subvermispora* [58], *Phlebia spp.* [59,60], *Physisporinus rivulosus* [61] and *Dichomitus squalens* [60] do exist that are specialised only on lignin degradation, as are pathogenic species such as *Fusarium solani f. sp. glycines* [62] as well.

Only lignin degrading enzymes occasionally termed ligninases are laccases (phenol oxidases) and peroxidases (lignin peroxidase (LiP), manganese peroxidase (MnP) [53,63]. Noteworthy, oxidative degradation of lignin using laccases is effectuated by additional use of small molecules, so-called mediators, that serve as the actual substrates for the laccases, and then move in activated state into the lignocellulosic network where they attack the lignin structure as such.

In course of the emerging efforts to substitute fossil-based materials with more sustainable alternatives, both the biotechnological valorisation of lignin using some of the enzymes just mentioned as well as its biodegradation are intensively studied. The interested reader is encouraged to consult the recent literature on lignin degradation for further information, e.g., on mechanistic details [64–67].

4. Elucidating Structural Features in Lignin

Independent of the lignin-type as such, a full structural elucidation is only possible by using a combination of different types of analyses techniques. The traditional C9-formula of lignins, determined via elemental analysis, represents just one key-figure to characterise lignins. Systematic degradation and chemical modification using solvolytic methods such as acidolysis [68] and thioacidolysis [69], or oxidative methods such as ozonolysis [70,71] and the alkaline nitrobenzene oxidation [72] contributed to determine structural features and compositions of lignins in terms of the H/G/S-ratios etc. Acidolysis and thioacidolysis target interunit bonding motifs and yield different C9-fragments that allow analyses and thus inference to the structural motifs originally present in a lignin sample. While the parent method acidolysis uses aqueous hydrochloric acid for degrading polymeric structures, thioacidolysis uses thioethanol in the presence of Lewis-acidic boron trifluoride etherate. By now, thioacidolysis has become *the* solvolytic method used for the structural determination of lignins due to an overall superior performance in terms of yields [27,73], and due to the possibility to combine it with a preceding alkylation in order to deduce additional information regarding the degree of etherification. Typical structural motifs obtained in thioacidolyses of lignins are depicted in Figure 5A.

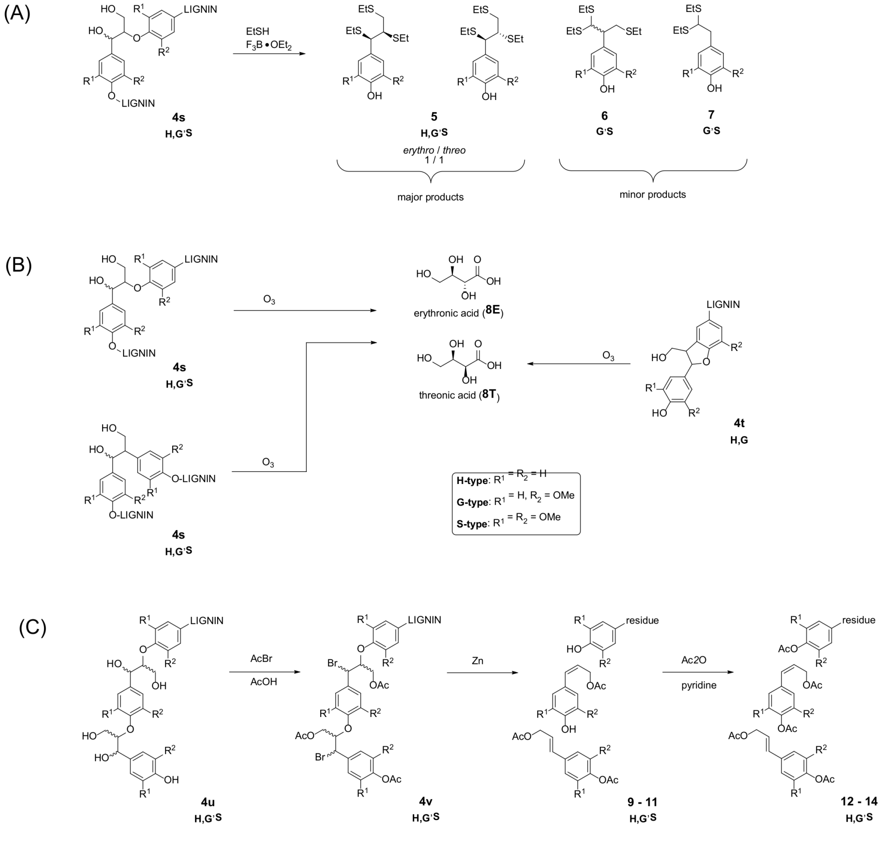

Figure 5. (**A**) Conversion of the most common interunit bonding motif arylglycerol-β-aryl ethers (β-*O*-4′) upon thioacidolysis. (**B**) Conversion of arylglycerol-β-aryl ethers (β-*O*-4′), diarylpropane (β-1) and phenylcoumaran (β-5′) motifs upon ozonolysis. (**C**) Conversion of arylglycerol-β-aryl ethers (β-*O*-4′) within the DFRC procedure.

Figure 5B shows the formation of erythronic acid and threonic acid as result of ozonolytically degrading the aromatic rings and other unsaturated moieties in lignin, e.g., stilbenes, while leaving the side-chains intact [13,74,75]. It has been shown that a skilful ozonolysis enables identification of interunit bonding motifs and, in addition, determination of the ratio between *threo*- and *erythro*-configured aliphatic moieties in lignins, which can give rise to stereochemical considerations.

In Figure 5C, the chemical transformations during a 'derivatisation followed by reductive cleavage' (DFRC) treatment of lignin are shown [76]; again, β-aryl ethers react preferentially to induce defined fragmentation for analysis. Important structural findings were possible using the DFRC method, particularly noteworthy is the fact that it is possible to proof the aforementioned racemic nature of lignin due to the mild chemistry that constitutes this method. This method also serves in combination with non-destructive advanced nuclear magnetic resonance (NMR) spectroscopy on lignins—excellent overviews have been written recently in Ref. [4] regarding NMR studies on lignins—and it can be used to determine whether a given lignin represents a linear or crosslinked polymer [22,28].

Combinations of refined degrading methods such as thioacidolysis and DFRC with non-destructive analysis tools such as NMR spectroscopy are used by default nowadays in lignin analysis. The most common, since most easily to be performed techniques comprise (i) size-exclusion chromatography for the determination of molecular mass key figures, such as the mean molecular weight, the weight-average molecular weight and the polydispersity index [77]; (ii) vibrational spectroscopy, especially Fourier-transform infrared (FT-IR) spectroscopy for delineating functional groups present in a lignin sample, albeit its drawback that it does not easily allow for quantification [78]; (iii) difference spectroscopy for the quantification of phenolic groups, ethylenic double bonds, non-condensed phenolic groups and phenylcoumarans, [79–81]; (iv) quantitative ^{13}C NMR for the elucidating the amount of methoxy groups, etc. [4,82,83]; (v) two-dimensional heterocorrelated NMR such as ^{1}H-^{13}C HSQC for determining interunit bonding motifs [4]; (vi) quantitative ^{31}P NMR on phosphitylated samples to determine both the nature and the abundance of the interunit bonding motifs [4,84]; (vii) electron paramagnetic resonance (EPR) or electron spin resonance (ESR) spectroscopy for qualitatively and quantitatively elucidating unpaired electrons [85]; and (viii) mass spectrometry, especially newer MALDI-TOF MS/MS methods, that seem to be capable of determining structural aspects and molecular mass key figures more accurately [36,44].

