**Recent advances in anaerobic bioconversion of lignocellulosic wastes – A review**

**Abstract**: This review explores recent advancements in the anaerobic bioconversion of lignocellulosic wastes into biogas, emphasizing its potential as a sustainable solution to the global energy crisis and environmental degradation. Lignocellulosic biomass, derived from agricultural residues, forestry byproducts, and organic waste, offers an abundant and renewable feedstock for bioenergy production. However, its complex structural composition—primarily cellulose, hemicellulose, and lignin—poses significant challenges to biodegradability in anaerobic digestion (AD) systems. To overcome this, a variety of pretreatment techniques have been developed to enhance substrate accessibility and improve biogas yields. This review categorizes and critically evaluates these methods, including physical, chemical, hydrothermal, biological, and hybrid pretreatments, focusing on their mechanisms, effectiveness, limitations, and impacts on methane production. Additionally, the paper discusses the role of co-digestion strategies, feedstock variability, and microbial dynamics in optimizing AD processes. By synthesizing current literature and technological developments, this review provides a comprehensive reference for researchers and practitioners seeking to advance efficient and economically viable bioenergy systems using lignocellulosic waste.

**1 Introduction**

**1.1 Energy Context**

Global energy consumption is projected to rise from 580 EJ in 2018 to nearly 700 EJ by 2050, driven by population growth and expanding industrial activities (Nations, 2022). Despite rapid growth in wind and solar installations, fossil-based sources—coal, oil, and natural gas—remain the backbone of global energy systems, accounting for over 75% of primary energy supply and contributing significantly to greenhouse gas emissions (Olabi and Abdelkareem, 2022). This continued reliance on carbon-intensive fuels exacerbates climate change and undermines sustainability objectives outlined by the Paris Agreement. Moreover, the production and disposal of agricultural and forestry residues present environmental challenges that demand sustainable valorization pathways (Islam et al., 2020). Addressing these interlinked issues requires integrated approaches that couple renewable energy generation with waste management, thereby fostering circular economy practices and reducing dependency on imported hydrocarbons.

**1.2 Generations of Biofuels**

Biofuel technologies have undergone three distinct developmental stages, each driven by evolving feedstock availability and environmental imperatives. First-generation biofuels utilize edible crops such as maize and sugarcane to produce ethanol or biodiesel; their deployment proved the technical viability of bioenergy but sparked concerns about food security and land-use change (Khan et al., 2022). The second generation, emerging in the early 2000s, shifted focus to lignocellulosic feedstocks—agricultural residues, forestry byproducts, and dedicated energy crops—to avoid direct competition with food resources. However, the inherent recalcitrance of lignocellulose posed challenges in pretreatment, hydrolysis, and fermentation, limiting commercial uptake (Zheng et al., 2017; Hagman et al., 2018). Recent progress, including novel reactor designs and enzyme cocktails, has begun to narrow this gap (Dahmen et al., 2019). Third-generation biofuels explore advanced systems such as algae-based lipids and microbial engineering to produce drop-in fuels; while promising high yields and reduced land footprint, these approaches remain at pilot or demonstration scale due to high capital and operational costs (Shrestha et al., 2021).

**1.3 Why Lignocellulose for Anaerobic Digestion**

Among alternative biofuel pathways, anaerobic digestion (AD) of lignocellulosic biomass holds particular promise by simultaneously generating renewable methane and valorizing waste streams. Globally, an estimated 180–200 billion tonnes of lignocellulosic residues are produced annually from agriculture and forestry (Gatto and Drago, 2021), yet only a fraction undergoes biological conversion. Under optimal AD conditions, these residues can yield up to 0.35–0.40 m³ of methane per kilogram of volatile solids, offering energy returns comparable to liquid biofuels (Nigam and Singh, 2011). Furthermore, AD produces nutrient-rich digestate that can be recycled as biofertilizer, closing nutrient loops and displacing synthetic fertilizers (Kamaraj et al., 2019). Despite these advantages, the rigid structure of lignocellulose—composed of cellulose, hemicellulose, and lignin—impedes microbial accessibility, necessitating pretreatment strategies to enhance biodegradability (Shahid et al., 2021; Islam et al., 2020). This review synthesizes existing physical, chemical, biological, and hybrid pretreatment methods, evaluating their impacts on methane yield, process economics, and environmental footprint. By consolidating recent techno-economic analyses alongside laboratory performance data, we aim to provide a roadmap for selecting appropriate pretreatment techniques to advance sustainable biogas production.

**2. Anaerobic Digestion of Lignocellulosic Biomass**

**2.1 Importance of Biogas in Renewable Energy Systems**

Biogas plays a significant role in meeting renewable energy goals and has potential applications in both energy production and transportation, especially with further technological advancements. Considerable research has been dedicated to various aspects of biogas systems, including its generation, production enhancement (Prabhu et al., 2021), and the optimization of process configurations (Mirmohamadsadeghi et al., 2019). One particularly efficient method of utilizing renewable biomass resources is the production of biogas from lignocellulosic materials such as forest residues, agricultural waste, energy crops, organic fractions of municipal solid waste, and various industrial wastes including wood, paper, and pulp.

**2.2 Anaerobic Co-Digestion and Challenges with Lignocellulosic Feedstocks**

In recent years, anaerobic co-digestion has gained momentum as a strategy to enhance methane yield and nutrient recovery while maintaining overall digester stability (Yang et al., 2021). Among the most abundant feedstocks for biogas production are agricultural crop residues, which when subjected to anaerobic digestion (AD), not only contribute to renewable energy generation but also aid in mitigating the environmental impact of agricultural waste. While considerable attention has been directed toward the digestion of manure, sludge, and food waste (Kumar et al., 2021), the anaerobic conversion of crop residues remains a challenge. This is primarily due to the complex physicochemical characteristics of lignocellulosic materials, which include high lignin and cellulose content, structural rigidity, low bulk density, poor fluid dynamics, a high carbon-to-nitrogen (C/N) ratio, and a deficiency in trace elements. These features, along with variable particle size and morphology, necessitate the development and implementation of tailored technologies to improve their biodegradability and conversion efficiency (Sun et al., 2021).

**2.3 The Role of Pre-treatment in Biogas Production**

While anaerobic digestion represents a promising renewable energy solution, the effective conversion of lignocellulosic biomass remains hindered by the structural complexity of its components—primarily cellulose, hemicellulose, and lignin. The overall conversion process typically includes three key stages: pre-treatment, enzymatic hydrolysis, and anaerobic digestion. Among these, pre-treatment is recognized as the most energy-intensive phase due to its role in disrupting the rigid lignocellulosic matrix, solubilizing hemicellulose, and partially removing or altering lignin content to enhance microbial accessibility (Poddar et al., 2021). Integrating pre-treatment into the AD workflow helps address inherent limitations in substrate digestibility and leads to improved biogas yields (Yu et al., 2019).

