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Waste Biomass Pretreatments for Biogas Yield Optimization and for the Extraction of Valuable High-Added-Value Products: Possible Combinations of the Two Processes toward a Biorefinery Purpose

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Abstract: Second- and third-generation biorefineries enable the sustainable management of biomasses within the framework of circular economy principles. This approach aims to minimize waste biomass while generating high-value molecules and bio-energy, such as biogas. Biogas production is achieved via anaerobic digestion, a process where microorganisms metabolize organic compounds in the absence of oxygen to primarily produce CO$_2$ and CH$_4$. The efficiency of this process is closely linked to the composition of the biomass and, sometimes, characteristics of the initial matrix can impede the process. To address these challenges, various pretreatments are employed to enhance digestion efficiency and mitigate issues associated with biomass complexity. However, the implementation of pretreatments can be energy-intensive and costly. The extraction of valuable molecules from biomass for various applications can represent a form of pretreatment. This extraction process selectively removes recalcitrant molecules such as lignin and cellulose, which can hinder biodegradation, thereby adding new value to the biomass. These extracted molecules not only contribute to improved anaerobic digestion efficiency but also offer potential economic benefits by serving as valuable inputs across diverse industrial sectors. This article presents a detailed state of the art of the most widespread biomass pretreatments and specifies when biomass is pretreated to improve the biogas yield and, in contrast, when it is treated to extract high-added-value products. Finally, in order to define if the same treatment can be simultaneously applied for both goals, an experimental section was dedicated to the production of biogas from untreated olive mill wastewater and the same biomass after being freeze-dried and after the extraction of polyphenols and flavonoids. The use of pretreated biomass effectively improved the biogas production yield: the untreated olive mill wastewater led to the production of 147 mL of biogas, while after freeze-drying and after polyphenols/flavonoids extraction, the production was, respectively, equal to 169 mL and 268 mL of biogas.

Keywords: biomass pretreatments; biogas; biomethane; biorefinery

1. Introduction

Today, it is crucial to apply the principles of a circular economy to manage biomasses effectively. This approach helps mitigate climate change and meet society’s growing demands for materials and energy [1].

Second- and third-generation biorefineries represent a significant advancement over their first-generation counterpart, as they exemplify the seamless integration of energy and
material production within a circular economy framework [2–5]. Biorefinery involves multiple process steps and utilizes various biomass resources, especially residual materials from different industries. This strategic utilization of biomass is crucial for minimizing landfill disposal, especially of organic waste, which poses significant environmental threats [6].

Within a biorefinery, the biomass is fractionated into its intermediates (i.e., proteins, carbohydrates, lignin, and fats) that are then processed into valuable compounds. Then, the residuals can be used for energy production such as bio-CH$_4$, biogas, biohydrogen, and biodiesel [7].

Biogas is produced through anaerobic digestion (AD) of the residual substrate and typically consists of 50–75% CH$_4$ and 25–50% CO$_2$ [8]. The AD process is driven by specific microorganisms under oxygen-free conditions and occurs in different stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis [9]. The composition of the substrate influences the efficiency of each stage, impacting the overall biogas yield, CH$_4$ content, and hydraulic retention time (HRT). Substrates rich in easily degradable compounds like carbohydrates, proteins, and lipids tend to yield biogas more rapidly compared to those containing lignin and lignocellulose, which require longer degradation times [10]. The complexity of the substrate’s structure thus plays a critical role in biogas production within the biorefinery context. For this reason, it is sometimes necessary to modify the structure of the initial biomass through pretreatments, to speed up the entire process increasing biogas yield and reducing the HRT [11]. The aim of pretreatment is to break down the substrate into less complex components and reduce particle size, facilitating microbial and enzymatic conversion by physical, chemical, biological, or combined means [12,13].

It is indeed crucial to evaluate the efficiency of pretreatments in terms of both energy-cost demand and the overall energy balance of the process.

From an AD perspective, the recovery of molecules with economic value within a biorefinery can be viewed as a form of pretreatment because it reduces the complexity of the initial substrate. For example, removing nanocrystalline cellulose can have various applications in polymer design [14], improving the initial substrate for AD as it represents a recalcitrant compound to the hydrolytic activity of microorganisms [15].

This approach integrates pretreatment with the production of marketable molecules, thereby addressing the challenges associated with treatment costs and adding more value to the initial biomass. By optimizing pretreatment methods to enhance the recovery of high-added-value compounds, biorefineries can improve efficiency, reduce energy demands, and increase the economic viability of the entire process [2,16].

This review aims to assess conventional and widely utilized pretreatment methods, alongside alternative green technologies, for extracting valuable molecules. The objective is to integrate both aspects, employing methodologies that serve as pretreatments to enhance biogas yield and bio-CH$_4$ concentration, while also enabling the recovery of marketable materials.

In the last section of this study, experimental outcomes are presented using various fractions of pretreated olive oil mill wastewater (OMWW), subsequently subjected to anaerobic digestion to evaluate biogas production within a biorefinery framework.

2. Pretreatments for Improving the Biogas Production Yield

2.1. Physical Pretreatment

Physical pretreatments lead to a decrease in biomass particle size and crystallinity using mechanical or wave energy. This reduction results in a lower polymerization grade, thereby enhancing the surface/area ratio. Consequently, this increases the contact area available for microorganisms, enzymes, and chemical agents.

This category of pretreatment can be categorized into mechanical, thermal, ultrasonic, and microwave radiation. The main disadvantage of these kinds of pretreatments is the high energy required to carry out the processes, which is usually higher than the theoretical energy content of the biomass [11].
Nevertheless, these methods are straightforward, as they do not involve chemicals and generate less waste compared to other pretreatment options, making them particularly appealing [12].

Mechanical pretreatments are widely employed to enhance the digestibility of raw biomass by acting on the polymerization and crystallinity of lignocellulosic materials. Milling technologies are commonly used as mechanical pretreatments and involve various types of mills as disk mills, planetary ball mills, hammer mills, and knife mills [13].

