CHAPTER 15

PROCESS TECHNIQUES FOR CONVERSION OF LIGNOCELLULOSIC BIOMASS TO BIOGAS

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Abstract

The urgent need to combat climate change and establish sustainable energy sources has propelled biofuel research and development. Biogas production through anaerobic digestion is widely recognized as a pivotal solution to divert biomass waste from landfills, reduce environmental pollution, and provide a carbon-neutral energy source for humanity. Second-generation feedstocks, specifically lignocellulosic biomass waste from the agricultural, forest, and timber industries, have emerged as the optimal alternative to foster economic growth without jeopardizing food security or misusing arable land. However, their intrinsic resistance hampers the complete extraction of their fermentable sugars, necessitating the exploration of diverse methods to facilitate the easy access of sugars for hydrolysis. Hence, this chapter delves into various lignocellulosic biomass pretreatment techniques employed to optimize easy access to fermentable sugars for valorization in biogas production.

Keywords: Biofuels; Green economy, Biogas, Lignocellulosic Biomass pretreatment.

15.1 Introduction

The ever-increasing demand for energy supply has been one of our time's most challenging global issues (Saud *et al.* 2020). As the world's population grows, industrialization expands, and economies thrive, the reliance on conventional fossil fuels, such as coal, oil, and natural gas, also increases. However, this heavy dependence on non-renewable energy sources has given rise to countless concerns, including climate change, environmental degradation, and energy security (Saud *et al.* 2020; IEA 2023; Hoang *et al.* 2023).

There is an urgent need to explore sustainable and environmentally friendly alternatives to fossil fuels to address these pressing challenges. Among the various renewable energy options, biofuels have attracted significant attention as a promising solution. Using organic matter such as agricultural residues, algae, and organic industrial waste, including water and food waste in biofuels, can significantly reduce greenhouse gas emissions, mitigate climate change impacts, and act as a waste management strategy. Furthermore, their renewable nature makes them an attractive prospect to reduce dependence on finite fossil fuel reserves (Davis *et al.* 2018).

Visionary pioneers such as Henry Ford, Rudolph Diesel, Harold Hibbert, Ernst Berl, and Kanaiyalal Manekial Munshi foresaw the eventual depletion of finite petroleum-based fuels due to civilization's growth and potential geopolitical conflicts. Recognizing this, they embarked on a quest to identify sustainable alternatives, culminating in a standard agreement that the future of fuels lies in Earth's abundant biomass resources (Kovarik 2013).

This collective insight led to the exploration of biofuels, with ethanol emerging as a prominent contender. The initial focus revolved around planting dedicated crops or rapidly regenerating trees, classified as first- and second-generation fuels, to produce biofuels like ethanol. However, as research progressed, it became evident that this approach posed risks to food security and contributed to detrimental land and water resource allocation (Subramaniam, Masron and Azman 2020; 2019).

In response, Kanaiyalal Manekial Munshi advocated a paradigm shift. He suggested that food crop cultivation for ethanol production should be prohibited, proposing a pivot toward using agricultural residues and other cellulosic waste to manufacture biofuels. As a result, the strategic selection of biomass feedstock became a crucial determinant in the efficiency, sustainability, and overall success of the biofuel production process (Kovarik 2013).

The decision about biomass feedstock substantially influences its availability, energy content, and composition, and has environmental implications. Consequently, meticulously evaluating multiple factors becomes imperative to pinpoint the most suitable biomass feedstock for a given biofuel production scenario. This underscores the necessity to customize and optimize the biofuel production process to seamlessly harmonize with the unique attributes of the available feedstock within a specific geographical region (Sumardiono *et al.* 2022a).

Biomethane, a gas derived from anaerobic digestion, has gained significant attention among researchers due to its potential to revolutionize the transition towards a circular economy. This versatile biofuel holds promise for widespread adoption, even among non-technical individuals in rural areas (Pilloni *et al.* 2021). However, the successful implementation of biogas production is based on the availability of biomass resources and the cost-effectiveness of their utilization. Lignocellulosic biomass stands out as a plentiful and sustainable feedstock for biogas production without posing a threat to food security or resource misuse (land, water, and energy) (Aro 2016; van Huyssteen, Thiam and Nonhebel 2023). However, the stubborn nature of lignocellulose poses a challenge in harnessing its full energy potential. Various pretreatment technologies have been developed to break down its recalcitrant structure to address this issue, facilitating hydrolysis and leading to higher biogas yields. Thus, this chapter aims to comprehensively review the various process techniques involved in the production of biogas from lignocellulosic biomass (Kumar and Sharma 2017; Kumari and Singh 2018).