A wide array of pre-treatment strategies has been explored to enhance the suitability of lignocellulosic biomass for AD. These include physical, thermal, chemical, biological, combined, and emerging pre-treatment technologies. Ongoing research has also extended to non-conventional and green pre-treatment methods suitable for biorefinery integration, as well as the application of nanotechnology, biological agents, and enzymatic enhancements to boost biomethane production. Each of these approaches aims to improve process efficiency while maintaining environmental and economic sustainability.

**2.4 Microbial Dynamics and Process Optimization in AD**

Roopnarain and Adeleke (2017) have clearly outlined the sequential stages involved in the anaerobic digestion process: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Figure 1). These biological transformations rely on complex microbial consortia, each adapted to a specific phase of degradation. The pivotal role of microorganisms in the breakdown of organic matter and the production of biogas is well recognized, as originally detailed by Chaiprasert (2011). Although biogas typically contains a lower methane concentration (50–60%) compared to liquefied petroleum gas (LPG) or compressed natural gas (CNG), global efforts continue to support its commercialization as a renewable, cost-effective, and environmentally preferable alternative to conventional fossil-based fuels (Garcia et al., 2019).

To maximize the efficiency of anaerobic digestion systems, it is imperative to apply scientific and engineering approaches that optimize methane recovery across a variety of feedstocks. Parameters such as substrate composition, operating conditions, and digester configuration play critical roles in determining overall system performance (Kumar et al., 2015). The natural degradation of organic matter highlights the ubiquity of microbial agents capable of breaking down diverse compounds, and a deeper understanding of these microbial dynamics, coupled with comprehensive feedstock characterization, offers an avenue for developing precise and sustainable solutions to current and future energy challenges. In this context, the present review examines anaerobic digestion strategies aimed at enhancing biogas production from lignocellulosic feedstocks.

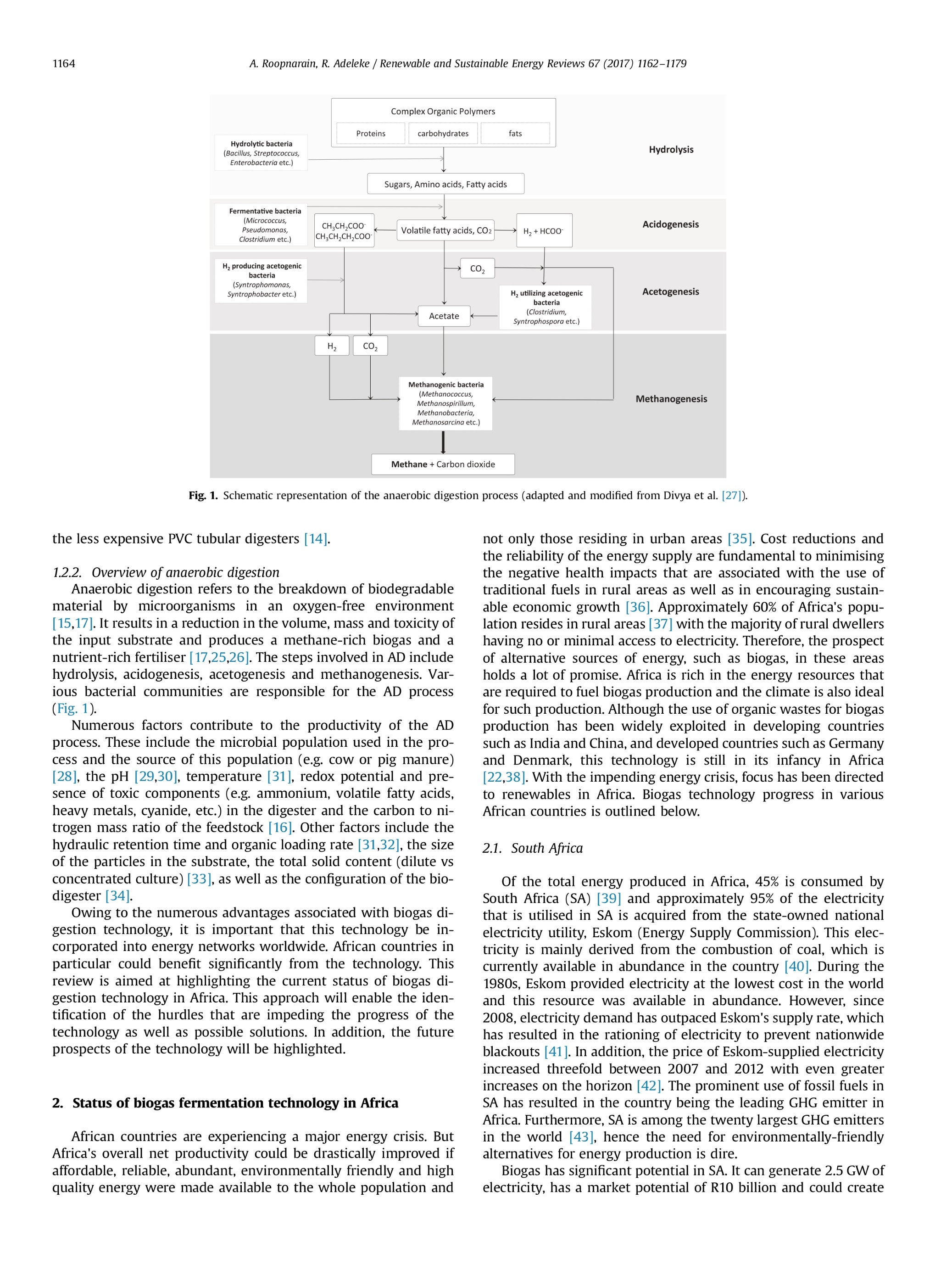


Fig. 1: Schematic representation of the anaerobic digestion process (Roopnarain, A., & Adeleke, R. (2017))

**2.5 Co-Digestion Strategies for Enhanced Biogas Production**

The global energy crisis necessitates the development of environmentally sustainable and scalable solutions. To achieve meaningful progress in commercialization and industry integration, innovation rooted in scientific research is essential. Traditionally, anaerobic digestion systems have focused on mono-substrate digestion; however, their limited efficiency across varying substrate types has prompted a shift toward co-digestion strategies. The co-digestion of multiple organic substrates has gained increasing attention as a viable approach to improve methane yields and process stability. Emerging evidence suggests that co-digestion of complementary biodegradable wastes results in significantly higher methane production compared to single-substrate digestion systems. Organic substrates inherently contain a range of macro- and micronutrients necessary for the growth and function of both aerobic and anaerobic microbial communities. Importantly, these nutrient profiles can vary based on species, cultivation conditions, and material maturity (Hagos et al., 2017).