Although FT-IR spectroscopy is quick and informative as such, and albeit the UV-based determination of especially the phenolic end-groups is quick and often practically useful, overall superior results are obtained using NMR spectroscopy-based analysis methods, since NMR spectroscopy allows obtaining both detailed structural information and quantitative data. The main difficulty hampering the analyses using NMR methods is the low solubility of most lignins in the typical NMR solvents such as deuterated chloroform ($CDCl_3$) or deuterated dimethyl sulfoxide (DMSO-d_6). Solubility issues can be circumvented by carefully acetylating the lignin prior to analyses [22,86], accepting the fact that the observed shifts are affected by the presence of the introduced acetates. Figure 6A shows a quick-quantitative ^{1}H-^{13}C-HSQC (QQ-HSQC) spectrum of acetylated Norway spruce MWL in DMSO-d_6 [86].

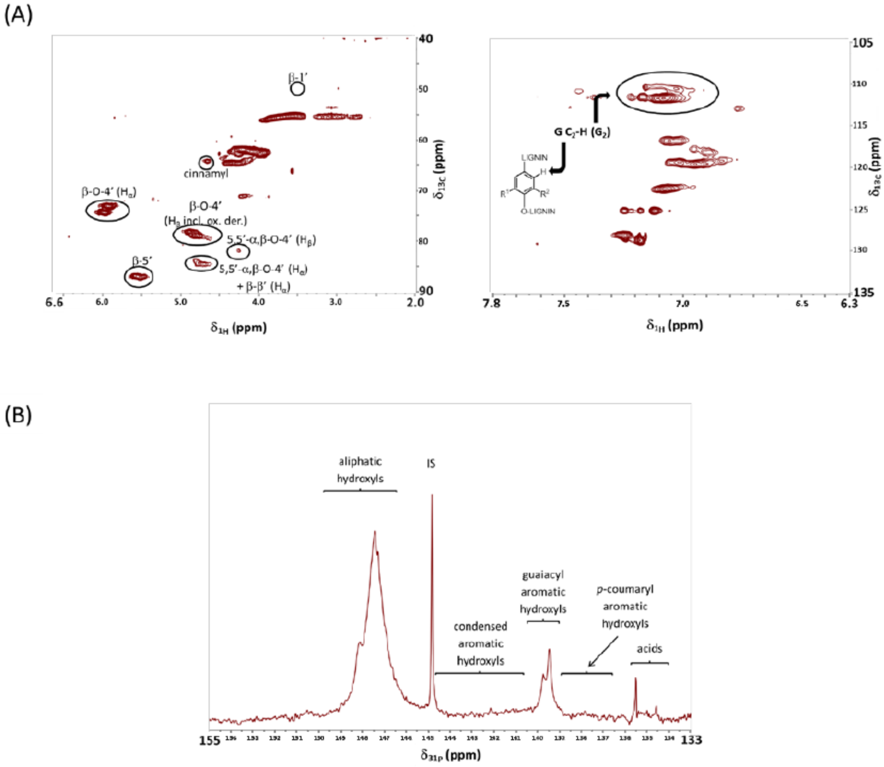

Figure 6. (**A**) Quantitative two-dimensional ^{1}H-^{13}C NMR (HSQC$_1$) of acetylated Norway spruce MWL in $CDCl_3$, aliphatic region (left) and aromatic region (right) [86]. (**B**) ^{31}P NMR of a phosphitylated sample of Norway spruce MWL in $CDCl_3$ in the presence of cholesterol as internal standard.

Different possibilities for the acquisition of quantitative HSQC spectra exist; often the available NMR equipment dictates the method that can be practically employed. In case a high-field (400 MHz or more) NMR machine equipped with a cryo-probe is available, either a quick-quantitative HSQC (QQ-HSQC) pulse sequence [87], or an approach consisting of a series of HSQC-measurements with incremented repetition times and mathematical backward-extrapolation, called $HSQC_0$ [88], can be adopted for the acquisition of lignin spectra [86]. While the results are comparable, the QQ-HSQC method has the drawback of being more difficult to be implemented, while the $HSQC_0$ method is significantly more time-consuming, and bears an additional error source due to the necessary extrapolation. In any case, quantification is achieved based on the intrinsic standard represented by the distinct shifts of the aromatic hydrogen atoms in 2-position, which can be easily detected, summed up, and used as reference. It is needless to say that the two-dimensional analyses should be accompanied by quantitative one-dimensional ^1H and ^{13}C NMR data. For obtaining these in case of lignins, again a careful adjustment of the pulse sequences and internal standards, especially in case of ^{13}C NMR, is necessary for obtaining viable data sets. Just as in case of the two-dimensional NMR studies, eventual solubility issues are conveniently evaded by working with acetylated samples [89].

Using an acetylated sample makes it impossible to determine the degree of natural esterification in the lignin sample. This characteristic number can be determined, however, by analysing the lignin sample before and after basic hydrolysis comparing the quantities of free hydroxyl groups. Both the number and the nature of free hydroxyl groups can be determined conveniently using ^{31}P NMR spectroscopy on phosphitylated lignins in the presence of an internal standard [84]. Figure 6B shows the phosphitylation reaction with 2-chloro-3,3,4,4-tetramethyl- 1,3,2-dioxophospholane [90] and a representative ^{31}P NMR spectrum of phosphitylated Norway spruce MWL in the presence of cholesterol as internal standard using a suitable pulse sequence for obtaining quantitative results. The different chemical environments around the phosphorous atoms lead to characteristic shifts, allowing for the differentiation and quantification. Typical ranges for the different groups distinguishable on the bases of the aforementioned phosphorous reagent are indicated in Figure 6B. During the phosphitylation, solubility improves significantly; for solubilising tenacious samples, ionic liquids can be used as solvents for ^{31}P NMR spectroscopy [91]. This quantitative analytical technique, perhaps the fastest, simplest and most reliable one for getting significant structural information about polyphenolic material, as withnessed by the huge amount of citations, is nowadays routinely used in many laboratories.

An alternative ^{31}P NMR-based protocol has been developed for the determination of quinones [92,93]. The determination of free radicals in lignin model studies was possible using a ^{31}P-containing spin trap reagent, such as, e.g., 5-diisopropoxyphosphoryl -5-methyl-1-pyrroline -*N*-oxide (DIPPMPO) [94,95]; these studies gave valuable insight in the mechanisms underlying enzymatic lignin degradation.