Qu Y. et al. (2024) [14] showed an increase in CH$_4$ production of up to 104.11% following ball-mill pretreatment of corn straw. This significant increase is attributed to greater volatile solid (VS) removal and cellulose degradation of the initial biomass. Moreover, the pretreatment improved the rheological characteristics of the digestion slurry, reducing the energy needed for stirring and resulting in a positive energy balance.

In another study by Arifan T. et al. [15] on pineapple peel, various mechanical pretreatments were applied, including chopping, blending, and grinding, that reduced the diameter of biomass particles by up to 1 cm, 0.5–1 cm, and 1 mm, respectively. Only the grinder pretreatment led to an improvement in CH$_4$ yield. This explains how the dimension of particles is an essential parameter to consider to improve biogas production. However, none of these pretreatments had any impact on reducing lignin content. Indeed, at temperatures lower than 180 °C without alkaline or strong acid conditions, the lignin degradation is negligible [16]. Mechanical pretreatments are not able to remove lignin and it is necessary to combine different methodologies to reduce its concentration.

Thermal treatments of biomass exploit the impact of heat, pressure, and time to modify the substrate structure, enhancing its biodegradability by breaking down chemical bonds within cellular components and increasing the solubility of the original matrix [17]. Different thermal pretreatments were examined by Saha et al. (2023) [18] in Lantana camara biomass. They subjected the biomass to different thermal treatments, i.e., hot air oven, autoclave, hot water bath, and microwave, applying different combinations of temperature and duration. Among these, the autoclave pretreatment at 110 °C for 80 min proved to be the most effective, resulting in increased CH$_4$ yield and accelerated hydrolysis during the process. In contrast, the production strongly decreased with the same treatment but at 120 °C. Park et al. (2017) [19] applied hydrothermal pretreatments on poultry slaughterhouse waste using a temperature range between 170 °C and 220 °C. The maximum efficiency of the process was obtained at 190 °C, a temperature at which particle size was reduced strongly, from 510 to 100 µm, and biogas production increased. Above 190 °C, no positive effects were evaluated. This can be explained by the fact that temperatures exceeding 190 °C promote the Maillard reaction, which transforms carbohydrates and amino acids into melanoidins, high-molecular-weight compounds that inhibit the anaerobic process [20].

Ultrasonic pretreatments utilize ultrasonic waves to break down cell walls or flocs within biomass [21]. During sonication, microbubbles are formed due to the high pressure applied to the liquid. This continuous compression leads to the implosion of microbubbles, releasing a significant amount of energy into the surrounding space, reaching temperatures of 5000 °K and pressures of 500 bar [22]. This type of pretreatment is commonly employed in wastewater plants for sewage sludge, where the high concentration of microbes is resistant to biodegradation, limiting the efficiency of anaerobic digestion [23].

Buller et al. (2022) [24] studied the impact of ultrasonic pretreatment on brewery spent grain. The biomass was initially sonicated for 1 h at an ultrasonic power of 53 W. The findings revealed a beneficial effect of sonication, leading to increased biogas production and improved biogas quality, with a higher CH$_4$ content. However, ultrasonic pretreatment is associated with high energy consumption, which represents the main drawback.

2.2. Chemical Pretreatment

Chemical pretreatments involve the use of various types of compounds that disrupt the biomass structure by interfering with the intra- and inter-polymer linkages of the
organic components. This disruption increases the biodegradability of the initial biomass but also reduces the hydraulic retention time (HRT) [25]. Examples of these compounds include acids, alkaline, organic, or inorganic compounds as well as ionic liquids.

Alkaline and acidic pretreatment makes lignin more accessible to hydrolytic enzymes, thereby increasing its biodegradability. This is due to the consequent disruption of the cell wall with hydroxyl radicals. The most-used compounds for alkaline pretreatment are sodium hydroxides, ammonia, and calcium hydroxide [26] whereas HCl, H$_2$SO$_4$, H$_3$PO$_4$, and HNO$_3$ are used for the acidic treatments [27]. Compared to acidic treatment, the alkaline one requires less caustic reagent and operates under milder process conditions. In contrast, acidic treatments result in higher process costs due to the expense of recovering the acid used and the need for corrosion-resistant equipment [28].

Reactions during alkaline treatment involve the partial dissolution of lignin and hemicellulose, as well as the saponification of intermolecular ester bonds [29]. This process effectively breaks down and disrupts the complex structure of biomass, making its components more susceptible to enzymatic degradation.

Liew et al. (2011) [30] utilized an alkaline pretreatment employing NaOH to enhance the biodegradability of lignocellulose derived from autumn leaves and to optimize AD processes. Three distinct concentrations of NaOH (2%, 3.5%, and 5%) were employed for this purpose. The most favorable outcomes were achieved with the 3.5% NaOH treatment, demonstrating a substantial increase in biogas production up to 24 times greater than the control condition. Additionally, this treatment resulted in significant degradation of cellulose (36%) and hemicellulose (34.9%), indicating improved accessibility and breakdown of these key components within the lignocellulosic structure. The negative aspect of this kind of pretreatment is that pH adjustments are usually needed after the treatment to reach the optimal pH for AD. This means more chemicals, higher costs, and environmental concerns.

Dai et al. (2018) [31] investigated the effects of alkaline and acidic pretreatments on rice straw biomass, utilizing varying concentrations of NaOH, HCl, and CO(NH$_2$)$_2$. The acidic treatment using HCl resulted in a reduction in hemicellulose content from 12.5% to 7%. Additionally, positive outcomes were observed in the bio-CH$_4$ yield (30 days HRT), showing increases of 157.4%, 4.3%, and 121%, respectively, for NaOH, HCl, and CO(NH$_2$)$_2$ pretreatments.