15.2 Feedstock

Throughout the lifecycle of agricultural endeavors, a plethora of residual materials is generated, encompassing a diverse range of waste streams. These residual materials notably comprise the non-edible, cellulosic-rich fractions of crops, including rice straw, corn stover, and sugar bagasse, alongside the organic amalgam of animal manure, formed through the amalgamation of animal excreta and straw or grass, often employed for bedding purposes. Additionally, the realm of agricultural waste extends to encompass the timber industry and yard residues, characterized predominantly by leaves, branches and woodchips (Sumardiono *et al.* 2022a).

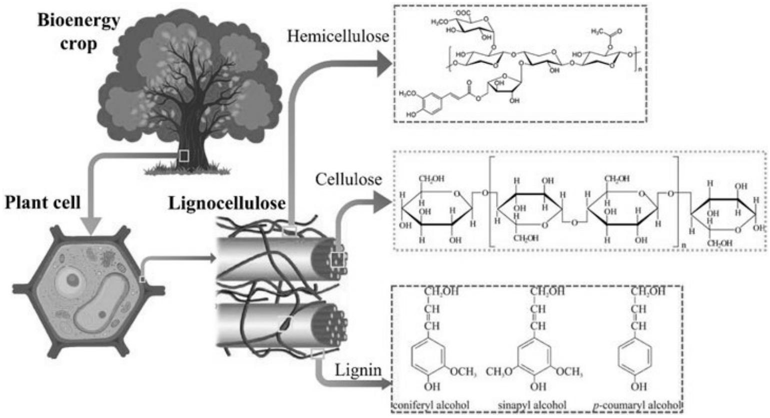

Figure 15.1 Lignocellulose composition (Sankaran *et al. 2021*).

The transition to second-generation feedstock in biofuel production has faced persistent challenges, mainly due to the complex composition of lignocellulose. Figure 15.1 highlights these materials' cellulose, hemicellulose, and lignin composition in addition to a small number of extractives. Ongoing development efforts are still needed to overcome these obstacles. The primary issue lies in the recalcitrant nature of most second-generation feedstock, which hinders their full energy potential extraction. Fermentable sugars that are crucial for biofuel production are trapped within the holocellulose, containing cellulose and hemicellulose. Unfortunately, the presence of rigid lignin, acting as a natural binder, makes it difficult to access and convert these sugars. The proportions of cellulose, hemicellulose, and lignin vary widely, typically 33-50%, 19-30%, and 5-25% respectively, depending on the biomass used (Sankaran *et al.* 2021; Rodionova *et al.* 2022; Singh, Srivastava and Shukla 2016). These variations add complexity to the conversion process, necessitating tailored approaches for efficient biofuel production.

Cellulose represents the principal carbohydrate component in lignocellulosic biomass, characterized by lengthy structural chains of β-glucose monomers linked to β-(1,4)-glycosidic bonds, forming microfibril bundles. These linear cellulose chains are held together by intermolecular and intramolecular hydrogen bonds, which result in varying degrees of polymerization (Sebayang *et al.* 2016). The hydrogen bonds create highly organized crystalline regions, making cellulose accessible to the activity of hydrolytic enzymes during biogas production. Conversely, less crystalline amorphous regions within the cellulose structure resist degradation. These amorphous regions present irregular bonding and chemical structures, making it challenging for enzymes to break down the cellulose molecules within these areas efficiently (Raj *et al.* 2015).

Hemicellulose, a vital component in plant cell walls, consists of branched chains of sugars, including hexoses (D-glucose, D-mannose, and D-galactose), pentoses (D-xylose and L-arabinose), and acetylated sugars (Sebayang *et al.* 2016). The main hemicellulose, xylan, is transformed into xylose during biomass hydrolysis, a process used in biofuel production (Rocha-Meneses *et al.* 2020). However, acetylation during galactose residue biosynthesis and by-products like acetic acid can hinder microbial growth in biofuel production. Precise temperature and retention time control during hemicellulose degradation are crucial to minimize by-product formation. The branched structure and low molecular weight of hemicellulose make it easily hydrolyzable, an essential attribute for biofuel production (Singh, Suhag and Dhaka 2015; Akhtar, Goyal and Goyal 2017).

Lignin is a complex, sizeable molecular structure formed primarily from three types of monomers: p-coumarin, sinapyl alcohols, and coniferyl. These monomers combine to create a highly interconnected and rigid structure (Rezania *et al.* 2020). Unlike cellulose and hemicellulose, lignin lacks a sugar-based framework and instead has a 3D structure with alkyl-aryl bonds, acting as an adhesive between cellulose and hemicellulose. However, lignin's presence often reduces the efficiency of enzymatic hydrolysis. This reduction occurs due to interactions like electrostatic, hydrophobic, and hydrogen bonds, which can cause lignin to bind to enzymes. Additionally, soluble chemicals derived from lignin may act as enzyme inhibitors, further affecting the hydrolysis process (Liu *et al.* 2021; Sumardiono *et al*. 2022b; Dai *et al.* 2019).