The phosphorous marker-based heteronuclear NMR spectroscopy is just one example for the use of non-lignin-inherent, NMR-active elements for the structural elucidation of lignins. While ^{31}P NMR is by far the most prominent one, ^{19}F NMR is used in order to identify functional groups via their interconversion into ^{19}F marker-containing derivatives; most often, ^{19}F NMR is used for the determination of carbonyl groups in lignin, via the in situ formation of trifluoromethyl ethers [96,97].

Much less commonly used heteronuclear NMR methods for lignin utilise ^{28}Si or ^{199}Hg NMR spectroscopy, in order to elucidate hydroxyl functionalities (^{28}Si) [98], and aromatic substitution patterns (^{199}Hg) [99]. ^{15}N NMR is less important for analysing lignins due to lack of nitrogen functionalities in standard lignins [100].

Analyses of the aforementioned mono- and two-dimensional NMR spectra is only possible based on extensive studies using monomeric, dimeric, and trimeric lignin models, which continuously help improving structural elucidation of lignins. In this respect it is important to identify natural impurities in lignins, such as tannins and lignans, which due to their chemical similarity cannot be always easily removed completely from isolated lignin.

Determining the key molecular weight figures of lignins can be as challenging as the determination of the structural motifs. Initial efforts used techniques such as vapor pressure osmometry [101] or cryoscopy [102], which exploit colligative properties of the polymers to determine average molecular weights of lignin. It was found, however, that these methods are too susceptible to influences emerging from lignin-solvent interactions.

A seemingly more promising way is the use of size exclusion chromatography (SEC) in form of gel permeation chromatography (GPC) [77,103–105]; however, this method is suffering from problems emerging from the diversity within the chemical structure of lignins: the structural differences between different lignins require theoretically a set of tailor-made standards for achieving a calibration of the SEC set-up that fits the characteristics of the lignin analyte. This is, however, simply not feasible. Efforts have been made in the past for estimating the error that is created when a standards made from a polymer such as polystyrene are used, in combination with only a number of synthetically derived lignin models, but this universal calibration did not lead to significantly superior results than a calibration only based on polystyrene standards, thus indicating that differences in the hydrodynamic volume of the two polymers cannot be the only issue to be considered [106]. Additional general problems arise from the low solubility of most lignin types in the solvents that are commonly used for SEC/GPC analyses, which makes it necessary to derivatise the sample; common derivatisations are acetobromination [107] or acetylation (and thus the same ones used for structural analysis via NMR), which cause ultimately the same structural changes discussed before [76]. Different detector-types are known to lead to different results: commonly used UV-based detectors and refractive index detectors were shown to perform less well compared to molecular weight sensitive detectors based on viscosimetry [108], or laser light scattering detectors [109–113]. Technical advances in the field of mass spectrometry made it possible to use MALDI-TOF mass analysis techniques for the determination of molar masses of lignin. Since this method should be independent from solubility issues and structural aspects, results are expected to be more accurate [114].

5. Mechanical and Thermal Properties Lignin

The interesting properties of lignin from a material's point of view are extensively investigated, and numerous efforts aim at exploiting mechanical and thermal characteristics in industrial applications [115]. With respect to such industrial applications, aspects such as flowability and processibility become important. The differences in structural characteristics among different lignins as discussed above naturally result in different macroscopic properties. As outlined in more detail above, isolated lignins display a rather broad distribution of chain lengths compared to other natural and synthetic polymers. The combination of these lignin-specific distribution of polymer-sizes and thus polymer characteristics, in addition to the presence of a large number of reactive functional groups and free radicals, makes it difficult to design general processes for lignin valorisations, especially with respect to the thermal behaviour of the lignin in the process. Several methods give insight into molecular motion and thermal stability of a polymer and to determine characteristic key figures such as the glass transition temperature (T_g) and the decomposition temperature. Thermal gravimetric analyses (TGA), differential scanning calorimetry (DSC), and dynamic mechanical analysis (DMA) / dynamic rheology are commonly used for determining these key figures, especially in studies aimed at tailoring lignins by fractionation as discussed below. Due to its structural and chemical characteristics, heating of lignin is accompanied by vaporisation of volatiles (including the water that might be more or less strongly bound to the hydroxyl groups), chemical reactions and thermal degradation, and thus results in a weight loss so, that TGA is most suitable for determining the physico–chemical properties of lignin with exception of T_g. Interpretation of TGA spectra of lignins, however, remains tedious, since the complexity of the natural polymer does not allow for quick conclusions. DSC represents the easiest way to get hold of T_g, but is often seen as being too simple as method, since non-negligible degrees of crosslinking—effectuated by radical combination reactions—have been observed during the initial DSC scan when temperatures are reached that are close to the T_g of the lignin, resulting in augmented molecular weights and polydispersity, so that the measured T_g is nothing but an artefact. These issues can be avoided using DMA analysis; T_g is determined here based on the analysis of the temperature-dependent evolutions of the storage (G′) and the loss (G″) moduli. As a general trend for a homogeneous series of lignin samples, i.e., a series of different batches of the same lignin, e.g., a Kraft lignin, it is found that T_g increases with increased molecular weight.

The successful correlation between molecular weight and key figures of the thermal stability seems to sustain efforts that aim at 'tailoring' a given lignin sample by simple fractionation in batches of narrower molecular weight distributions [116]. These fractionations can be done using consecutive rounds of extraction with organic solvents [117–119], fractionated precipitation [23,120–122], and ultrafiltration [123–128]. Most noteworthy is, however, the fact that it remains difficult to strictly correlate *structural* features with physico-chemcial characteristics of the fractionated lignin samples even in case of successful fractionations.

A series of studies investigated the processibility of kraft lignin in extruder-based applications: while the original kraft lignin used in the study proofed to be useless due to its thermal characteristics, a targeted functionalisation or a simple specific blocking of the phenolic hydroxyl groups, e.g., via formation of methyl ethers, led to a significant improvement of the thermal stability [129–132].

6. Chemical Valorisation of Lignin

Chemical valorisation of lignin is difficult. The structural features and the dense distribution of functional groups present a chemical challenge for 'synthesising' lignin-based materials. The situation is additionally complicated by the low solubility of the polymer and the tedious analytics that are necessary to validate any structural change beyond the simple conversion of the different hydroxyl groups. Nevertheless, the need for sustainable and biodegradable materials justifies the numerous efforts that were made and are still being made. In the context of this book chapter, the aim of the following paragraphs can thus only be to give a general overview and to point to the references that allow further insight.

Generally speaking, oxidative valorisation of lignin aims at the production of more complex aromatic compounds, and platform chemicals for the industrial production of fine chemicals. Reductive degradation, in contrast, aims at a general reduction of functionality and at the formation of simple bulk aromatic compounds or less defined small molecules for bio-oils or fuels.