The utilization of organic components serves as a pretreatment method to enhance the digestibility of biomass. Organic solvents such as methanol, ethanol, ethylene glycol, acetone, or their combinations are employed for this purpose. These solvents can be used in conjunction with high temperatures or catalysts like HCl, H$_2$SO$_4$, oxalic acid, or salicylic acid [32]. Through this method, lignin is isolated from lignocellulosic biomass and can be recovered as a by-product [33]. Mirmohamadsadeghi et al. (2014) [34] conducted a pretreatment study using ethanol with H$_2$SO$_4$ as a catalyst on three different lignocellulosic biomasses: elmwood (a hardwood), pinewood (a softwood), and rice straw. The pretreatment was particularly effective on softwood, showing significant increases in biogas yields of 84%, 73%, and 32% for pinewood, elmwood, and rice straw, respectively.

In chemical pretreatments, ionic liquids are utilized for biomass valorization. Ionic liquids are salts that have a melting point of 100 °C or lower, remaining in liquid form at room temperature. They are particularly effective for degrading cellulose because they can disrupt the hydrogen bonds between different polysaccharide chains, a capability not typically found in common solvents [35]. This reduction in cellulose compactness makes carbohydrates more accessible to microorganisms during the hydrolysis phase.

For instance, researchers [36] achieved positive results using N-methylmorpholine-N-oxide on forest residue and barley straw. They observed a 100% increase in CH$_4$ yield following treatment with this ionic liquid for 3.5 h at 90 °C.

Chemical pretreatments are effective methods for increasing bio-CH$_4$ yield in AD. However, they have certain negative aspects related to the use of chemicals, which can be environmentally unfriendly and quite expensive [37,38], although there are recovery methods that can make the process more sustainable [39–41].
2.3. Biological Pretreatment

Biological pretreatment methods are gaining attention due to their distinctive features. These methods employ microorganisms such as bacteria and/or fungi, in combination with enzymes. The primary goal of these pretreatment approaches is to break down insoluble materials like cellulose or lignin without the need for chemical additives, making them energy-efficient and environmentally friendly [42].

Implementing a two-phase AD system can be considered a form of pretreatment. This approach optimizes the initial stages of AD specifically: hydrolysis and acidogenesis. Enhancing these stages is crucial as it leads to greater breakdown of the initial material, resulting in increased biogas production overall.

In their study, Paranjpe et al. (2023) [43] compared the efficiency of single-step anaerobic digestion with a two-step AD process using a mixed biomass of sewage sludge and food waste. In the two-step reactor setup, mesophilic conditions were maintained throughout both stages but with different pH levels: pH 5–6 for the hydrolysis and acidogenesis phases and pH 6.5–8 for the methanogenic phase.

As expected, the results of the study demonstrated that implementing a two-step AD system led to increased biogas production and higher yield of bio-CH$_4$ compared to the single-step process. Specifically, the two-step system achieved a cumulative biogas yield of 1914.8 mL whereas the single-step process yielded 1349.4 mL of biogas. This significant difference highlights the effectiveness of the two-step approach in enhancing overall biogas production and bio-CH$_4$ yield.

Another option for biologically treating biomass is through anaerobic pretreatment, where a mixed culture of aerobic and anaerobic microorganisms work together to degrade organic substances, producing CO$_2$, H$_2$O, and nitrates. This transformation facilitates easier accessibility to the organic matter, particularly targeting the more recalcitrant components for breakdown. This approach can enhance subsequent treatment processes by making the organic material more accessible and easier to degrade.

Huilinir et al. (2017) [44] employed aerobic pretreatment to enhance CH$_4$ production from sewage sludge. Before the AD, they subjected the sludge to 48 h of aerobic pretreatment, maintaining an airflow of 0.35 vvm at 35°C. This procedure resulted in an approximately 30% increase in bio-CH$_4$ yield.

Other pretreatment methods involve the use of enzymes to enhance the hydrolysis rate during AD. The hydrolysis phase is crucial for the effectiveness of AD, as during this phase, proteins, lipids, and carbohydrates are broken down into smaller molecules, making them available for subsequent steps such as acidogenesis, acetogenesis, and methanogenesis. During AD, specific anaerobic microorganisms like Streptococci, Bacteroides, Clostridia, and Enterobacteriaceae produce essential enzymes such as lipases, proteases, and amylases [45]. Nevertheless, to enhance the efficacy of this phase, specific enzymes can be supplemented directly into the biomass. To support this, Kendir Çakmak et al. (2020) [46] investigated the effect of enzymatic pretreatment on an initial biomass of Porphyridium cruentum, a red microalgae, on biogas production. They utilized a protease and an enzyme mix, which resulted in a 33% and 30.5% increase in biogas production, respectively. Under protease treatment, they applied both mesophilic and thermophilic conditions, achieving 77% and 100% improvements in bio-CH$_4$ production, respectively. Unfortunately, the high cost of enzymes poses a significant challenge that needs to be addressed in order to make this pretreatment viable at an industrial scale [47].

As mentioned previously, fungi can be employed in biomass pretreatments, especially for lignin degradation. One advantageous aspect of using fungi is that they do not require chemicals or high-energy inputs. These fungi belong to various groups, including white-rot, brown-rot, and soft-rot fungi. White-rot fungi, in particular, are well known for their selective degradation of lignin [48] due to the secretion of ligninolytic enzymes such as lignin peroxidase, manganese peroxidase, and laccase [49]. In their study, Alexandropoulou et al. (2017) [50] evaluated lignin degradation and the impact on bio-CH$_4$ production of fungal treatments using two different fungal strains, Leiotrametes menziesii and Abortiporus...
biennis. The initial biomass was willow sawdust and the lignin degradation increased by 17.1% for the A. biennis and 30.5% for the L. menziesii. For bio CH₄ production, they obtained an increase of 43% compared to the control in the case of A. biennis.