Anaerobic co-digestion has become a widely adopted method for improving biogas output in digesters. Numerous studies have focused on combining livestock manure with other organic materials to boost methane production rates (Salminen and Rintala, 2002). By enabling the simultaneous digestion of two or more substrates, co-digestion offers a strategic advantage over mono-digestion in terms of methane yield and economic feasibility (El-Mashad and Zhang, 2010; Li et al., 2010; Siddique and Wahid, 2018). This method allows better balancing of carbon-to-nitrogen (C/N) ratios, enhanced buffering capacity, and dilution of potential toxic compounds.

For instance, a study conducted by Yong et al. (2015) in China examined the co-digestion of food waste and straw. Utilizing a 1-liter enclosed reactor maintained at 35°C with a total organic load of 5 g VS/L, they assessed various food waste–straw mixtures. The optimal ratio of food waste to straw (5:1) achieved a peak methane production yield (MPY) of 0.392 m³/kg-VS, representing increases of 39.5% and 149.7% compared to individual digestion of food waste and straw, respectively. Gas production (GP) and methane content reached 0.58 m³/kg-VS and 67.62%, respectively. The study further analyzed particle size effects and recommended a straw size of 0.3–1 mm as optimal for maximizing energy efficiency and cost-effectiveness (Almomani and Bhosale, 2020).

In a separate investigation, Almomani and Bhosale (2020) explored the anaerobic co-digestion of agricultural solid wastes (ASWs) and cow dung (CD), as well as the impact of sodium bicarbonate (NaHCO₃) alkalinity treatment. Their results demonstrated that a 60:40 ASW to CD ratio yielded the highest cumulative methane production (CMP) of 297.99 NL/kg-VS. Notably, the addition of NaHCO₃ at a dose of 1.0 g/gVS enhanced the CMP to 386.3 NL/kg-VS, highlighting the potential of alkalinity regulation in improving process efficiency.

**3. Feedstock and Biomass Composition**

**3.1 Feedstock for Biogas Production**

The exploration and utilization of organic wastes have gained considerable momentum due to advances in sustainable development, particularly in the domains of renewable energy production and waste management. While anaerobic digestion systems were originally employed for the treatment of animal and vegetable wastes, their application has now broadened significantly. Today, biogas can be generated from a diverse array of feedstocks, including agricultural, industrial, and municipal waste streams. The quality and stability of biogas production depend heavily on the properties of the feedstock. Parameters such as moisture content, volatile solids concentration, nutrient availability, and particle size play a significant role in influencing the efficiency and stability of the anaerobic digestion process (Dobre et al., 2014).

To enhance the effectiveness of feedstock utilization, advanced processing strategies have been introduced. Technological innovations such as optimized feedstock selection, impurity removal systems, co-digestion trials, and pre-treatment technologies have shown great potential in improving biogas yield and process efficiency (Brown and Li, 2013; Almomani et al., 2019). These advancements are crucial for maximizing energy recovery from a broad range of organic waste resources.

**3.2 Composition of Lignocellulosic Biomass**

Lignocellulosic biomass is composed primarily of three major biopolymers: cellulose (typically 80–90%), hemicellulose (10–50%), and lignin (5–35%), along with minor components such as proteins, ash, and pectin (Xu and Li, 2017). The relative proportions of these components vary significantly depending on the biomass source, as illustrated in Figure 2.

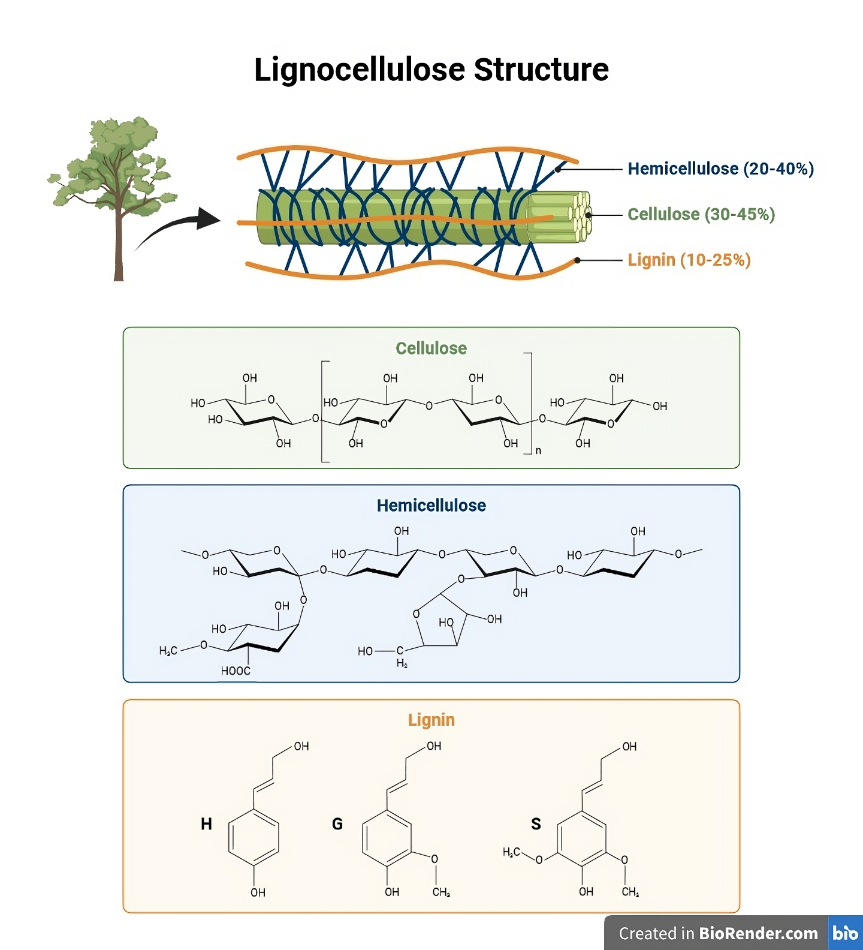


Fig.2: Structure of a biomass in general

Cellulose, the most abundant polymer, is a linear polysaccharide made up of D-glucose subunits linked through β-(1,4)-glycosidic bonds. This polymer forms a crucial part of the structural framework in plant cell walls (Kumar et al., 2017). Though cellulose is generally insoluble in water under neutral conditions, it can be dissolved in specific solvents such as ionic liquids (ILs) and N-methylmorpholine-N-oxide (NMMO), which aid in its processing and application (Kumar et al., 2020). Cellulose is valued for its biocompatibility, hydrophilicity, stereoregularity, and potential applications in fuel production and the development of bio-based chemicals (Jedvert and Heinze, 2017).