15.3 Pretreatment Technologies

Pretreatment is pivotal for the conversion of lignocellulosic biomass into biofuels to be economically viable. This is because of the intricate nature of the structure of lignocellulosic biomass. It should also be emphasized that the efficiency of pretreatment is a critical factor in achieving higher biofuel yields (Kumar and Sharma 2017). The primary objectives of the pretreatment involve increasing the surface area of the biomass by reducing its particle size and dissolving hemicellulose and/or lignin. These goals can be accomplished through chemical or physical modifications of the lignocellulosic biomass structure. Thus, enhancing the accessibility of microorganisms or their enzymes to the cellulose surface improves cellulose hydrolysis, as shown in Figure 15.2 (Muley and Boldor 2017; Kumari and Singh 2018).

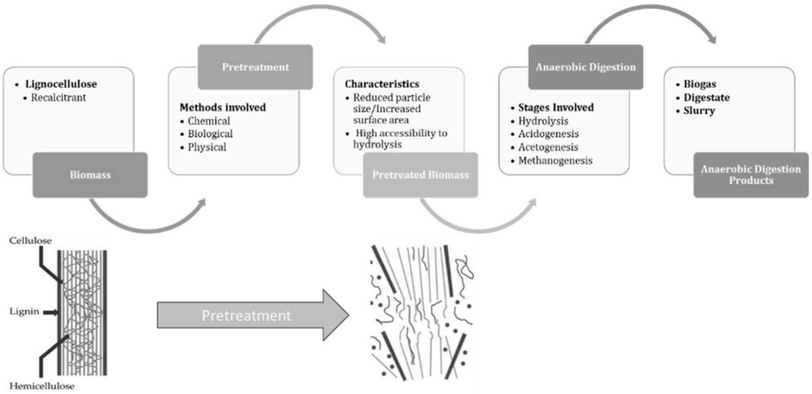

Figure 15.2 Effects of biomass pretreatment.

Various methods aimed at improving the intricate structure of lignocellulosic biomass have been designed and developed. These include the physical, chemical, and biological pretreatment methods. In some cases, these methods are combined to compensate for the shortfalls of the other (Kumari and Singh 2018; Das et al. 2021).

15.3.1 Physical Methods

Physical pretreatment, the most ancient technique in biomass pretreatment for biofuel production, focuses on the initial modification of raw biomass to enhance its suitability for subsequent conversion processes. This pivotal step primarily involves reducing particle size and densifying the biomass. Several distinct methods fall under this category, each with unique merits and considerations (Awasthi *et al.* 2023).

15.3.1.1 Chipping, Grinding and Milling

Chipping, grinding, and milling are the most prevalent and extensively adopted approaches to meticulously reduce particle dimensions and crystallinity, preparing the substrate for downstream processing. These methods are usually used together, depending on the feedstock type. Chipping decreases biomass size to 10-30 mm, while grinding and milling can reduce particle size to as little as 0.2 mm. Nonetheless, research suggests that decreasing biomass particles to smaller than 0.4 mm has no meaningful effect on the pace and yield of hydrolysis (Kumar and Sharma 2017). These effectiveness of these methods hinges on the feedstock's intrinsic characteristics and moisture content, whether dry or wet. Certain feedstocks exhibit differential susceptibility to pulverization, depending on their hydration state. However, it is imperative to recognize that the energy-intensive nature of the machinery used translates to escalated overall conversion costs. This underlines the urgency of advancing machinery design, optimizing energy consumption, and achieving universal applicability across diverse feedstock types and conditions (Das *et al.* 2023; Kumar 2017). The impact of particle size reduction (20, 1.0, 0.15, and 0.075 mm) was investigated on rice straw, revealing notable effects. Anaerobic digestion results demonstrated that volatile fatty acids (VFAs) increased as particle size decreased, consequently enhancing methane production. Specifically, methane production per size reduction (from 20 to 0.075 mm) ranged from 107 to 197 mLg-1 VS—moreover, smaller particle sizes facilitated quicker substrate degradation than larger particles (Dai *et al.* 2019).

Similarly, another study conducted size reductions of 1 mm and 4 mm on various straws, including barley, rye, and wheat, and obtained analogous outcomes. However, the study also highlighted that despite subjecting the straws to the same conditions, barley straw generated significantly higher biogas than wheat and rye straw. This difference was attributed to the varying lignin content found in lower concentrations in barley straw (Álvaro *et al.* 2023). This difference indicates that biomass pretreatment needs to be optimized for every substrate.