7. Oxidative Modifications

Oxidative valorisation of lignin can be achieved by different means that range from purely chemical ones to biotechnology. These routes have recently been reviewed in detail [45,133], but shall be mentioned here as well in a wider context.

7.1. Metal-Free Oxidations

4-acetamido-TEMPO (5 mol %; TEMPO = 2,2,6,6-tetramethylpiperidine-*N*-oxyl) proofed to be a versatile catalyst when used in combination with HNO_3 and

HCl (10 mol % each) for the chemoselective aerobic oxidation of secondary benzylic alcohols within lignin model compounds and lignins [134]. The resulting aryl ketones can then serve as substrates for further catalytic cleavages; this step can be done photolytically, as has been demonstrated recently [135].

7.2. Organometallic Catalysts

One of the structurally least-complex metal-based catalysts for activating both molecular oxygen or hydrogen peroxide is methyltrioxo rhenium (MTO) [136,137]. Two peroxorhenium intermediates, a mono-peroxo η^2-complex ([MeRe(O_2)O_2] (**15**)), and a *bis*-peroxo η^2-complex ([MeRe$(O_2)_2$O] (**16**)) are involved in the activation of hydrogen peroxide (Figure 7A) [138]. In order to improve, and fine-tune catalyst performance, polystyrene or poly(4-vinylpyridine) beads have been used as support, or as material for encapsulation in order to immobilised MTO (Figure 7B). Oxygen transfer from these peroxo-complexes to the substrate occurs via a concerted mechanism that includes a butterfly-like transition state, **17**, which prevents the formation of intermediate radical species (Figure 7A). The concerted mechanism makes MTO a rather unique reagent for the oxidative valorisation of lignin, since it allows a more controlled chemistry by suppressing the presence of various species of very reactive oxygen-based radicals in the reaction mixture. MTO-activated hydrogen peroxide proofed to be a versatile catalyst for various lignin samples; its activity was explicitly shown for lignin model compounds, including challenging substrate such as activated phenols and methoxybenzenes [139–141]. The polymer-supported variant oxidised both phenolic and non-phenolic lignin model compounds, and due to the lowered Lewis acidity of the MTO catalyst upon immobilisation, also aliphatic groups can be oxidised while side-reactions targeting the aromatic rings and leading to over-oxidation can be suppressed.

Cobalt salen ([Co(salen)]) complexes (**21**) were shown to be stable in aqueous reaction media [142] and could thus be successfully exploited in oxidative lignin transformations. The oxidation proceeds mechanistically via the initial formation of a phenoxy-radical, which reacts with molecular oxygen to ultimately form oxidised lignin derivatives and models (Figure 7B) [143–146]. More recently, a bulky heterocyclic nitrogen base was incorporated in a new Co-containing Schiff base-based catalyst which was able to convert both S- and G-type lignin models in high yields; the conversion of S- and G-type structures is essential for an effective use of lignin as a chemical feedstock, and remained elusive before using other [Co(salen)] complexes [147].

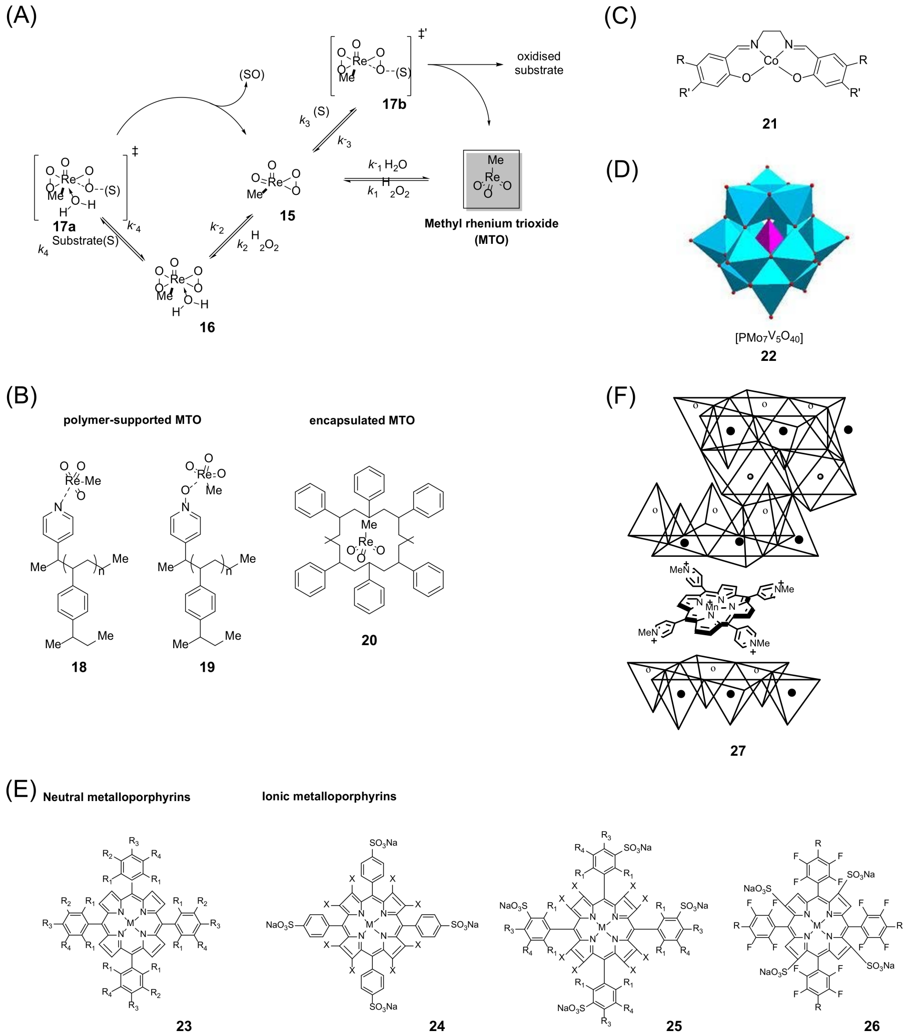

Figure 7. Organometallic catalysts for the oxidative valorisation of lignin: (**A**) Methyltrioxo rhenium (MTO) and mechanistic details of radical oxidation. (**B**) Polymer-supported versions of MTO. (**C**) Polyoxometalate (POM). (**D**) General structure of salen complexes. (**E**) Neutral and ionic metalloporphyrins. (**F**) Schematic illustrating a polymer-supported metalloporphyrin. For details and references, see main text.

Keggin-type polyoxometalates (POMs, **22**) (Figure 7C) with the general formula $[PM^1_xM^2_{12-x}O_{40}]$ can activate hydrogen peroxide and molecular oxygen for the oxidative valorisation of lignin and its various model compounds. Different protocols, including the catalytic use of POMs have been developed as "green" alternatives for the oxidative valorisation of lignin [148–151]. A couple of dedicated review articles exist in the archival literature regarding this topic [149,152]. Phenolic lignin model compounds were shown to be oxidised by C-C bond cleavage between the aryl ring and the adjacent hydroxy-substituted carbon atom using vanadium catalyst **45** (Figure 7) [153]. Labelling experiments indicated key mechanistic differences that serve to explain the differences in activity with respect to a different vanadium catalyst that favoured C-O bond cleavages [154].