However, fungal pretreatment is associated with a significant drawback related to the duration of treatment. This process often requires an extended period (up to a month). To address this issue, one approach could involve combining different pretreatment methods. This combination could help reduce the time required for fungal pretreatment while also lowering the energy costs and chemical inputs associated with other pretreatment techniques mentioned earlier.

3. Recovery of Valuable Products from Biomasses

The constant growth of the population is leading to the constantly increasing production of agricultural, forestry, and food processing-derived waste biomasses [51,52]. The most abundant and widely produced agricultural waste biomasses are rice straw, corn straw, sugarcane bagasse, and wheat straw [53]. In the Mediterranean basin alone, approximately 30 Mt of residues from the olive oil industry are produced yearly [54].

Depending on the type of biomass waste, a huge amount of biochemicals and valuable products can be produced. Together with the food processing wastes, these residuals can be optimized for the production of pectic oligosaccharides [55], bioactive compounds (flavonoids, phenolic acids, and carotenoids), polymers [56], and so on. Agricultural and forestry biomasses can be exploited for the recovery of cellulose and hemicellulose. The first step to go through for the production of biopolymers, biochemicals, or biofuels consists of the deconstruction of biomasses into constituent sugars, which can then be fermented or transformed for the production of building blocks. Cellulose and hemicellulose are commonly hydrolyzed to monomeric sugars via acid or enzymatic hydrolysis [57]. Acid hydrolysis allows us to reach up to 90% sugar recovery efficiency from cellulose and hemicellulose sugars. It is mainly carried out with sulfuric acid or hydrochloric acid. Acid hydrolysis presents several drawbacks: toxic products are often produced; the remaining acid needs to be deactivated with lime, with the consequent production of calcium sulfate; and specific alloys are required due to corrosion processes associated with the used acids. All these factors result in the relatively high costs of the whole process.

The most promising alternative, in order to achieve a more sustainable production, consists of enzyme technology [58]. Enzymes are highly effective for the decomposition of residual biomass into its primary components, which can be then transformed into building blocks or chemicals [59].

Acid and enzymatic hydrolysis can be applied to biomass, especially to the cellulose and hemicellulose components, for the production of monomeric sugars [57].

The recovery from biomass of its constituent sugars allows the production of commodity chemicals, biofuels, and biopolymers. The US-DOE (United States Department of Energy) has identified 12 sugar-derived building blocks, such as glycerol, sorbitol, xylitol, and others [51]. The production chain of sugar must consider an initial pretreatment section and following refinement of the produced sugars for the removal of inhibitors produced during pretreatment and saccharification processes, such as carboxylic acids and phenolic compounds [60].

Lignocellulosic residuals can be exploited for the production of oligosaccharides, which consist of a promising source of prebiotics. The interest in these short-chain carbohydrates stays in their non-degradability in the presence of enzymes. Moreover, these compounds show high stability at relatively low pH and elevated temperatures [61]. The hydrolysis of heterogeneous hemicellulose allows the production of oligosaccharides. The main production techniques are direct autohydrolysis and chemical/thermal pretreatments followed by acid or enzymatic hydrolysis [62]. The most requested oligosaccharides are fructooligosaccharides, lactosucrose, xylooligosaccharides, isomaltooligosaccharides, galactooligosaccharides, and soybean oligosaccharides [63].
Biological residuals can be also used for the production of biofuels and bioenergy [64,65]. Bioethanol consists of a liquid fuel, which is also used as a base chemical for the synthesis of ethylene and ethylene glycol and for the production of derivative polymers, such as polyethylene and polyethylene terephthalate [66]. Bioethanol is produced via saccharification and fermentation processes or with separate hydrolysis and fermentation [67]. The microbial fermentation of monomeric sugars (acetone-butanol process) allows the production of biobutanol, a fuel showing high compatibility with combustion engines [68].

Solvent-based extractions and microwave and ultrasound treatments are largely applied for the recovery of bioactive molecules from residual biomasses [69]. Flavonoids and polyphenolic compounds are widely requested in the pharmaceutical, food, and cosmetic industries [70]. In particular, polyphenols are recognized for their anti-inflammatory and anti-proliferation properties [71].

Finally, biomasses rich in cellulose can be exploited for the production of nanocellulose [72], a completely biodegradable material with high mechanical strength and low permeability [73].

The next section deals with the description of the main procedures adopted for producing valuable products from residual biomasses commonly used in biorefinery chains.

4. Pretreatments for Recovery of Valuable Products from Residual Biomasses

According to the concept of biorefinery, agro-industrial residual biomasses cannot be directly destined to AD for biogas and bio-CH$_4$ production. These raw materials can firstly be pretreated, in order to extract the numerous valuable products in them contained. Finally, the residual sub-products can be processed via AD, in order to obtain energy and to produce fertilizers with the residual solids. Waste biomasses have to be first processed for the separation of cellulose from the lignocellulosic matrix [74]. The recovery of valuable molecules is crucial for the economic sustainability of the biorefinery chain; therefore, the treatments applied for this process will also be discussed in this paragraph. The recovery of cellulose from biomasses can be carried out according to several different procedures, in order to consider all of the possible variables that can affect the process [75]. Indeed, different aspects influence cellulose removal such as the type of raw material, solid-to-liquid ratio, main size of particles, pressure, temperature, pH, solvents required, and others [76].

The first treatment process is referred to as “biomass conditioning” and includes size reduction, dirt and pigment removal, drying and sieving, and others [77]. These treatments allow for improved efficiency of microbial and enzymatic action, thus favoring the recovery of cellulose. After conditioning, residual biomasses are further treated to solubilize lignin and hemicellulose and to produce cellulose fibers [75]. After the removal of lignin and hemicellulose, purification processes are often required. The main strategies for lignin and hemicellulose separation comprehend deep eutectic solvents [78], acid/alkaline pretreatments [79], ozone/peroxide bleaching [80], organosolv [81], steam explosion [82], ultrasound-assisted treatments [83], high-pressure treatments [84], microwaves [85], supercritical fluids [86], cold plasma [87] biological treatments [88], and/or a combination of them. In the following paragraphs, the most widespread and applied pretreatments are summarized in their main characteristics.