Hemicellulose, the second principal component, comprises a branched heteropolymer consisting of several sugar monomers, including xylan, galactomannan, glucuronoxylan, glucomannan, arabinoxylan, and xyloglucan. These are linked by glycosidic bonds in a more amorphous and less polymerized structure compared to cellulose. Due to its relatively low crystallinity, hemicellulose is more readily hydrolyzed and is widely utilized in industrial applications such as hydrogels and drug delivery systems (Liu et al., 2019).

Lignin is a highly complex aromatic polymer derived from the radical coupling of three main monolignols: p-hydroxycinnamyl, coniferyl, and sinapyl alcohols. These precursors generate the structural phenylpropanoid units known as p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) groups, respectively (Sluiter et al., 2008). Lignin contributes mechanical strength and rigidity to plant tissues and constitutes 15–40% of the dry weight of plant biomass such as wood and straw. It is significantly more resistant to microbial and enzymatic degradation compared to cellulose and hemicellulose. As one of the most abundant organic polymers in nature, lignin plays essential roles in plant structure and carbon cycling. It also finds numerous applications in agriculture and industry. For instance, in the paper industry, lignin must be removed during the pulping process to produce high-quality paper (Echresh et al., 2019).

**3.3 Biomass Recalcitrance and Pre-treatment**

One of the primary challenges in converting lignocellulosic biomass to bioenergy is its inherent recalcitrance, which refers to the structural resistance to chemical and biological degradation. Effective utilization of lignocellulosic feedstocks requires overcoming obstacles such as the crystallinity of cellulose, the high lignin content, and the complex, heterogeneous architecture of plant cell walls. Pretreatment processes play a critical role in disrupting this rigid structure. Through mechanisms such as lignin depolymerization, hemicellulose solubilization, and reduction in cellulose crystallinity, pretreatment enhances substrate accessibility and digestibility (Zoghlami and Paës, 2019).

Over the past few decades, numerous pretreatment strategies have been developed to address these challenges. As depicted in Figure 3, these methods are broadly categorized into physical, chemical, biological, and combined approaches. The selection of an appropriate pretreatment technique is closely tied to the feedstock composition, particularly the relative abundance of cellulose, hemicellulose, and lignin (Dadhah et al., 2017). Each method targets specific structural barriers, and their effectiveness can vary depending on the biomass source. In subsequent sections, various pretreatment techniques will be discussed in detail, examining their impacts on the separation and conversion of lignocellulosic components (Baruah et al., 2018).