The performance of the different catalysts with respect to the most common interunit bonding motif aryl glycerol-β-aryl ethers (β-*O*-4′) is shown in Figure 8.

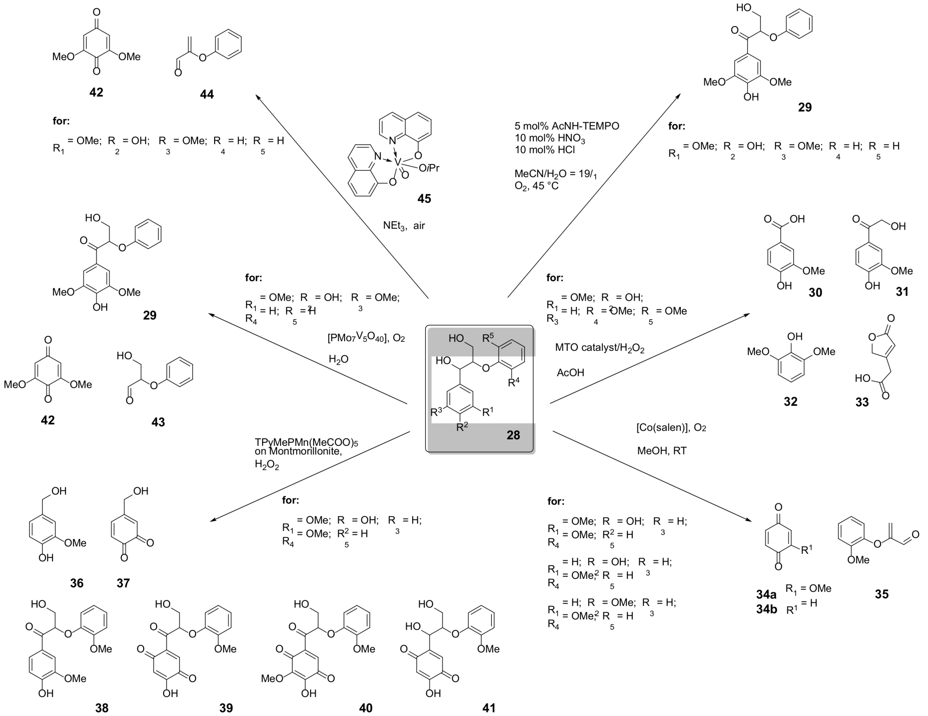

Figure 8. Conversion of the most common interunit bonding motif aryl glycerol-β-aryl ethers (β-*O*-4′) upon oxidative treatment with different organometallic catalysts and under metal-free conditions. References are given in discussion in the main text.

7.3. Biomimetic Catalysts

Synthetic metalloporphyrins represent biomimetic systems for both LiP- and MnP-containing highly oxidised metallo-oxo species similar to those found in, e.g., LiP I and LiP II [53]. Highly functionalized porphyrins such as those shown as structures **23–26** in Figure 7, bearing aryl substituents in the *meso*-positions of the heme motif, represent tuneable catalyst systems that oxidised both lignin model compounds and lignin. In contrast to the enzymes using the heme-type metal complexes, the metalloporphyrins can tolerate an excess of hydrogen peroxide, and are thus exhibiting a higher industrial potential. This feature is further improved by immobilising these catalysts on solid supports such as silica gel [155], naturally occurring clays [156], or artificial polymers [157], while maintaining effectiveness using mediators. Important findings have been reviewed before in dedicated articles [158,159].

7.4. Biotechnological Approaches

The industrial use of lignolytic enzymes that were mentioned above in course of the biodegradation of lignin has recently been discussed [160]. Nevertheless, for the sake of completeness, the most important ones shall be briefly discussed here as well.

The multicopper oxidase laccase (EC 1.10.3.2) oxidises suitable substrates under concomitant reduction of oxygen to water. Radical cationic species are generated starting from a phenolic substrate within an outer-sphere electron-transfer mechanism. Subsequent deprotonation then yields a phenoxy radical, which initiates the depolymerisation of lignin following an *exo*-depolymerisation mechanism [63,161–163]. Whether a given substrate is oxidised by laccases is not only a question of steric matching according to the oversimplified lock-and-key model, but also a question of oxidation potential. Although different laccases are known that exhibit very different redox potentials, ranging from $E^\circ = 0.43$ V (tree laccase from *Rhus vernicifera*) to 0.78 V (fungal laccase from *Polyporus versicolor*) [164], a laccase suitable to oxidise blocked phenolic substrates has not yet been identified. However, a combination of laccases and a radical mediator species, such as 1-hydroxybenzotriazole (HBT) [165], *N*-hydroxyacetanilide (NHA) [166], violuric acid or 2,2′-azinobis-3-ethyl-benzthiazoline-6-sulfonate (ABTS), respectively, can serve to oxidise blocked phenolic substrates in an 'indirect' way (Figure 9B) [167,168]. Noteworthy, initial studies indicated that the use of mediators cannot only boost activity as a whole, but also holds the possibility of actively influencing the mechanistic pathways, and thus controlling also product distributions. (Figure 9A): oxidation presumably does no longer occur via an electron transfer process in these cases, but via a hydrogen atom abstraction process [169].

Manganese peroxidases oxidatively depolymerise lignin under concomitant reduction of molecular oxygen or hydrogen peroxide to water [170]. Activation of molecular oxygen or hydrogen peroxide is achieved via a two-step process, in which an iron protoporphyrin IX activates the oxidant first for the oxidation of the manganese co-factor from Mn(II) to Mn(III) [171]. Carboxylic acid anions then chelate the Mn(III)-centre, forming a small, freely diffusible species functioning as oxidant for depolymerizing oxidatively lignin and lignin model compounds—comparable to the activated mediator in the laccase-mediator systems [172,173]. The reactive manganese species is rather selectively attacking methyl and methylene groups in *para*-position to the phenolic hydroxyl-groups in lignin. Analyses of the residual lignin suggest an *exo*-depolymerisation mechanism.

Large scale industrial use of the aforementioned enzymes, but not only of these enzymes, is, however, still a problem, with noteworthy exception of laccases. Drawbacks are related to the costs connected to enzyme production, the industrial use including necessary recovery steps, and eventual substitution of used enzymes due to inevitable loss of activity upon consecutive use [174]. Support of enzymes turned out to be a suitable mean to render them more applicable in industrial transformations. In this respect, both the immobilisation and the encapsulation of laccase and other enzymes, either alone or in combinations, have been reported [175–185]. The combination of enzymes in connection with the effect of the solid support on their mechanism is another versatile option for directing reaction outcomes [186].