15.3.1.2 Extrusion

Extrusion is a mechanical pretreatment method that uses pressure and heat to operate on the processed material via shear forces. This allows the material to heat up and become malleable, allowing it to be molded and expanded through a nozzle. The rotational speed of the extruder screws generates shear forces and pressure. Both single-screw and twin-screw extruders are used in this technique. The extruder chamber may reach about 300°C, and the pressure varies according to the equipment settings. The length of the plasticizing system and the speed at which the extruder screw revolves determine how long biomass remains inside the extruder chamber. Lignin in biomass is broken down, while cellulose and hemicelluloses are released, aiding in the efficient conversion (Kumar and Sharma 2017; Kupryaniuk *et al.* 2020). A study investigated the extrusion-cooking of corn straw using a single screw extruder with varying screw speeds (70, 90, and 110 rpm), and initial moisture content (25% and 40%). The extruder screw speed influenced the temperature range, with the highest being 126–150°C at 70 rpm. The study evaluated energy consumption and the potential use of this process in an agricultural biogas plant. Laboratory testing of biogas and methane efficiency after extrusion revealed that corn straw pretreated at 110 rpm with 25% moisture produced the most favorable methane and biogas production efficiency (51.63%) compared to untreated straw (49.57%) (Kupryaniuk *et al.* 2020).

15.3.1.3 Pulsed Electric Field

Pulsed Electric Field (PEF) is a non-thermal technique that disrupts plant structures by forming pores in cell membranes. Beyond enhancing cell membrane permeability and expediting the breakdown of polysaccharides within lignocellulosic materials, PEF offers further benefits like brief processing duration and reduced energy usage (Szwarc, Nowicka and Zieliński 2023). These induced pores facilitate subsequent disruptive actions by agents, further deconstructing cellulose into its constituent sugars (Safavi and Unnthorsson 2017). PEF pretreatment resulted in a 16% methane yield increase compared to untreated maize silage. This effect was achieved using an electric field intensity of 20 kV/cm and a frequency of 10 kHz for 180 seconds (Szwarc and Szwarc 2020). Similarly, the methane production from PEF-pretreated rapeseed straw exceeded that of raw straw by 14%. This enhancement was accomplished using an electric pulse of 40 kV, pulse width of 50 μs, and frequency of 5 kHz for 5 minutes (Szwarc and Głowacka 2021).

15.3.1.4 Ultrasound

The pretreatment of biomass using ultrasound involves subjecting biomass material to ultrasonic waves. This process induces cavitation, the rapid formation and collapse of bubbles within a liquid. Cavitation generates high temperatures and pressures, leading to physical and chemical changes in the biomass. These changes make the biomass more amenable to subsequent processes, such as enzymatic hydrolysis or fermentation, and enhance the overall efficiency of biomass conversion into biofuels or other valuable products (Kisielewska *et al.* 2020; Das *et al.* 2021). In a particular study, the methane production from a cornstalk and dairy manure mixture subjected to ultrasonic pretreatment exhibited a substantial 69.7% increase when an ultrasonic energy input of 284.1 kJ/kg TS was applied (Zou *et al.* 2016).

15.3.1.5 Microwave

Microwave treatment constitutes another noteworthy avenue within mechanical pretreatment. This method orchestrates the disintegration of lignocellulosic components by subjecting the substrate to elevated temperatures induced by microwave irradiation. The heightened solubility of lignocellulose biomass induced by this procedure is affected by its ability to change the orientation of the substrate's polar compounds' dipoles (Pellera and Gidarakos 2017; Wang *et al.* 2021).

There are thermal and non-thermal methods. Thermal processing accomplishes this treatment through heating-induced molecular motion, whereas non-thermal processing relies on direct interactions between microwaves and polar molecules to induce changes in dipole moments or behaviors (Wang *et al.* 2021). The irradiation frequency used in microwave pretreatment is between 300 MHz and 300 GHz with a wavelength that ranges between 0.001 to 1 M. Various factors such as the substrate type, duration, temperature, and power influence the effect that microwave irradiation exerts on the substrate (Pellera and Gidarakos 2017). The benefits of pre-treating lignocellulosic feedstock using microwave irradiation techniques include easy operation, minimal energy consumption, rapid and efficient heating capabilities, limited inhibitor formation, and the ability to disrupt the structural arrangement of the cellulose components (Kumar and Sharma 2017).

In their 2021 study, Wang *et al.* investigated the impact of temperature on corn straw's lignocellulose structure for biomethane production using microwave irradiation. They applied varying conditions, including microwave power ranging from 0 to 1600W, a frequency of 2.45 GHz, pressure varying from 0 to 6MPa, a treatment time of 10 minutes, and temperatures of 120, 150, 180 and 210°C. Their findings revealed that elevating the irradiation temperature adequately removed lignin but reduced cellulose and hemicellulose concentrations, ultimately resulting in the highest methane production at 150°C, yielding 73.08% more methane than the untreated sample (Wang *et al.* 2021).