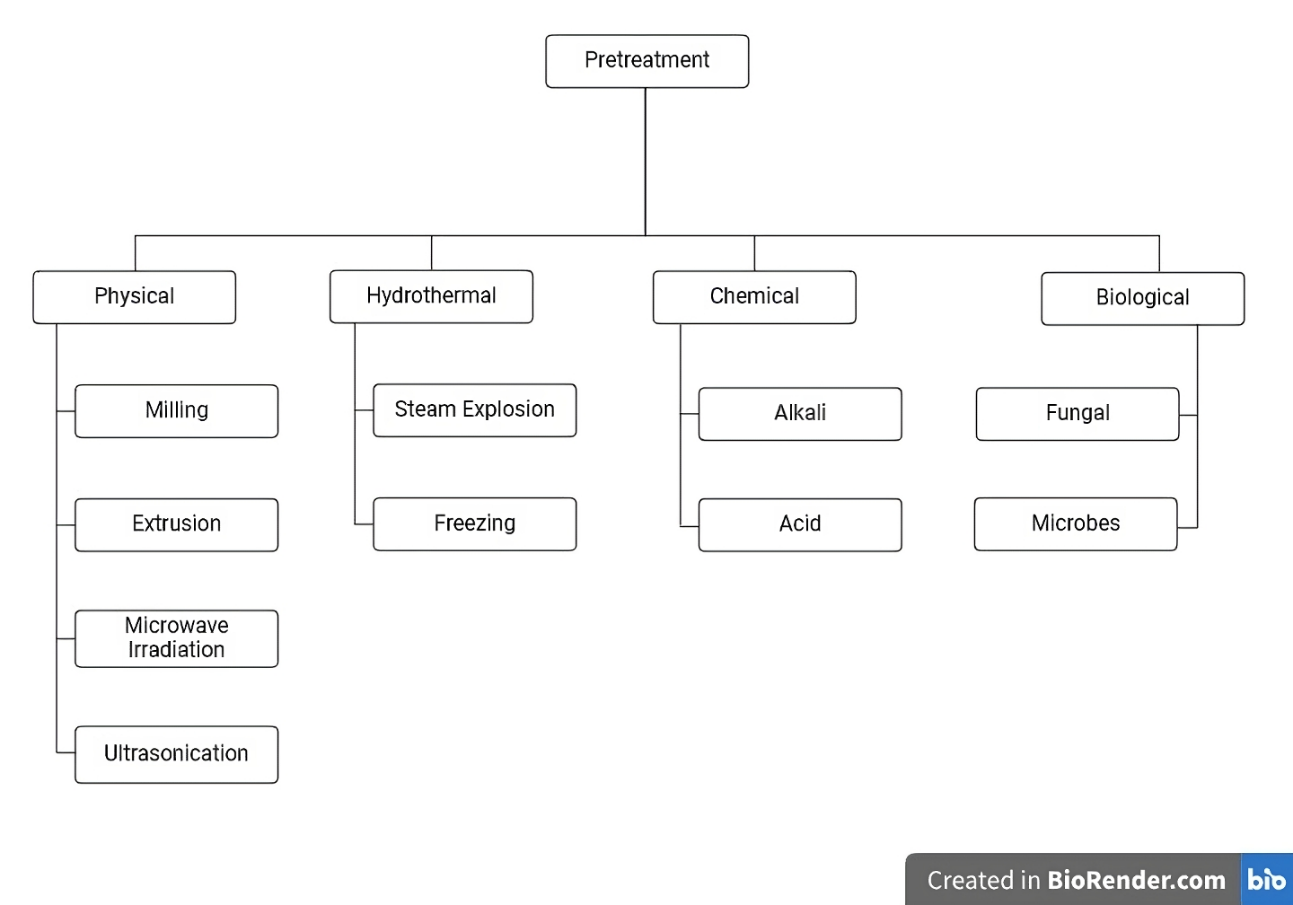


Fig. 3: Different lignocellulosic biomass pretreatment techniques

**Table 1: Different lignocellulosic biomass pretreatment methods, their effects and drawbacks.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Pretreatment Method** | **Process** | **Effect on biomass** | **Drawback** | **Refererence** |
| Physical | Milling | Increases the surface area and accessibility of lignocellulosic biomass for subsequent enzymatic hydrolysis | One drawback is the potential for increased energy consumption during the milling process.  Top of Form | Dahunsi, 2019; Mayer-Laigle et al., 2018; Baruah et al., 2018; Mankar et al., 2021; Sitotaw et al., 2021; Zhang et al., 2019 |
| Extrusion | Disrupts lignocellulosic biomass structure, improving enzymatic accessibility for bioconversion | One drawback is the potential for high energy consumption and equipment costs.  Top of Form | Hjorth et al., 2011; Konan et al., 2022; Duque et al., 2017; Victorin et al., 2020 |
| Microwave Irradiation | Enhances lignocellulosic biomass enzymatic digestibility by breaking down structural barriers | One drawback is uneven heating leading to localized hot spots in the biomass.  Top of Form | Aguilar-Reynosa et al., 2017; Calcio Gaudino et al., 2019; Li et al., 2016 |
| Ultrasonication | Increases lignocellulosic biomass enzymatic digestibility by disrupting its structure at a molecular level | high equipment cost associated with ultrasonication devices | Pilli et al., 2011; Dhandayutha- pani et al., 2021 |
| Hydrothermal | Steam explosion | Enhances lignocellulosic biomass enzymatic digestibility by breaking down hemicellulose and lignin barriers | One drawback is the potential for formation of inhibitors like furfural and acetic acid, which can hinder downstream processes and high energy consumption associated with the explosion process.  Top of Form | Zheng et al., 2021; Sun and Cheng, 2002; Lam, 2011; Menardo et al., 2013; Datar et al., 2007 |
| Freezing | Disrupts lignocellulosic biomass structure, facilitating enzymatic hydrolysis | One drawback is the potential for cell wall damage due to ice crystal formation, affecting biomass integrity | Rooni et al., 2017; Guan et al., 2021; Smichi et al., 2016; Cano et al., 2015; Yang et al., 2021; Jan et al., 2008 |
| Chemical | Alkali | Delignifies lignocellulosic biomass, improving enzymatic hydrolysis efficiency | One drawback is the potential for lignin redistribution and formation of alkali-resistant compounds, limiting enzymatic digestibility | Badiei et al., 2014; Rabelo et al., 2009; Park and Kim, 2012. |
| Acid | Solubilizes hemicellulose and enhances enzymatic digestibility of lignocellulosic biomass | generation of inhibitors like furfural and hydroxymethylfurfural, which can inhibit downstream fermentation processes | Guan et al., 2021; Monlau et al., 2013; Syaichurrozi et al., 2019; Taherdanak et al., 2016; Tsai et al., 2021. |
| Biological | Fungal | Enzymatically degrades lignocellulosic biomass, increasing its digestibility for bioconversion processes | requirement for specific fungal strains, which may limit its applicability and efficiency. | Zheng et al., 2014; Taherzadeh and Karimi, 2008 |
| Microbes | Enhances lignocellulosic biomass degradation and enzymatic accessibility for bioconversion | One drawback is the potential for longer processing times and the need for strict control of microbial conditions | Zhang et al., 2019; Badiei et al., 2014, Taherzadeh and Karimi, 2008 |

**4. Pretreatment**

Pretreatment is a crucial step in the anaerobic digestion of lignocellulosic biomass due to the natural recalcitrance of its structural components. The primary goal of pretreatment is to break down the complex architecture of lignin, cellulose, and hemicellulose, thereby enhancing microbial accessibility and improving biogas yields. Pretreatment methods are generally categorized as physical, chemical, biological, or a combination of these approaches (Table 1).

**4.1 Physical Pretreatment**

Physical pretreatment involves the application of mechanical processes such as chipping, grinding, milling, extrusion, and cavitation to alter the physical characteristics of lignocellulosic biomass. The primary aim is to disrupt the plant cell wall’s waxy surface and lignin encrustation, thus increasing the substrate's surface area. This facilitates greater microbial accessibility and accelerates the breakdown of cellulose and hemicellulose during anaerobic digestion (Akhtar et al., 2016).

**4.1.1 Milling**

Milling employs various mechanical forces—impact, compression, friction, and shear—to induce both physical and chemical alterations in the biomass structure. This process effectively reduces particle size, lowers the degree of polymerization, and decreases cellulose crystallinity, thereby weakening the rigid plant matrix (Dahunsi, 2019). Often performed as an initial step in pretreatment, milling accelerates fermentation by allowing faster enzymatic or microbial degradation of substrates. Moreover, finely milled particles exhibit enhanced buffering capacity, which can help resist acidification during digestion (Mayer-Laigle et al., 2018).

The advantages of milling include its chemical-free operation and room-temperature processing, which eliminates costs associated with chemical procurement and post-treatment washing or filtration. However, the method is energy-intensive and requires high-capacity mechanical equipment, making it expensive and less feasible at scale unless integrated with other techniques (Baruah et al., 2018; Mankar et al., 2021).

Multiple milling technologies are employed in lignocellulosic preprocessing, such as two-roll milling, rod milling, ball milling, wet disk milling, hammer milling, centrifugal milling, and colloid milling. The appropriate milling method depends on factors including biomass type, moisture content, target particle size, and the intended downstream application (Sitotaw et al., 2021; Zhang et al., 2019).

Among these, wet disk milling is particularly noted for its lower energy consumption (Baruah et al., 2018). This method utilizes two grooved, counter-rotating discs—or a combination of a stationary and a rotating disc moving in opposite directions—to create shear forces that break down biomass in a continuous flow. In scenarios where chemical agents are applied concurrently, the system must be built with corrosion-resistant materials. Although wet disk milling is efficient, it has minimal effect on the crystallinity and polymerization degree of lignocellulose (Rajendran et al., 2018). Additionally, it requires a large volume of water to maintain low solid loading, which can be a limitation.

Ball milling is considered one of the most robust and effective methods for mechanical pretreatment. It significantly reduces particle size while decreasing both the crystallinity and polymerization degree of cellulose. However, its high energy consumption, costly equipment, and lack of lignin removal capabilities make it economically impractical as a standalone technique. Consequently, ball milling is often paired with other pretreatment methods to achieve more comprehensive biomass breakdown (Rizal et al., 2018).

**4.1.2 Extrusion**

Extrusion is a physical pretreatment technique that integrates multiple mechanical actions into a single continuous process. In this method, lignocellulosic biomass is fed into one end of the extruder and transported along a barrel by a rotating screw. As the material advances, it experiences friction against the screw and barrel, which generates heat and promotes mixing and shearing. The barrel typically contains distinct zones, including a compression zone at the midpoint and an expansion or wear zone near the outlet. Upon release of pressure, significant abrasion occurs, weakening the structural integrity of the biomass (Hjorth et al., 2011; Konan et al., 2022). This abrasion facilitates the depolymerization of macromolecules, improving biodegradability through both external and internal friction (Duque et al., 2017).