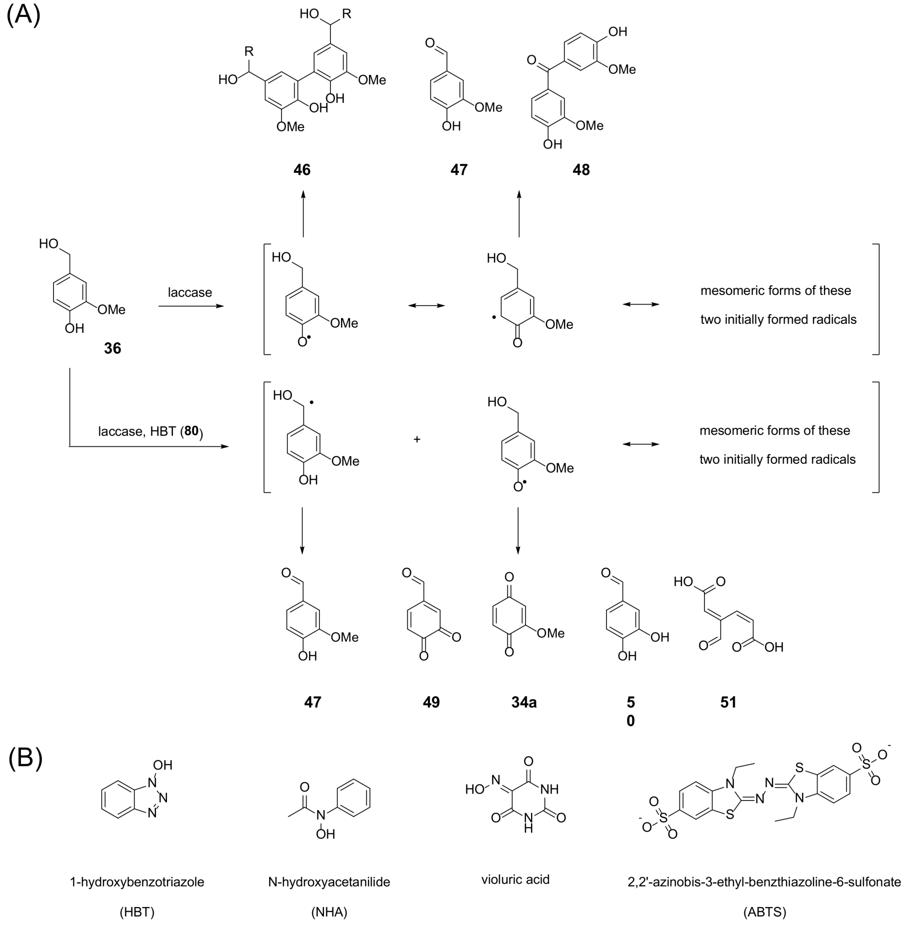

Figure 9. (**A**) Mechanistic implications of the use of mediators. (**B**) Common mediators.

7.5. Reductive Modifications

Reductive modification of lignin has been a research subject for decades [187], and due to the aforementioned rather destructive nature of this method, only a few recent examples are listed here, mainly for the sake of overall completeness of this chapter. It shall be noted explicitly, that a reductive valorisation of lignin is suitable only for the production of low-value applications, since the concomitant removal of versatile functional groups does not go together with the generation of functionalised materials that offer interaction points with other materials [154,188].

7.6. Metal-Free Reductions

Hydrosilanes were shown to convert aryl ethers to hydrolysable silyl ethers in the presence of $B(C_6F_5)_3$ in a general process that was used to convert model lignin compounds to both aryl silyl ethers and alkanes.

Softwood lignin itself could only be partially solubilized (30%) but hardwood lignin was efficiently reductively decomposed by the mono-functional hydrosilane $HMe_2SiOSiMe_3$ [189].

Lignin could be essentially entirely dissolved under moderate temperatures and pressures using ethanol/water mixtures. The molecular weights of the dissolved lignins were shown to be reduced (GPC and NMR studies) [190].

7.7. Heterogenic Transition Metal Catalysts

Cleavage and hydrodeoxygenation (HDO) of carbon-oxygen bonds in lignin model compounds were achieved using a Pd/Zn synergistic catalysis at high temperature (150 °C) and high hydrogen pressure (20 bar) (Figure 10A) [191]. The catalyst system showed a high selectivity and was recyclable. Tests on whole lignins, however, were not reported.

A heterogeneous nickel catalyst for the selective hydrogenolysis of aryl ethers to arenes and alcohols generated in situ from soluble nickel precursor $Ni(COD)_2$ or $Ni(CH_2TMS)_2(TMEDA)$ (COD—cyclooctadiene; TMS—trimethylsilyl; TMEDA—tetramethyl ethylendiamine) in the presence of a base additive (Figure 10A) [192]. The catalyst selectively cleaves C-O bonds in aryl ether models of lignin without hydrogenation of aromatic rings, and it operates at loadings down to 0.25 mol % at atmospheric hydrogen.

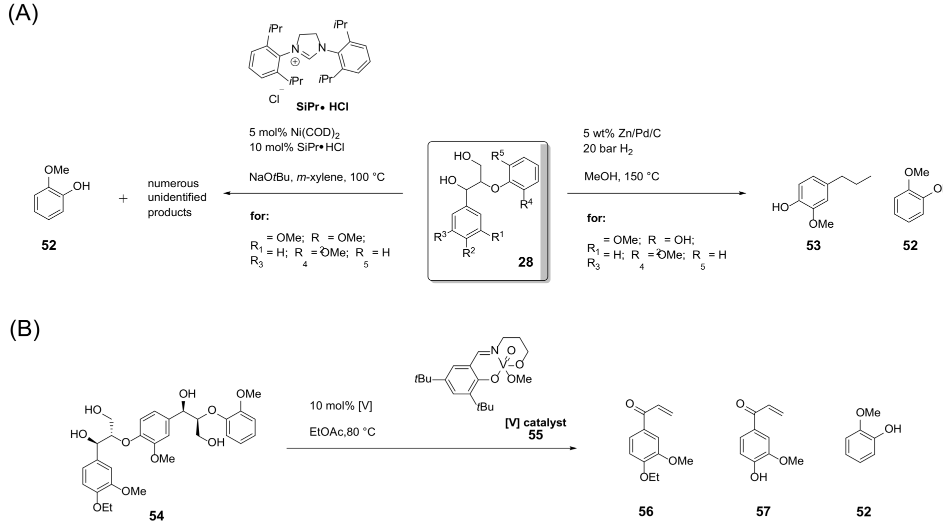

Figure 10. (**A**) Conversion of the most common interunit bonding motif aryl glycerol-β-aryl ethers (β-*O*-4′) upon reductive treatment with different organometallic catalysts. (**B**) Vanadium-catalysed reductive cleavage of a trimeric lignin model. References are given in the discussion in the main text.