15.3.2 Chemical Methods

Chemical biomass pretreatment for biofuel generation focuses on lignin breakdown, increasing the reactivity of biomass components, and increasing the accessibility of complex carbohydrates for enzymatic and microbiological conversion. These goals increase biofuel yields and make biofuel manufacturing methods more efficient. The chemical pretreatment methods include using alkali, acids, and solvent pretreatments.

15.3.2.1 Alkali Pretreatment

Alkali pretreatment involves utilizing essential solutions like NaOH and KOH to disrupt the interconnections between lignocellulose components. This process aims to cleave the ester bond that crosslinks lignin and xylan, ultimately promoting the breakdown of lignocellulosic structures. The outcome of alkali treatment is a dignified substrate characterized by increased porosity and augmented surface area. Consequently, these alterations facilitate enhanced enzymatic hydrolysis. Notably, this technique yields intact cellulose, unlike acid-based treatments that tend to fragment the cellulose chains. One of the advantages of alkali treatment is the potential for operation at relatively lower temperatures. However, it is essential to acknowledge that this method is time-intensive. (Das *et al.* 2023). A study focused on the pretreatment of hazelnut husk using a 4% NaOH solution reported a substantial 162.2% increase in methane production compared to the untreated sample (Şenol 2020). In another investigation, which involved pre-treating wheat straw with a 1.6% NaOH solution for 24 hours, a noteworthy 15% rise in methane production was observed (Mancini *et al.* 2018).

15.3.2.2 Acid Pretreatment

The acid pretreatment breaks down the hemicellulose into its constituent sugar monomers, or oligomers by cleaving van der Waals forces, covalent bonds, and hydrogen bonds that hold the biomass components together (Li *et al.* 2010). Depending on the acid concentration in the acid solution, this pretreatment can be classified as either a concentrated or diluted acid pretreatment process. In brief, the primary advantage of concentrated acid pretreatment, compared to dilute acid pretreatment, lies in its operation at significantly lower temperatures (albeit over more extended periods), leading to reduced operational costs. On the flip side, the process equipment is generally more expensive due to the necessity for corrosion-resistant materials. Furthermore, in dilute acid pretreatment, where higher operational temperatures are used, the production of sugar degradation compounds is more prevalent. Some of these compounds, including formic acid, furan-2-carbaldehyde and 5-hydroxymethylfurfural, and levulinic acid, have been identified as potential inhibitors of the growth of methanogenic microorganisms used during anaerobic digestion (Hendriks and Zeeman 2009; Solarte-Toro *et al.* 2019).

In a recent investigation, corn stover underwent pretreatment with a 6% H3PO4 solution and was subsequently subjected to anaerobic digestion. The outcomes revealed a 40.75% surge in biogas production compared to the untreated sample (Tian *et al.* 2016). In a separate study, salvinia molesta was treated with 4% H2SO4, resulting in an impressive 81.78% increase in biogas production (Syaichurrozi *et al.* 2019).

15.3.2.3 Solvent Pretreatment

Solvent pretreatment technologies are pivotal in biomass-to-biofuel conversion, offering a critical pathway to enhance overall efficiency and yield. This method involves strategically applying specialized solvents to transform the structure of lignocellulosic biomass, dismantling the intricate network of cellulose, hemicellulose, and lignin. By selectively dissolving lignin and inducing alterations in the crystalline configuration of cellulose, solvent pretreatment substantially enhances the accessibility of enzymes or catalysts during the subsequent hydrolysis phase. Consequently, this process leads to an intensified release of sugars and their subsequent conversion into biofuels. An array of solvents, ranging from conventional options like water to advanced choices such as ionic liquids, organic solvents, and supercritical CO2, present both advantages and challenges that influence the degree of biomass delignification and solubilization achieved. Beyond the augmentation of biofuel yields, solvent pretreatment also diminishes the necessity for more rigorous conditions in downstream processes, contributing to a more sustainable and economically feasible framework for biofuel production (Das *et al.* 2023).