The effectiveness of extrusion as a pretreatment has been demonstrated in multiple studies. For example, extrusion of rice straw resulted in a 32.5% and 72.2% increase in methane production when compared to milled and untreated rice straw, respectively (Tsapekos et al., 2015). Similarly, Kozlowski et al. (2019) reported an increase in biogas and methane production by 7.50% and 8.51%, respectively, following extrusion pretreatment. Despite these improvements in yield, the application of extrusion in full-scale biogas plants has faced economic challenges. In particular, studies in Poland have shown that the energy demand of the extrusion process renders it economically unjustifiable in certain operational contexts.

Although extrusion can be conducted under relatively mild conditions—such as low pH, moderate temperatures, and short retention times—it remains constrained by its high energy consumption and the significant capital cost of the required machinery (Victorin et al., 2020).

**4.1.3 Microwave Irradiation**

Microwave irradiation is another physical pretreatment method that uses non-ionizing electromagnetic radiation, typically in the frequency range of 300 to 300,000 MHz with wavelengths between 1 mm and 1 m, to deliver energy to the biomass (Huang et al., 2016). This form of non-conventional heating has been extensively applied to the treatment of lignocellulosic materials. The mechanism involves dipole rotation and ionic conduction, which result in dielectric polarization and subsequent molecular collisions. These interactions rapidly and uniformly heat the material, disrupt its structural integrity, and enhance its susceptibility to microbial degradation (Aguilar-Reynosa et al., 2017; Calcio Gaudino et al., 2019).

Microwave irradiation may be applied under atmospheric or high-pressure conditions. High-pressure microwave pretreatment is conducted in closed reactors and typically operates at temperatures ranging from 150°C to 250°C (Li et al., 2016). In a comparative study, Huang et al. (2016) evaluated microwave pyrolysis across seven types of biomass, including corn stover, rice straw, rice husk, sugarcane bagasse, sugarcane peel, coffee grounds, and bamboo. The results demonstrated that microwave pyrolysis effectively converts biomass into bioenergy and green materials. Both temperature and heating rate were found to correlate linearly with microwave power levels up to 250 W. Compared to conventional thermal methods, microwave pretreatment is notably faster, consumes less energy, and results in greater weight loss due to more aggressive thermal decomposition. It also exhibits distinct kinetic parameters and reaction dynamics.

However, a key limitation of microwave irradiation lies in the inherently low dielectric loss factor of dry biomass, which limits its ability to absorb microwave energy effectively. This challenge persists until sufficient char is generated to improve microwave coupling efficiency (Salema et al., 2017).

**4.1.4 Ultrasonication**

Ultrasonication is a modern and highly effective mechanical pretreatment technique that has demonstrated considerable potential in enhancing the biodegradability of biomass, particularly sludge. This method involves the application of high-frequency ultrasonic waves that disrupt the physical structure, alter chemical bonds, and influence the biological accessibility of organic matter. The effectiveness of ultrasonication depends on both the physicochemical properties of the biomass and key operational parameters such as frequency, amplitude, and duration of sonication. In full-scale applications, ultrasonication has resulted in up to a 50% increase in biogas production. Energy balance analyses have further indicated a favorable energy return, with an average net energy gain to electricity use ratio of 2.5 (Pilli et al., 2011).

In addition to sludge treatment, ultrasonication has shown promising results with microalgal biomass. Dhandayuthapani et al. (2021) conducted a study using defatted green microalgae biomass derived from *Chlorella sorokiniana* NITTS3 as a substrate for ethanol fermentation by *Saccharomyces cerevisiae* NITTS1. Ultrasonic pretreatment of the defatted biomass led to a significant increase in ethanol production, with yields rising by 25.83 g/L compared to untreated biomass. Analysis of the hydrolysate obtained after sonication revealed elevated concentrations of simple sugars, notably glucose and xylose, which are readily fermentable by yeast. Optimal fermentation conditions—30°C, pH 4, and agitation at 200 rpm—resulted in an ethanol yield of 52.10 ± 0.12 g/L, corresponding to 86.70 ± 0.52 mg of bioethanol per gram of dry moldy bran.

These findings underscore the versatility of ultrasonication as a pretreatment approach not only for improving anaerobic digestion but also for enhancing fermentative biofuel production by increasing substrate accessibility and sugar release.

**4.2 Hydrothermal Pretreatment**

Hydrothermal pretreatment (HTP) is recognized as one of the most environmentally sustainable methods for biomass processing due to its reliance solely on water and lignocellulosic substrate, without the need for chemical additives. Key parameters influencing the efficiency of HTP include temperature and residence time, both of which play critical roles in disrupting the rigid lignocellulosic structure (Guan et al., 2021). During HTP, biomass—such as rice straw—is subjected to high-pressure, high-temperature water, which facilitates cellulose hydration and enables the partial removal of hemicellulose and lignin. Importantly, this process avoids the need for corrosive chemicals or specialized hydrolysis reactors made from corrosion-resistant materials.

Unlike several other pretreatment techniques, HTP can be performed without prior size reduction of biomass on a commercial scale, leading to substantial energy savings in preprocessing (Kumari and Singh, 2018). Furthermore, the process generates fewer residues requiring neutralization and uses fewer chemicals for hydrolysate conditioning. By increasing the accessible surface area of cellulose, HTP improves its enzymatic digestibility (Sato et al., 2021).

In a comparative study, Chandra et al. (2012) evaluated the performance of untreated and pretreated rice straw substrates using NaOH and hydrothermal pretreatments. Their findings showed that NaOH-pretreated biomass achieved the highest biogas and methane yields, though HTP-treated straw also outperformed untreated samples. Similarly, Hashemi et al. (2019) demonstrated that hydrothermal pretreatment improved biogas production from safflower straw, with the best methane yields observed under the mildest pretreatment conditions. These findings emphasize the efficiency of HTP in enhancing substrate digestibility and methane production, especially under optimized process conditions.

**4.2.1 Steam Explosion**

Steam explosion, initially developed by Mason in 1926, is a widely adopted hydrothermal pretreatment technique that utilizes high-pressure saturated steam to disrupt biomass structure. The process typically involves exposing biomass particles to temperatures between 160°C and 260°C for a short duration, followed by rapid decompression. This sudden pressure release causes the biomass to undergo explosive decompression, which mechanically breaks down the cell walls, hydrolyzes hemicellulose, and alters the structure of lignin (Zheng et al., 2021; Sun and Cheng, 2002).