7.8. Organometallic Catalysts

The conversion of lignin into low-boiling arenes instead of high-boiling phenols was realised by depolymerising lignin under simultaneous conversion of phenols into arenes using a tandem reaction catalysed by a tailored nickel-centred soluble organometallic catalyst [193]. Earlier studies using homogeneous Ni-catalyses for these purposes were not tried on lignin itself [194]. Another earlier study accomplished roughly 40 years ago on lignin model compounds used a homogeneous nickel–hydride complex [188]; selectivity in this system depended highly on the substrate specificities.

A vanadium species has also been reported to reductively degrade lignin by selectively attacking the C-O bonds of the aryl ethers forming the lignin backbone (Figure 10B) [154,195]. The studies were accompanied by a detailed structural analysis of the lignin that remained after catalysis: data confirm the selectivity of the catalyst. The experiments did reveal, however, that also the history of the lignin has an influence on the effectiveness of the transformation.

8. Applications of Lignin

A huge amount of applications of lignin exist and have been reported in both academic publications and patents, and recent reviews and monographs give excellent overviews [196]. Reported applications range from easy-to-be-realised uses of lignin as, e.g., filler, to more advanced applications in which the properties of lignin are used more distinctively. Industrial use of lignin, however, also includes its targeted destruction, as, e.g., for the production of bulk chemicals such as DMSO or vanillin [197,198]. Possible routes for the conversion of lignin to platform chemicals for the production of fine chemicals have been already outlined in the section on the chemical valorisation of lignin. In the following paragraphs, the use of lignin in low cost applications is just briefly touched, and more emphasis is placed on the use of lignin in nanomaterials and in pharmaceutical applications.

8.1. Lignin as Bulk Material in Co-Polymer Applications

Lignin has been used as low cost hydroxyl component for the formation of polyurethanes (PU) and polyesters (PE) [199–201]. Given the structural differences of various lignins, different qualities of PU are available for different applications [202–205]. As different lignins come with different distributions of aliphatic vs. aromatic hydroxyl groups, different chain lengths, etc., their use as component in polyurethane formation requires optimisation and careful PU characterisation. Generally, lignins represent the less elastic part of a PU.

Since lignin carries alcoholic and acidic functionalities at the same time, it has been used for the formation of PE [206], using application-depending stability-introducing acyl chlorides. It is obvious that the application will ultimately dictate which lignin is the most suitable one, but current research results underline generally the suitability of lignin as polymeric component for PEs [199].

Oxypropylation of lignin is used to adjust molecular weight characteristics of a lignin sample, and thus also to adjust the characteristics of a copolymer in which this modified lignins are incorporated, e.g., PU and PE formations [207,208].

Other polyblends incorporating lignin have been extensively studied, and were recently concisely reviewed [209]. Among them are phenol-formaldehyde adhesives [210]. The incorporation of lignin here allowed to reduce the amount of phenol by up to 60%, and to introduce new characteristics into the glue matrix, such as conductivity. Also lignin-epoxy adhesives were realised and studied extensively [211,212].

In order to increase their mechanical strength, light stability and partial biodegradability, polyolefins were blended with lignin [209,213,214]. As for other lignin applications, the quality of the lignin starting material had a great impact on the stability of the blends; different lignin types gave rise to different blends, whose characteristics additionally changed with varying amounts of lignin. UV-absorbing films have been reported, for example [215], as well as low-cost films with up to 50% lignosulfonate content [216].

Poly(vinyl chloride) (PVC) has been blended with lignin, driven by the same ideas of adjusting thermal strength and light stability [217–219]. Although the functional groups present in the two starting materials can easily undergo proton-donor/proton-acceptor interactions, complete miscibility is not automatically granted. Successfully grafted PVC-lignin blends showed an improvement of polymer characteristics in terms of yield and breaking strength.

Other lignin-containing blends have been realised using poly(vinyl acetate) (PVAc), poly(vinyl alcohol) (PVA), polysaccharides, e.g., starch and gums, etc. In all applications, the limiting factor is the solubility and the thermal stability of the lignin used [209].

Another possibility to make use of favourable thermal characteristics of lignin is its incorporation in the production of thermoplastics [129,220].

The mechanical strength of lignin is best exploited in the production of lignin-based carbon fibres, which represent a research object since the first example was reported in 1969 [221]. Lignin-based carbon fibres are obtained, in turn, upon carbonisation of lignin fibres that are formed upon melt-spinning [222,223] or electro-spinning; especially in the latter case, lignins are, however, usually still mixed with other polymeric materials such as polyethylene oxide (PEO or PEOX), [224,225] polyacrylonitrile (PAN) [226–228] or other plasticisers [229,230] to yield significant results. Only few reports exist detailing pure lignin fibers, that could eventually serve as carbon fibre precursors [231,232].

8.2. Applications in Nanomaterials

8.2.1. Capsules

Micro- and nanoparticles, micelles or capsules represent promising structures with respect to pharmaceutical applications. Recently, lignins were used to produce capsules capable of encapsulating either hydrophobic [233,234] or hydrophilic drugs [235]. Lignin capsules for hosting hydrophobic substances were produced by exploiting the amphiphilic character of kraft lignin: when dissolved in oil-in-water emulsions, the lignin polymers preferentially located at the oil-water interface, where they could be further intermolecularly crosslinked to form capsules [233]. This crosslinking was achieved using ultrasonification for radical activation, or by reacting diglycidyl ether-terminated poly(ethylene glycol) with the lignin polymers. The capsules could be filled with hydrophobic molecules, which in turn could be released upon addition of surfactants. The fundamental principle could be extended to other lignin types as well, realising a pH-dependent release mechanism for entrapped actives [234].

When treating lignin-containing aqueous solutions that were emulsified by the aid of a surfactant in an organic phase comprised of cyclohexane containing 2,4-toluene diisocyanate, a lignin-based polyurethane was formed at the interface between aqueous and organic phase [235]. Hydrophilic substances could be encapsulated upon formation, and subsequently released upon enzymatic degradation of the lignin shell.

8.2.2. Nanoparticles

Emulsion-based techniques can also be used to constitute lignin-based beads, simply by dispersing the lignin-containing phase: suspensions of black liquor in a mixture of oil and chlorobenzene in the presence of an emulsifier allowed the polymerisation of the lignin by crosslinking lignin oligomeric and polymeric chains using epichlorhydrin; spherical beads with diameters between 300 and 450 μm are obtained [236]. Less defined bead sizes were obtained when an alkaline lignin solution was dispersed in 1,2-dichloroethane and the lignin intermolecularly crosslinked in the aqueous droplets—either by mediation of epichlorhydrin [237] or by radical polymerisation processes involving acrylic acid moieties that were attached to the lignin before [238]. Attaching further functionalisation to these beads, e.g., sulfonhydrazine groups [237,238], made them attractive for applications in the wine industry, particularly for removing carbonyl-bearing compounds responsible for binding of sulphur dioxide in wines [239]. Lignin predissolved in tetrahydrofurane (THF)-water mixtures that were then poured in an excess of water precipitated in form of stable suspensions of nano-particles [240]; this way of lignin nano-particle generation was used in proof-of-concept studies as dug carriers [241].