15.3.2.4 Organosolv Pretreatment

Organosolv pretreatment is a versatile and adaptable process involving diverse combinations of organic or aqueous solvents, including, but not limited to, methanol, ethanol, acetone, ethylene glycol, and tetrahydrofurfuryl alcohol. This method primarily aims to dissolve lignin, a pivotal step that enhances the accessibility of cellulose and hemicellulose for ensuing conversion procedures. A distinctive advantage of the organosolv approach, setting it apart from alternative pretreatment methods, is its capability to yield pure lignin as a valuable by-product. Unlike other techniques that may lead to lignin degradation or the production of impurities, organosolv offers the potential to recover lignin in a form well-suited for various industrial applications. Nevertheless, a key consideration in organosolv pretreatment is the effective removal of solvents. Solvents can impede enzymatic hydrolysis and fermentation processes in downstream stages. To address this concern, a preference is given to low-molecular-weight solvents, such as ethanol and methanol. These solvents are favored due to their lower boiling points and simplified extraction, streamlining solvent recovery. The risk of solvent-related inhibitory effects on subsequent biochemical processes is minimized by employing solvents that can be readily separated from the treated biomass (Merklein, Fong and Deng 2016; Mancini *et al.* 2018). When wheat straw underwent pretreatment using this method with 50% ethanol as an organic solvent at 180°C for one hour, the subsequent anaerobic digestion yielded a remarkable 15% increase in methane production compared to the untreated sample (Mancini *et al.* 2018).

15.3.2.5 Ionic Liquids

Ionic liquids are environmentally friendly substances composed of a single cation and a single anion, with melting points below 100°C (Lei *et al.* 2017; Sriariyanun *et al.* 2022). These remarkable compounds can degrade lignin and disrupt the crystalline structure of cellulose. Their mechanism involves specifically targeting the hydrogen bonding within lignocellulose, resulting in the facilitation of enzymatic hydrolysis. A distinctive feature of many ionic liquids is their potential for recovery and reuse, reducing their overall consumption. This characteristic contributes to their heightened environmental sustainability compared to other solvents. Notably, imidazolium salts, a class of ionic liquids, are widely employed in biomass pretreatment due to their effectiveness (Sriariyanun *et al.* 2022; Abed *et al.* 2023). Mancini *et al.* (2018) observed an 11% increase in methane yield by pre-treating wheat straw with 85% N-methylmorpholine-N-oxide for three hours at 120°C (Mancini *et al.* 2018).

15.3.2.6 Deep Eutectic Solvents

Deep eutectic solvents (DES) represent a greener alternative than ionic liquids, boasting distinct physicochemical attributes such as non-toxicity, inertness toward water, and facile synthesis. DES consists of binary or ternary mixtures of hydrogen bond acceptors and donors, making them acid- or alkali-based. These solvents offer a unique advantage due to their abundance of intramolecular hydrogen bonds, which facilitates the cleavage of hydrogen bonds within biomass samples. This solubility enhancement leads to heightened conversion rates. In acidic DES, the hydrogen bond donor assists in breaking down the hydrogen bonds. However, the application of DES can result in the condensation of lignin, which is unfavorable for its subsequent utilization. Conversely, alkali-based DES exhibits pronounced delignification effects, yielding lignin fractions with well-retained substructures highly suitable for valorization. Among alkali DES, Akanolamine alkali DES has demonstrated superior efficacy as a delignification solvent (Abed *et al.* 2023; Chen and Mu, 2019; Miftah *et al.* 2022).

15.3.3 Biological Methods

Biological pretreatment of biomass is by far the most cost-effective pretreatment method because it uses less energy, and no chemicals are required. However, these methods are time-consuming. This pretreatment employs microorganisms, such as fungi, and enzymes to delignify the lignocellulose (Shrestha *et al.* 2017). During biological pretreatment, cellulose and hemicellulose are typically converted into individual sugar units through the action of cellulolytic and hemicellulolytic microorganisms, making it easy for downstream biofuel conversion processes. Each fungus and enzyme are different from the next because they are substrate specific, that is, they are lignocellulosic polymer specific (Sharma *et al.* 2019).

15.3.3.1 Fungal Pretreatment

Fungi, renowned microorganisms, are well-acknowledged for their proficiency in engaging with and decomposing lignocellulosic materials via their enzymatic capabilities. The breakdown of lignocellulose can be achieved through either intracellular or extracellular mechanisms. The extracellular mechanism employs two enzyme categories: hydrolytic enzymes, responsible for polysaccharide breakdown, and oxidative enzymes, tasked with lignin degradation (Andlar *et al.* 2018).

Three primary categories of fungi are distinguished for their prowess in lignocellulose degradation: white, brown, and soft rot fungi, each employing distinct mechanisms (Andlar *et al.* 2018; Isroi *et al.* 2011). Soft-rot fungi are classified as ascomycetes, whereas brown and white rot fungi belong to the basidiomycete group (Andlar *et al.* 2018).