During steam explosion, organic acids—such as acetic acid—are often released from the biomass itself and can aid in hemicellulose hydrolysis. In addition, water at elevated temperatures contributes to hydrolysis due to its mildly acidic behavior. The combined physical and chemical action of steam explosion significantly improves the degradability and bioconversion potential of lignocellulosic materials.

Several studies have investigated the fuel characteristics and biogas potential of steam-exploded biomass. Lam (2011) reported that steam-exploded wood displayed favorable fuel properties, such as high heating value, low moisture uptake, and excellent pelletizing qualities. Pellets produced from steam-exploded biomass exhibited higher density and structural integrity compared to untreated wood. Similarly, Biswas et al. (2011) found that steam-exploded salix biomass exhibited lower alkali metal content and improved physical properties, including enhanced density, impact resistance, and abrasion resistance. Although char reactivity showed a slight decline at higher temperatures and longer residence times, ash fusion behavior remained largely unaffected.

Steam explosion can be implemented in both continuous and batch reactor configurations. Continuous systems are typically preferred for industrial-scale applications, while batch reactors are commonly used in research settings. The technique has been successfully applied to a wide range of biomass types, including wheat straw, corn stalks, Miscanthus, hardwoods, softwoods, sugarcane bagasse, and food processing residues such as citrus pulp and potato waste, as well as aquatic biomass like seaweed.

Several studies have reported significant improvements in methane yields following steam explosion pretreatment. For example, methane production from wheat straw has been shown to increase by 20–30% compared to untreated biomass (Menardo et al., 2013; Datar et al., 2007). These enhancements affirm steam explosion as a robust and scalable pretreatment strategy for improving the anaerobic digestibility of lignocellulosic feedstocks.

**4.2.2 Freezing**

Freezing is a simple yet effective hydrothermal pretreatment method that utilizes repeated freeze–thaw cycles to disrupt the lignocellulosic matrix and improve biomass digestibility. As described by Rooni et al. (2017), the process involves mixing milled lignocellulosic biomass with water and subjecting it to sub-zero temperatures, typically as low as –18°C, followed by thawing at ambient temperature (approximately 22°C). This freeze–thaw process is usually repeated across multiple cycles to maximize structural damage. The mechanical stress caused by the expansion of water during freezing increases the internal water content of plant stalks and results in the expansion and rupture of cell walls. Consequently, the lignocellulosic structure becomes more porous, enhancing the accessible surface area for enzymatic or microbial action (Guan et al., 2021).

The effectiveness of freezing–thawing pretreatment in isolating cellulose-rich fractions has been confirmed across a range of biomass types. For example, Li et al. (2010) reported cellulose yields between 75% and 77% from bamboo subjected to this pretreatment. Similarly, Smichi et al. (2016) demonstrated that freezing–thawing was the most effective method among several tested for achieving the highest glucose concentration from *Juncus maritimus*. These improvements are attributed to enhanced substrate permeability and structural loosening.

Beyond plant biomass, freezing–thawing pretreatment has also been investigated for its effects on sludge biodegradability and subsequent anaerobic digestion. Studies by Cano et al. (2015) and Yang et al. (2021), have shown promising results, particularly in enhancing sludge solubilization and organic matter availability. In one notable study, Rooni et al. (2017) examined the freezing pretreatment of barley straw for bioethanol production. Their laboratory-scale experiments revealed that applying four freeze–thaw cycles resulted in a maximum hydrolysis efficiency of 19.42%, highlighting the potential of this low-cost and chemical-free method.

While additional work, such as that by Li et al. (2019), may further clarify the scope and limitations of freezing–thawing pretreatment, the existing evidence supports its utility as an environmentally benign and operationally simple approach to enhance biomass conversion efficiency.

**4.3 Chemical Pretreatment**

Chemical pretreatment techniques involve the application of acids, alkalis, oxidants, or solvents to disrupt the structural integrity of lignocellulosic biomass. These methods aim to improve the digestibility of cellulose and hemicellulose by altering or removing lignin, decrystallizing cellulose, and solubilizing hemicellulose. Chemical pretreatments are particularly effective when integrated with physical or thermal strategies and can significantly enhance enzymatic hydrolysis and microbial degradation in downstream processes.

**4.3.1 Alkali Pretreatment**

Alkali pretreatment involves the immersion of lignocellulosic biomass in alkaline solutions such as sodium hydroxide (NaOH), potassium hydroxide (KOH), ammonium hydroxide (NH₄OH), or lime (Ca(OH)₂). This process modifies the structural components of the biomass by disrupting ester and ether linkages in lignin and between lignin and carbohydrates. It also results in partial decrystallization of cellulose and facilitates the solvation and removal of hemicellulose. A subsequent neutralization step helps to separate lignin from inhibitory compounds, reducing the formation of fermentation inhibitors and improving overall biodegradability. Compared to other chemical pretreatment methods, alkali pretreatment typically operates under milder temperature and pressure conditions (Badiei et al., 2014).

This method enhances microbial accessibility to lignin by disrupting its crosslinkages within the plant cell wall matrix, thereby facilitating more efficient enzymatic or microbial degradation. Alkali pretreatment has demonstrated broad efficacy across various biomass types, including maize stover, switchgrass, bagasse, wheat straw, rice straw, hardwoods, and softwoods.

One specific variant, lime pretreatment, uses CO₂ for in situ neutralization, which eliminates the need for a solid-liquid separation step. It is considered cost-effective and energy-efficient compared to other alkaline techniques. Additionally, CaCO₃ can be recovered via CO₂ precipitation (Rabelo et al., 2009). While alkali pretreatment generally incurs high operational costs—mainly due to the use of chemical catalysts—it generates fewer inhibitory compounds and can be combined with other methods for high-solids biomass treatment. The approach has shown significant potential for improving sugar yields from various lignocellulosic sources.

Park and Kim (2012) investigated the effectiveness of alkali pretreatment on eucalyptus residue for ethanol production. Their study revealed that enzymatic digestibility of pretreated biomass was five times greater than that of untreated eucalyptus. Moreover, a percolation-based pretreatment process resulted in more than double the degree of delignification and a twelvefold increase in enzymatic digestibility compared to soaking-based methods, demonstrating the efficiency of alkali pretreatment in facilitating biomass conversion for biofuel applications.

**4.3.2 Acid Pretreatment**

Acid pretreatment is another widely used chemical method for enhancing the digestibility of lignocellulosic biomass. This approach employs mineral and organic acids such as sulfuric acid (H₂SO₄), hydrochloric acid (HCl), acetic acid (CH₃COOH), and phosphoric acid (H₃PO₄) to disrupt the biomass matrix. These acids effectively hydrolyze hemicellulose and, to a lesser extent, cellulose, thereby increasing the accessibility of carbohydrates for enzymatic or microbial digestion (Guan et al., 2021). Acid pretreatment is applicable using either concentrated or dilute acid solutions. While concentrated acids demonstrate high efficacy in depolymerizing structural carbohydrates, their corrosiveness, toxicity, and associated recovery costs limit their practical application at scale.