4.1. Deep Eutectic Solvents

Deep eutectic solvents represent a sustainable alternative to the use of ionic liquids for pretreatments of lignocellulosic biomass and delignification. These compounds are synthesized from ammonium salts and hydrogen bond donors, such as amines, alcohols, and carboxylic acids [89,90]. The combination of Lewis or Bronsted acids and bases containing anions and cations produces mixtures containing hydrogen bond acceptors and hydrogen bond donors [91]. Deep eutectic solvents are classified into different types; however, they are commonly denoted with the nomenclature $\text{Cat}^+X^-$zY, where $\text{Cat}^+$ can be ammonium, phosphonium, and sulfonium ion, Y is the acid/base, and z is the element that reacts with X$^-$ [92].
Solvent referred to as Type I results from the combination of quaternary ammonium salts and metal halides. These compounds have hygroscopic properties, which limit their application. Type II solvents include species resulting from the combination of metal halides and salts. Choline chloride (ChCl), carboxylic acids, and amides constitute the Type III solvents. This latter group offers several advantages, such as biodegradability and inexpensiveness. Type IV solvents consist of metal chloride hydrate and hydrogen-bonded donors, while Type V contains non-ionic components, such as molecular hydrogen-bonded acceptors/donors [93]. The main properties to consider in deep eutectic solvents are density, viscosity, conductivity, freezing point, and pH. The density is generally higher than water and approximately within 1.3–1.6 g/cm$^3$ [94]. Mainly due to the high tendency to produce hydrogen bonds, these species show high viscosity, usually higher than 100 mPas [95]. Conversely, the higher the viscosity, the lower the conductivity [96]. The freezing point may vary significantly as a function of the specific solvent; on average, it can range from 50 $^\circ$C to 80 $^\circ$C [97,98]. Probably the most important property to consider for pretreatments of biomass and/or extraction of useful molecules is pH. Acidic conditions favor the extraction of lipids, while basic conditions are required for the extraction of pigments and other valuable molecules [99,100].

4.2. Acid Pretreatments

Acid pretreatment allows the disruption of the cellulosic biomass by cleavage of glucosidic bonds [101]. It solubilizes hemicellulose and partially also lignin, facilitating the processing of cellulose from enzymes [102]. The most commonly used inorganic acids for this scope of application are phosphoric acid, nitric acid, hydrochloric acid, and sulphuric acid [103]. Conversely, the most diffused organic acids are citric acid, acetic acid, and oxalic acid.

The variables to control during acid pretreatment are the acid concentration, reaction time, temperature, and solid-to-liquid ratio [104]. Concentrated acids lead to higher sugar recovery yields from lignocellulosic biomasses but, at the same time, transform hemicellulosic sugars into toxic compounds (i.e., furfural and acetic acid), which inhibit microbial growth [105]. Conversely, dilute acid pretreatment presents lower toxicity and is less corrosive.

The process is carried out in stainless steel reactors, where the lignocellulosic biomass is heated for a time period depending on the operating temperature [106]. The acid is injected only after the target temperature has been reached. Finally, the resulting biomass is treated to be dewatered. The final products will mainly consist of hydrolyzed hemicellulose sugars [107].

In order to reduce the production of toxic substances, responsible for the lowering of the enzymatic activity, some detoxification strategies have been defined, such as electrodialysis and adsorption resins. Unfortunately, all these strategies lower the final amount of sugars in the hydrolysate [108].

In addition to the formation of toxic compounds and corrosivity, a further critical aspect related to acid pretreatment, is the high acid recovery cost.

4.3. Alkaline Pretreatments

Alkaline pretreatment is used to solubilize lignin and part of hemicellulose. It is also useful for reducing the crystallinity of cellulose. Alkaline pretreatment is the most applied technique in pulping processes [109]. The dissolution of lignin and hemicellulose depends on the breakdown of the intermolecular ester bonds existing between lignin and hemicellulose molecules. The most used alkali compounds are sodium hydroxide, ammonia, calcium hydroxide, and sodium carbonate.

The main parameters to control are the alkaline concentration (and typology of alkali used), reaction time, temperature, and selection of the process intensification method. Alkaline pretreatment makes cellulose more accessible for enzymatic hydrolysis and favors the conversion of hemicellulose into sugars.
Among the main disadvantages of such a treatment, the non-selectivity of alkaline should be considered, which causes the loss of part of cellulose and hemicellulose components. Alkaline pretreatment often shows low environmental sustainability and excessively high pH, which can hinder the activity of cellulase enzymes during the hydrolysis step. Dilute acid pretreatment and alkaline pretreatment are often combined together to separate lignin and hemicellulose sugars from the lignocellulosic component, thus favoring their digestibility and fermentability [110].

4.4. Organosolvent

Organic solvents are used to solubilize and remove lignin from lignocellulosic biomass [111]. Organosolvents can be used in their pure form or in aqueous solutions and allow the separation of the lignocellulosic biomass into lignin, cellulose, and hemicellulose, with a high purity degree. If compared with the previously described chemicals, these solvents can be easily recovered and reused; therefore, organosolvents are referred to as an environmentally sustainable option. The most widespread solvents belonging to this group are acetone, ethanol, methanol, and ethylene glycol. Lignocellulosic biomass is generally brought to 150–200 °C; then, it is mixed with solvents. The solubilization of the lignocellulosic component is often carried out in the presence of catalysts [112].