Soft-rot fungi specialize in degrading surface layer plant polysaccharides, leading to wood darkening and softening. This process is facilitated by producing laccases and peroxidases, primarily contributing to lignin modifications. While these enzymatic processes are less specific than their counterparts in white-rot and brown-rot fungi, the precise mechanisms underlying lignocellulose degradation by soft-rot fungi remain an area of limited scientific understanding. Brown-rot fungi, on the other hand, are adept at rapidly metabolizing cellulose and hemicellulose while making minor modifications to lignin. They do not possess lignin-degrading enzymes but rely on small reactive molecules to depolymerize lignin. In advanced stages of degradation, the remaining wood takes on a cubical appearance with a brownish hue, primarily due to the accumulation of oxidized lignin (Andlar *et al.* 2018).

In contrast, lignin presents a complex challenge in selecting efficient fungal strains. White-rot fungi, aptly named for the characteristic white residues they leave on biomass after colonization, represent the most diverse basidiomycetes used for lignin pretreatment. They play a pivotal role in lignin disintegration and are considered natural lignin-degrading microorganisms. These fungi produce a range of ligninolytic enzymes, including laccases, lignin peroxidases, and manganese peroxidases (Isroi *et al.* 2011; Mayans *et al.* 2023; Zhao *et al.* 2020).

Non-selective fungi simultaneously degrade all lignocellulose components, resulting in the loss of fermentable sugars for downstream processes. In contrast, selective fungi primarily release hemicellulolytic enzymes and rely on sugars derived from hemicellulose as their primary carbon source for growth and metabolism, thereby preserving cellulose. For the breaking down of more lignin while safeguarding more significant amounts of cellulose and hemicellulose to enhance biomass digestibility, it is advisable to employ white-rot fungi with heightened lignin selectivity and reduced holocellulose selectivity, thereby delignifying the lignocellulose without compromising holocellulose (X. Zhao *et al.* 2020). In a 10-day pretreatment of rice straw to enhance biogas production, two distinct fungal strains, *Trichoderma Longibrachiatum* (TL) and *Pycnoporus Sanguineus* (PS), were used. The study showed that these strains significantly reduced the chemical oxygen demand of the biomass, with reductions of 71.43% for TL and 64.70% for PS. Furthermore, during anaerobic digestion, the pretreated biomass produced a substantial increase in biogas production, yielding 20.79% more biogas for TL and 17.85% more biogas for PS compared to untreated samples (Rani and Dhoble 2023).

15.3.3.2 Enzymatic Pretreatment

Commercial enzymes are used during the enzymatic pretreatment process to break down lignocellulosic materials. The primary enzymes employed for this purpose are lactases and manganese peroxidase, which are well-known for breaking down cellulose, hemicellulose, and lignin. Lactase and manganese peroxidase enzymes assist in deleting fermentable sugars within the LC biomass, enhancing methane production (Amin *et al.* 2017; Yunqin, Dehan, and Lishang 2010). However, a drawback of enzymatic pretreatment is the prohibitive cost of obtaining these enzymes (Amin *et al.* 2017). For instance, when paper and pulp sludge were subjected to pretreatment using the enzyme endoglucanase laccase, there was a 34% increase in methane yield (Yunqin *et al.* 2010).

In another study, waste sugar beet pulp, characterized by a particle size of 0.25 cm, and spent hops were subjected to an enzymatic pretreatment process involving endoglucanase, xylanase, and pectinase enzymes. A 10% w/v dry matter suspension of sugar beet pulp and spent hops was combined with these enzymes, and the hydrolysis process was conducted at an elevated temperature of 50°C. Upon subsequent anaerobic digestion, these enzymatically treated samples demonstrated a significant enhancement in biogas production, yielding approximately 19% and 13% more biogas compared to their untreated counterparts (Ziemiński, Romanowska and Kowalska 2012).

15.3.4 Physicochemical Methods

Physicochemical pretreatment techniques represent a distinct category of biomass pretreatment methods that synergistically employ both chemical and physical processes to facilitate the breakdown of lignin and hemicellulose.

15.3.4.1 Steam Explosion

Steam explosion is a widely used physicochemical pretreatment method because of its ability to delignify the substrate, hydrolyze hemicellulose, and reduce the cellulose's crystallinity. Additionally, this method is eco-friendly as it does not use chemicals. Generally, the steam explosion biomass pretreatment method disrupts the lignocellulose by employing high-temperature steam at elevated pressure for a set time (maximum of 60 minutes) (Siddhu *et al.* 2016; Lizasoain *et al.* 2017; J. Singh, Suhag, and Dhaka 2015). This is followed by a rapid reduction in pressure to atmospheric levels or lower, causing the biomass to decompress into individual fibers and fiber bundles explosively. The explosion phenomenon is initiated by the internal moisture evaporation within the biomass cells and the abrupt drop in pressure surrounding the biomass (Lizasoain *et al.* 2017).