The effectiveness of acid pretreatment has been demonstrated in multiple studies. Monlau et al. (2013) optimized methane production from sunflower oil cake by applying the highest effective concentration of H₂SO₄ at 17°C, resulting in enhanced methane yields. Similarly, Syaichurrozi et al. (2019) examined the effects of sulfuric acid pretreatment on *Salvinia molesta* biomass. Their results indicated that increasing the acid concentration from 2% to 6% (v/v) over a two-day batch process at ambient temperature and pressure improved biogas yield. This was attributed to lignin solubilization and an increase in nitrogen-free extract content. Taherdanak et al. (2016) further demonstrated that dilute sulfuric acid pretreatment of wheat plants significantly enhanced methane yield, lignin removal, and xylan degradation during anaerobic digestion.

Despite its efficacy, acid pretreatment presents several challenges. The low pH of acid-treated biomass requires neutralization prior to anaerobic digestion, typically achieved through alkali addition or extensive water washing. Moreover, under high-temperature conditions, acid pretreatment can generate toxic degradation byproducts such as 5-hydroxymethylfurfural (HMF), furfural, and phenolic compounds. These compounds are known to inhibit microbial activity and reduce biogas production efficiency. Consequently, additional steps such as detoxification or thorough washing are often necessary to remove these inhibitors and ensure the safety and effectiveness of downstream anaerobic digestion processes (Tsai et al., 2021).

**4.4. Biological Pretreatment**

Biological pretreatment utilizes naturally occurring microorganisms—primarily fungi and bacterial consortia—to break down complex lignocellulosic structures under mild operational conditions. This approach is environmentally friendly and energy-efficient, offering a sustainable alternative to chemical and thermal methods. By targeting structural components such as lignin and hemicellulose, biological pretreatment enhances the accessibility of cellulose, thereby improving the efficiency of subsequent anaerobic digestion and biogas production.

**4.4.1 Fungal Pretreatment**

Fungal pretreatment aims to exploit specific fungi capable of selectively degrading lignin and hemicellulose while minimizing cellulose loss. Since cellulose is more resistant to fungal degradation, its preservation is crucial for downstream biogas production. The degradation of lignin and hemicellulose increases the porosity of biomass, thereby improving enzymatic and microbial access to cellulose and enhancing digestibility during anaerobic digestion (Zheng et al., 2014). Among the various fungi studied, white-rot fungi have shown the highest effectiveness due to their ligninolytic enzyme systems. These fungi possess the ability to modify lignin structure through the secretion of oxidative enzymes such as lignin peroxidase, manganese peroxidase, and laccase. Fungal pretreatment is generally conducted under sterilized or semi-sterilized conditions to control microbial competition and ensure consistent degradation performance (Taherzadeh and Karimi, 2008).

**4.4.2 Microbial Pretreatment**

In contrast to isolated fungal treatments, microbial consortia pretreatment employs diverse microbial communities—often sourced from natural habitats such as decaying biomass or compost piles—to degrade lignocellulosic materials. These consortia are typically more versatile and capable of degrading cellulose and hemicellulose in addition to lignin. Zhang et al. (2019) developed a thermophilic microbial consortium by enriching microbes from decaying straw and landfill environments. When applied to cassava residues at 55°C in the presence of distillery effluent, this consortium resulted in a 96% increase in methane yield over untreated residues after 12 hours of pretreatment.

Badiei et al. (2014) introduced a microbial enrichment group (MEG) system through multi-generation selection, achieving a 25% improvement in biogas yield from cotton stalks after a 7-day pretreatment at 35.2°C. However, not all microbial combinations are effective. For example, mixing garden waste compost and fungus from maize silage and applying the mixture to manure biofibers did not result in significant improvements in methane production.

Biological pretreatment strategies also extend to the use of formulated microbial agents, such as freeze-dried powders that combine pure cultures of yeast and cellulolytic bacteria. In one study, maize straw inoculated with this microbial formulation and incubated for 15 days at ambient temperature exhibited a 75% increase in methane yield and a 34.6% reduction in digestion time. These results highlight the importance of microbial synergy and environmental adaptation in successful pretreatment design.

Ongoing research also focuses on using microbes and enzymes to facilitate targeted degradation pathways. In addition to lignin removal, biological agents can be tailored to reduce specific inhibitory or antimicrobial compounds present in biomass. Fungal species such as *Sporotrichum*, *Aspergillus*, *Fusarium*, and *Penicillium* have been employed in solid-state fermentation processes to increase feedstock availability and reduce antimicrobial content, thereby further enhancing biogas yield (Taherzadeh and Karimi, 2008).

**5. Conclusion**

Anaerobic bioconversion of lignocellulosic wastes represents a promising and sustainable approach to addressing global energy demands and mitigating environmental degradation caused by fossil fuel dependence. With a rapidly growing population and intensifying ecological challenges, advancements in bioenergy technologies offer a viable pathway to reduce greenhouse gas emissions and transition toward a circular, low-carbon economy. Anaerobic digestion processes, when optimized for lignocellulosic biomass, can effectively convert agricultural residues and organic waste into renewable biogas, contributing to both energy security and environmental conservation.

A wide range of pretreatment techniques has been developed to enhance the efficiency of lignocellulosic bioconversion. These methods focus on improving substrate accessibility by altering factors such as cellulose crystallinity, surface area, and the protective effects of lignin and hemicellulose. Targeted disruption of these structural barriers enhances enzymatic hydrolysis, thereby improving biogas yields. Additionally, maximizing the utilization of hemicellulose components offers an opportunity to further reduce production costs and improve the overall energy balance of biogas systems. Given their performance, many of these pretreatment strategies hold strong potential for industrial-scale implementation.

However, despite significant progress, several challenges must still be addressed to enable widespread adoption of these technologies. Key issues include the variability of feedstock composition, process inefficiencies, high operational costs, and the management of byproducts such as digestate. Furthermore, ensuring the economic viability of these systems remains a priority, particularly in regions lacking established renewable energy infrastructure.

To fully realize the potential of anaerobic bioconversion, future research should prioritize technological innovation, integrated process optimization, and the development of robust microbial consortia tailored to diverse feedstocks. Equally important is the role of policy frameworks and economic incentives in accelerating the deployment of these technologies at scale. By fostering continued innovation and supporting the transition to bio-based energy systems, we can contribute meaningfully to global sustainability goals and create a cleaner, more resilient energy future for generations to come.

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