The main parameters to take into consideration are the reaction time, temperature, biomass particle size, and type of catalyst. The smaller the size of the particles, the higher the penetration of solvents. Temperature and reaction time define the cellulose yield. Acid catalysts are often used, such as acetic acid and sulfuric acid; their presence promotes the process but their concentration cannot exceed specific threshold values, in order to avoid the degradation of cellulose. Acid catalysts also favor delignification and improve the sugar yield [113].

Despite the numerous advantages, organosolvents are highly expensive and their recovery consists of an energy-intensive process. For that reason, their usage is still limited if compared with traditional solvents.

4.5. Steam Explosion

In steam explosion-based treatments, the lignocellulosic biomass is initially exposed to high-pressure saturated steam and then quickly depressurized, in order to cause the explosive decompression of the raw material and the consequent breaking down of lignin [114]. Steam explosion methods ensure high cellulose accessibility, elevated sugar yield, and high enzymatic digestibility [115]. Moreover, steam explosion is cost-effective and environmentally sustainable [116,117].

Steam explosion is carried out at pressures ranging from 0.69 to 4.83 MPa and temperatures from 160 °C to 260 °C [118]. The fast drop in pressure produces shear stress and causes the disruption of hydrogen bonds, favoring the enzymatic hydrolysis of hemicellulose and cellulose [119]. Steam explosion treatment allows the obtainment of solid residues containing only cellulose and lignin [120]. The process also favors the release of acids from biomass, such as acetic acid, formic acid, and levulinic acid [121]. The main parameters to control are the temperature and reaction time [122], which are crucial for the breaking down of hemicellulose and lignin and the hydrolysis of cellulose. Unfortunately, the application of such a method also presents some disadvantages, such as the incomplete rupture of the structure linking lignin and carbohydrates, the degradation of hemicellulosic sugars, and the undesired production of inhibitors capable of reducing the efficiency of the following fermentation process [123].

4.6. Ultrasound-Assisted Treatments

Ultrasounds are considered one of the most promising solutions for the intensification of processes such as lignin degradation, cellulose conversion, and sugar production [124]. The term ultrasound-assisted treatment refers to acoustic waves oscillating at frequencies above 16 kHz [125], capable of creating compression and rarefaction cycles when
Oscillating in liquid phases [126]. In particular, if rarefaction cycles are intense enough to overcome the molecular bound of solvents, they lead to the formation of cavities, which are immediately filled by the diffused gas or the vapor of solvents [127]. The application of ultrasounds generates cavitation phenomena [128]. Due to the collapse of gas and vapor bubbles, the temperature may locally increase by about 10 °C/s and the pressure may reach 2000 atm [129]. The cavitation effect improves the dissolution of solid particles [130]; moreover, the collapse of bubbles favors the formation of radical species [131], which enhances the activity of reagents.

Ultrasounds have been widely explored as a strategy for improving the efficiency of pretreatment processes, thanks to their capability of creating turbulence, microjets, and shock waves in biomass [132]. It was proven that, when exposed to ultrasounds, the lignocellulosic material shows higher solubility than in quiescent conditions [133]. The main advantages of ultrasound-assisted techniques are the lower reaction time, the lower operating temperatures, and the possibility of working at room pressure [134]. Moreover, the use of ultrasounds also generates sonocapillarity and sonoerosion, which help in solubilizing lignin and reducing the crystallinity of cellulose.

4.7. Microwaves

When lignocellulosic biomass is exposed to microwaves, the molecular collisions due to dielectric polarization favor its breakdown [135]. The realignment of polar molecules in biomass is responsible for the internal production of heat [136]. Therefore, microwaves cause volumetric generation of heat and, for that reason, differ from the conventional heating techniques, which firstly interest the external surface of materials and only later their internal regions. For biomass pretreatments, the most used solvent is water [137]; the ionic conduction of H\(^+\) contributes to the dielectric loss factor. Residues with low pH inevitably show higher H\(^+\) density and can be easily heated. For that reason, the humidity of biomass is a key parameter to consider when using microwaves.

In the presence of water, lignin, cellulose, and hemicellulose show intrinsic polarity and are consequently suitable for being treated with microwaves [138]. In addition to the humidity of biomass, the main parameters to consider are electromagnetic wave propagation within the medium, wavelength, and depth of penetration.

4.8. High Pressure Treatments

The treatments belonging to this group are divided into high hydrostatic pressure processes and ultrahigh-pressure processes. In these treatments, the biomass is subjected to high pressures, ranging from 100 to 800 MPa, with or without providing additional heat [139]. High-pressure techniques are capable of enhancing the accessibility of lignocellulosic components and are often applied before enzymatic hydrolysis [140]. The process is based on two assumptions: (i) the Le Chatelier Principle, affirming that pressure promotes all the structural reactions and causes a reduction in volume, and (ii) the Isostatic Principle, stating that the distribution of pressure is proportional in all the portions of the material, independently from its shape.

High-pressure treatments were proven to improve the cellulose pretreatment yield, in particular acting on its crystallinity, favoring the reduction in particle size and enhancing the structural disintegration of biomasses [141].

The main advantage of high-pressure treatments is the high efficiency in breaking down lignin. However, these methods are still excessively energy-intensive. Moreover, the need to obtain extremely high pressures leads to high costs and problems related to scalability.

4.9. Supercritical Fluids

The definition “supercritical fluid” refers to any substance at conditions above its critical pressure and temperature. These contemporary substances have the properties of liquids, such as density, and the properties of gases, such as compressibility [142]. The
most widespread supercritical fluid is carbon dioxide, which, since it has a low critical temperature (31.1 °C) and critical pressure (7.36 MPa), is reusable and is relatively non-toxic and environmentally friendly.

Supercritical fluids are highly effective in delignification processes [143,144]. The main parameters to consider are the pretreatment time, temperature, pressure, and moisture content. With regard to moisture, the combination of water with supercritical carbon dioxide leads to the production of carbonic acid. The resulting acid environment favors the hemicellulose hydrolysis [145].