15.3.4.2 Hydrothermal Pretreatment

In contrast to the steam explosion technique, hydrothermal pretreatment operates by utilizing the nearly supercritical temperatures of water to break down lignocellulosic materials. During this process, pressurized water infiltrates the biomass, causing the cell walls to lose their structure and removing a significant portion of hemicellulose and a fraction of the lignin. When exposed to elevated temperatures, water alters the pH and facilitates the release of O-acetyl, acetic, and uronic acids from hemicellulose

(Fernández-Cegrí *et al.* 2012; Martín *et al.* 2022). Despite its high efficiency in methane conversion, it is essential to note that hydrothermal pretreatment is energy-intensive (Karrabi *et al.* 2023). In a study by (Phuttaro *et al.*2019) the hydrothermal pretreatment of Napier grass at a temperature of 175°C for a 15-minute incubation period resulted in a remarkable 35% increase in methane production yield.

15.3.4.3 Ammonium-Based Pretreatment

Researchers worldwide have investigated using anhydrous ammonia for the pretreatment of lignocellulosic biomass in biofuel production. Some of the methods that employ ammonia for lignocellulose pre-treatment include ammonia fiber explosion (AFEX), soaking in aqueous ammonia (SAA), and ammonia recycle percolation (ARP) (C. Zhao, Shao and Chundawat 2020). The benefits associated with these approaches encompass enhanced wettability and increased surface area in the pretreated biomass. Moreover, most of the ammonia used in these processes can be recovered, with any unrecovered ammonia serving as a nutrient source for microorganisms during hydrolysis. Additionally, these methods promote cellulose recrystallization and effectively preserve both hemicellulose and cellulose with minimal to no degradation (Campbell *et al.* 2013; Chundawat *et al.* 2007; Mosier *et al.* 2005; Rabemanolontsoa and Saka, 2016; Teymouri *et al.* 2005; Zhao *et al.* 2020). The disadvantages of these methods include the costs associated with ammonia recycling, and there are safety and environmental concerns associated with handling and storing ammonia on-site in both pilot and industrial-scale facilities (Sendich *et al.* 2008).

The ammonia fiber explosion (AFEX) is a physicochemical treatment where lignocellulose is pretreated in a similar process to steam explosion. In AFEX, lignocellulosic biomass undergoes a steaming process involving liquid ammonia, mild temperature, and high pressure for a specified duration, followed by a rapid reduction in pressure. The application of pressurized ammonia induces swelling in the biomass, leading to an increase in its accessible surface area and a simultaneous decrease in cellulose crystallinity (Rabemanolontsoa and Saka 2016; C. Zhao, Shao, and Chundawat 2020; Mosier *et al.* 2005). Typically, this process involves introducing biomass to liquid ammonia at 1–2 kg of ammonia per kg of dry biomass at temperatures ranging from 60–90°C, with a residence time of 30 minutes and pressures between 40 and 50 atm—the sudden release of pressure results in ammonia evaporation, causing a drop in the system's temperature. During AFEX pretreatment, only a small fraction of hemicellulose degrades into soluble oligomers, and the lignin distribution remains unchanged, though its structure is altered. Consequently, the biomass's water-holding capacity and digestibility increases.

SAA, a modified AFEX variant, treats biomass in a batch reactor at moderate temperatures (25–60°C). While mild process conditions reduce the formation of sugar degradation products and fermentation inhibitors, the treatment duration can extend from 10 to 60 days. In SAA pretreatment, delignification is highly selective, leaving most of the carbohydrates in the solids, as it minimizes interaction with hemicellulose. This increases surface area and pore size, with minimal sugar loss after water washing (Kim, Lee and Kim 2016; Kim *et al.* 2003; Kim and Lee 2005).

ARP, conversely, involves the percolation of aqueous ammonia (10–15%) through biomass in a flow-through column reactor at elevated temperatures (150–190°C). The flow rate ranges from 1–5 mL/min, with 10–120 minutes residence times, after which the ammonia is either recycled or recovered. The reactor system must be slightly pressurized to prevent evaporation (Kim, Lee and Kim 2016).

15.4 Conclusion

The stubborn nature of lignocellulosic biomass presents a significant obstacle for producing biofuels through anaerobic digestion. Various methods have been created for preparing the biomass to overcome this challenge. However, these approaches often come with significant expenses, whether it be the cost of acquiring the necessary equipment, the substantial energy needed for the process, or the time it takes, which can sometimes limit progress. As a result, there is a pressing need to design and develop cost-efficient, time-saving, and energy-conserving equipment for reducing biomass size. One promising avenue is the genetic modification of microorganisms capable of efficiently removing lignin from lignocellulose, thus reducing the time required for biological pretreatment. If this can be achieved at a high level, biological pretreatment may even replace the traditional mechanical pretreatment used for size reduction, ultimately making biofuel conversion more economically viable.

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