Using a solution of lignin in ethylene glycol, the formation of lignin nanoparticles was achieved by gradually acidifying (hydrochloric acid) the solution [242].

A simple physical method based on ultrasonic irradiation can be used to build nanoparticles from lignins such as commercially available wheat straw lignin and Sarkanda grass lignin. It has been shown that the compositional and structural changes of the lignin in the produced nanoparticles were not significantly modified by the intensity of the ultrasound applied, but depended mainly on the nature of lignin itself [243].

When alkaline lignin solutions were added to cationic polyelectrolyte solutions of poly(diallyldimethylammonium chloride) (PDDAC), the cationic polymer was adsorbed on the lignin aggregates due to the display of overall negative surfaces stemming from the alkaline conditions chosen for solubilising the lignin. Similar to the process of creating LbL-protections for enzymes (*vide supra*), the now positively charged surface of the lignin-PDDAC hybrids interacted with negatively charged natural latex rubber particles to associate further in solution, resulting in natural rubber materials reinforced with finely dispersed nano-sized lignin particles. These new lignin-based hybrid-particles are characterised by improved mechanical and thermal properties [244].

Subjection of nanoparticles to crosslinking agents such as glutaraldehyde resulted in nanoparticles that showed useful stabilities over a wide pH-range when re-dispersed in water. Interestingly, the nanoparticles did not display cytotoxic activity when yeast or microalgae were exposed to them. Gradually adding water to a solution of acetylated lignin in THF resulted in the formation of nano-sized colloidal spheres that were water-dispersible and stable up to pH 12 [245]. Higher pH-values unblocked the phenols via hydrolysis of the acetic acid esters, causing higher degrees of electrostatic repulsion that effectively inhibited colloidisation.

8.2.3. Micro- and Nano-Porous Materials

Porous materials called aerogels are seen as promising materials for a range of applications, e.g., insulating material, adsorbents, catalysts, etc. [246,247]. Aerogels are obtainable via a sequence of sol-gel polymerisation of a resin, exchange of solvents and final drying. Most often, the resins used are of polyphenolic type, and as indicated during the discussion of the lower-value applications, lignin has been considered a potential source of phenols for this kind of resins.

Replacing part of resorcinol by lignin is enough for producing resorcinol-lignin-formaldehyde resins in aqueous solutions of sodium hydroxide [248].

Phenol-lignin-formaldehyde and tannin-lignin-formaldehyde resins have been produced using similar formulation approaches [249,250]. The maximum amount of lignin as replacement for the phenolic part could not exceed 50%. In any case, the replacement of simple phenols by lignin led to the formation of macropores (>50 nm) on the expense of mesopores (2–50 nm). Even if this caused an increase in the overall porosity and consequently a decrease in the bulk density, the total surface area was reduced, thus limiting the final material properties, such as the adsorption capacity or the insulating performances [248–250]. Aerogels based solely on lignin crosslinked by means of glycidyl-terminated oligo(ethylene glycol) or (propylene glycol) have been reported more recently [251]. More effective gel formations were observed in case of long-chain crosslinkers, and it was hypothesized that this is due to a more effective linking of lignin chains in this case.

Blocks of porous polymeric lignin-hybrids could be obtained using black liquor directly via the High Internal Phase Emulsion (HIPE) technique [252]. Epichlorhydrin proofed to be useful once more as crosslinker also in the presence of a surfactant: after the addition of oil, the mixture was heated to initiate epichlorhydrin-mediated crosslinking of lignin. Depending on the specifics of the oil and the surfactants used, monoliths of different porosity were obtained, exhibiting medium void sizes between 5 and 20 μm [252].

8.3. Pharmaceutical Applications

With respect to a use in pharmaceutical applications which normally come with significantly higher hurdles concerning safety and biocompatibility requirements, lignin is still under-utilised. New research is following some pioneering achievements, in which lignins have been used (i) as component for semi-interpenetrating networks (hydrogels) [253]; (ii) as anti-oxidative ingredients [254]; (iii) UV-absorbing component in films [255]; and for the generation of capsules for drug delivery approaches [233,256].

Anti-viral activities of lignin derivatives, also against HIV, have been investigated and reported [257,258].

Lignin is considered an interesting material with respect to anti-tumour treatments [259]: in several studies (i) polyphenols with structural motifs found in lignin, (ii) mixtures of the monomers lignin is composed of, or (iii) small aromatic molecules that could be the enzymatic degradation products of lignin were investigated, in order to evaluate the response of tumour development and growth and the effects on underlying and connected signalling pathways to these substances [260,261]. Often, the cytotoxic activity of lignin and lignin derivatives is the result of interactions of lignin with other substances such as ascorbic acid [262], or lignin containing natural complexes such as lignin–carbohydrate complexes (LCCs) [263].

9. Concluding Remarks

Despite great advances in the research on and with lignin, important questions are still unanswered, or have to be revisited in light of new findings. Possibilities do exist to convert lignin in valuable chemicals and materials, ranging from bulk applications to specialised nano-materials. Inclusion of these techniques into industrial processes will be one of the immediate next challenges, and more work is needed to compile a convincing process for lignin valorisation that starts with the biorefinery delivering a quality lignin suitable for further advanced transformations and ends with truly lignin-based, meaning mainly lignin-based high value products [264].

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

Abbreviations

The following abbreviations are used in this manuscript:

MWL	milled wood lignin
KL	kraft lignin
DHP	dehydrogenation polymers
LiP	lignin peroxidase
MnP	manganese peroxidase
DFRC	derivatisation followed by reductive cleavage
UV	ultra-violet
FT-IR	Fourier-transform infrared
NMR	nuclear magnetic resonance
HSQC	heteronuclear single quantum coherence
EPR	electron paramagnetic resonance
ESR	electron spin resonance

DMSO	dimethyl sulfoxide
DIPPMPO	5-diisopropoxyphosphoryl-5-methyl-1-pyrroline-*N*-oxide
TGA	thermal gravimetric analyses
DSC	differential scanning calorimetry
DMA	dynamic mechanical analysis
T_g	glass transition temperature
TEMPO	2,2,6,6-tetramethylpiperidine-*N*-oxyl
MTO	methyltrioxorhenium
POM	polyoxometalates
HBT	1-hydroxybenzotriazole
NHA	*N*-hydroxyacetanilide
ABTS	2,2′-azinobis-3-ethyl-benzthiazoline-6-sulfonate
COD	cyclooctadiene
TMS	trimethylsilyl
TMEDA	tetramethylethylendiamine
PU	polyurethanes
PE	polyesters
PVC	poly(vinyl chloride)
PVAc	poly(vinyl acetate)
PVA	poly(vinyl alcohol)
PDDAC	poly(diallyldimethylammonium chloride)
HIPE	high internal phase emulsion

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