4.10. Non-Thermal Plasma

Plasma is produced via ionization of gas (electrons, ions, excited atoms, and molecules) [146]. It is considered the fourth state of matter and is divided into high-temperature and low-temperature plasma [147]. High-temperature plasma is used in nuclear applications, while low-temperature plasma is further divided into thermal and non-thermal plasma. The main difference, between these two latter groups, lies in the temperature of the gas, which ranges from 11,600 to 116,000 K in thermal plasma, which is equal to the room value for non-thermal plasma.

Non-thermal plasma can be advantageously used during biomass pretreatments. Catalysts produced with the support of non-thermal plasma show tighter lattices and have excellent performances in terms of activity and selectivity [148,149]. When applied in pretreatments of lignocellulosic biomass, non-thermal plasma improves the separation of cellulose, hemicellulose, and lignin and ensures the preservation of their structural integrity. During pyrolysis, non-thermal plasma favors the conversion of reactants and increases the H/C ratio [150,151]. In gasification processes, it favors the breaking down of macromolecules and the production of hydrogen and carbon monoxide [152,153].

4.11. Biological Pretreatment

This group of treatments is based on the use of enzymes and microorganisms for deconstructing lignocellulosic biomass [154]. Enzymes can be used for solubilizing lignin and hemicellulose components while keeping cellulose intact [155]. Microorganisms such as fungi and bacteria are capable of promoting the fermentation of biomass and also to enhance the enzymatic hydrolysis [156]. Biological pretreatment offers key advantages; it is completely environmentally friendly and does not lead to the production of hazardous chemicals. However, the use of biological microorganisms implies the need for the careful consideration of numerous process variables. The process efficiency is strongly dependent on the species involved in the treatment, which must be consequently selected with accuracy. Moreover, the following parameters must be taken into account: particle size, temperature, moisture, aeration, reaction time, pH, availability of nutrients (carbon and nitrogen), and others [154].

The main limitation of biological pretreatment is the lower efficiency and the time needed to carry out their action, if compared with the traditional chemical treatments, which is strongly affected by any variations in the parameters previously mentioned. Moreover, enzymes and microorganisms are often more expensive than the common chemical substances used for biomass pretreatment.

Finally, the same chemical can be used for various typologies of biomasses, while, in the case of biological pretreatment, each kind of biomass needs a specific recipe of microorganisms to be correctly processed.

5. Dual Function Pretreatments

As stated in the previous sections, several pretreatments are developed to maximize the production of biogas/bio-CH₄ or to extract valuable products from residual biomasses. This section aims to experimentally prove that some pretreatments, used for the extraction of high-added-value products, are also capable of improving the biogas production yield of the residual biomass.
Biogas was produced with three different raw biomasses, consisting of untreated olive mill wastewater (OM) and the same material after being freeze-dried (OML) and after being treated for the recovery of bioactive molecules, such as polyphenols and flavonoids (OMPF). The procedures used for the production of OML have already been described in the literature [157,158]. As far as OMPF production is concerned, it involves an initial defatting step with organic solvents followed by freeze-drying; polyphenols and flavonoids are then extracted with a solution containing MeOH, HCOOH, and MeCN followed by the centrifugation phase and supernatant recovering.

Biogas was produced in small-scale batch bioreactors with an internal volume equal to 250 mL and was constantly kept at mesophilic conditions (37 °C) within a climatic room. Figure 1 shows one of the bioreactors used for the experimentation.

![Bioreactor](image)

Figure 1. Picture representing the bioreactor. From right to left, the first bottle contains the inoculum mixed with the residual biomass to test; the second bottle is the water tank used to measure the quantity of biogas produced according to the volumetric method; and finally, the third bottle exclusively serves to avoid the dispersion of water ejected during the process.

Each reactor was filled with 75 mL of inoculum, or a digestate directly produced in the laboratory, whose main properties are summarized in Table 1.

Table 1. Main properties of the inoculum (TOC: total organic carbon; TKN: total Kjeldahl nitrogen; WEOC: water extractable organic C; WEN: water extractable N; DM: dry matter basis).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Inoculum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture [%]</td>
<td>88.08</td>
</tr>
<tr>
<td>pH</td>
<td>8.76</td>
</tr>
<tr>
<td>TOC [% on DM]</td>
<td>56.2</td>
</tr>
<tr>
<td>TKN [% on DM]</td>
<td>5.8</td>
</tr>
<tr>
<td>Total P [g/kg of DM]</td>
<td>3.3</td>
</tr>
<tr>
<td>Total K [g/kg of DM]</td>
<td>80.11</td>
</tr>
<tr>
<td>WEOC [g/kg of DM]</td>
<td>117.06</td>
</tr>
<tr>
<td>WEN [g/kg of DM]</td>
<td>71.89</td>
</tr>
</tbody>
</table>
The dried organic matter was then weighed. The quantity of biomass added to the system was calculated as 33.3 wt% of the dried content of inoculum.

To calculate the dried organic matter of both the inoculum and the residual, the volatile solids were measured according to the standard procedure [159,160]. Two grams of each sample were distributed on a ceramic crucible and then inserted into a muffle furnace. The temperature was gradually brought to 550°C and kept at this value until the complete incineration of the sample was achieved. The residues were then transferred to the desiccator and, finally, weighed. Each sample was analyzed in triplicate.

Similarly, the moisture content was also defined according to the official procedure [161].

Finally, a one-way ANOVA test was used to define the significance of differences existing between the sample mean values. The level of significance was set at 0.05 (or less).

Results

Biogas was produced with the three following mixtures:

(1) Inoculum (¾) and OM (¼);
(2) Inoculum (¾) and OML (¼);
(3) Inoculum (¾) and OMPF (¼).

Finally, a one-way ANOVA test was used to define the significance of differences existing between the sample mean values. The level of significance was set at 0.05 (or less).

In terms of dry matter, each reactor was filled with 8.58 g of inoculum and approximately 2.86 g of dry biomass. According to the production time previously obtained with the same procedure and stated in [157], the production of biogas was carried out for 11 days. The daily biogas production for each sample is indicated in Table 2. Figure 2 shows the cumulative production of biogas and allows us to understand the beneficial effect due to pretreatment carried out for freeze drying and for polyphenol/flavonoid extraction.

Figure 2. Cumulative biogas production (mL) of the anaerobic batch reactors containing ¾ inoculum and ¼ of untreated olive mill wastewater (OM), freeze-dried OM (OML), and residual OM after the extraction of polyphenols and flavonoids (OMPF).
Table 2. Daily biogas production (mL) of the anaerobic batch reactors containing ¾ inoculum and ¼ of, respectively, untreated olive mill wastewater (OM), freeze dried OM (OML) and residual OM after the extraction of polyphenols and flavonoids (OMPF).

<table>
<thead>
<tr>
<th>Day n°</th>
<th>OM [mL]</th>
<th>OML [mL]</th>
<th>OMPF [mL]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>18</td>
<td>39</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>61</td>
<td>31</td>
</tr>
<tr>
<td>3</td>
<td>11</td>
<td>45</td>
<td>16</td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>46</td>
<td>22</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>30</td>
<td>12</td>
</tr>
<tr>
<td>6</td>
<td>14</td>
<td>4</td>
<td>17</td>
</tr>
<tr>
<td>7</td>
<td>12</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>8</td>
<td>17</td>
<td>24</td>
<td>11</td>
</tr>
<tr>
<td>9</td>
<td>24</td>
<td>13</td>
<td>7</td>
</tr>
<tr>
<td>10</td>
<td>6</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>4</td>
<td>13</td>
<td>2</td>
</tr>
</tbody>
</table>

These results confirm the feasibility of using biomasses previously pretreated for valuable product extraction/production and then used for producing biogas. All the samples started producing biogas immediately; however, the bioreactors containing pretreated OM showed a higher yield. The sample containing OML showed higher biogas production since the second day of the test, while the sample with OMPF immediately assumed higher production if compared with the untreated OM. Based on the diagram of Figure 2, the sample containing OML reached the best performance in terms of the total quantity of biogas achieved. In particular, the following final quantities were reached: untreated OM 147 mL; OMPF 169 mL; and OML 268 mL. In terms of production time, the sample containing OMPF showed the best results: the maximum production was reached after 9 days, while the other samples showed a continuously growing trend in the time period selected for the experimentation.

The freeze-drying of OM allowed for the improvement in the production of biogas, while the extraction of polyphenols and flavonoids reduced the production period. In both the situations tested, the initial pretreatments, finalized to the extraction/production of high-added-value products, also enhanced the biogas yield.

The quantity of biogas produced was finally evaluated per unit of total solid (TS) and volatile solid (VS) of the different samples. The results are resumed in Table 3 and are shown as mL of biogas produced per gram.

Table 3. Biogas produced per unit of total solid (TS) and volatile solid (VS) for the different samples tested or untreated olive mill wastewater (OM), freeze-dried OM (OML), and residual OM after the extraction of polyphenols and flavonoids (OMPF).

<table>
<thead>
<tr>
<th></th>
<th>OM</th>
<th>OML</th>
<th>OMPF</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS [%]</td>
<td>14</td>
<td>98</td>
<td>98.5</td>
</tr>
<tr>
<td>VS [mg/L]</td>
<td>162</td>
<td>298</td>
<td>188</td>
</tr>
</tbody>
</table>

These results support the concept of biorefinery well, where biogas and bio-CH$_4$ only represent the last step of a series of treatments aimed at exploiting the residues of agro-industrial biomasses as much as possible.

While the production of biogas/bio-CH$_4$ and its optimization process have been widely explored and documented in the literature, this latter field, or the application of “dual function” pretreatments, aimed at first extracting valuable products and then producing biogas, needs to be further investigated since its feasibility is strongly dependent on the type of biomass considered, the pretreatment selected, and their combination.
6. Conclusions

Residual agro-industrial biomasses are considered a huge and still not adequately exploited source of valuable products and energy. This article collects and describes the main biomass pretreatments defined for these two targets. Biomass pretreatments allow the separation of biomass into its main components (i.e., cellulose, hemicellulose, and lignin) and the extraction of biochemicals such as polyphenols, flavonoids, and others; moreover, they also serve to prepare biomass for AD, in order to enhance the productivity of biomass treatment. Two review sections are dedicated to the classification and description of the main pretreatments used to improve the biogas production yield and to obtain valuable products from agro-industrial wastes. For each technique, the main potentialities and the disadvantages are discussed.

The second half of this study aims to carry out the proposal of using the same pretreatment to first obtain useful products and then optimize the biogas yield from the same biomass, driving the transition toward the so-called “third-generation biorefinery”. For perspective, biogas was experimentally produced with three matrices made of an appropriate inoculum mixed with untreated olive mill wastewater and the same biomass after being freeze-dried and after the extraction of polyphenols and flavonoids, respectively. The experiments were carried out in lab-scale apparatuses, which allowed us to calculate the quantity of biogas produced with the volumetric method. The results confirmed that the use of pretreated biomass enhanced the biogas production yield: the untreated OM led to the production of 147 mL of biogas, while OML and OMPF produced 169 mL and 268 mL of biogas, respectively.

The bibliographic research and the experimental results produced in this study confirmed that the advantageous coupling of the two different strategies for valorizing agro-industrial residuals is a function of the type of biomass processed and the specific treatment considered; therefore, a huge number of possible combinations exists and only a portion of them is suitable for this dual function and needs to be detected with accuracy. While the production of biogas/bio-\(\text{CH}_4\) from residual biomasses has already been widely explored, more scientific efforts are still needed to detect the couples of biomass/pretreatment suitable for both high-added-value product extraction and high-efficiency biogas production.

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

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