Review Article

Anaerobic Digestion: Technology for Biogas as a Source of Renewable Energy from Biomass—A Review

Biswanath Saha, Nida Arshad and Malinee Sriariyanun*
Biorefinery and Process Automation Engineering Center, Department of Chemical and Process Engineering, The Sirindhorn Thai-German International Graduate School of Engineering, King Mongkut's University of Technology North Bangkok, Thailand

Wawat Rodiahwati
Research Centre for Process and Manufacturing Technology, Indonesia National Research and Innovation Agency, Jakarta, Indonesia

Marttin Paulraj Gundupalli
School of Bio Sciences and Technology, Vellore Institute of Technology, Vellore, Tamil Nadu, India

* Corresponding author. E-mail: macintous@gmail.com DOI: 10.14416/j.asep.2025.07.008
Received: 14 May 2025; Revised: 25 June 2025; Accepted: 9 July 2025; Published online: 17 July 2025

Abstract
Anaerobic digestion (AD) is a conventional method for converting biomass into renewable energy, gaining renewed interest in recent years due to its potential for sustainable energy production. While the fundamental principles of AD are well-established, modern research primarily focuses on optimizing the process under various conditions to enhance efficiency and yield. This study provides a comprehensive assessment of AD, exploring the impact of pretreatment methods, inhibitors, and key parameters affecting its performance. Special emphasis is placed on substrates containing lignin or bacterial cells, which are identified as the most adaptable for pretreatment strategies aimed at improving AD efficiency. The analysis further evaluates existing methods for assessing improvements in AD across different systems, highlighting current challenges and the potential for developing enhanced evaluation techniques. The findings underscore the importance of exploring alternative renewable energy sources beyond fossil fuels, with AD serving as a promising solution. Understanding the interplay between pretreatment, process parameters, and inhibitor management is essential for advancing AD technology and achieving economically viable outcomes.

Keywords: Anaerobic digestion, Biomass, Biorefinery, Inhibitor, Inoculum, Pretreatment

1 Introduction

Increasing the usage of fossil fuels decreases the energy storage of carbon energy, coal, natural gas, and petroleum, while also increasing the danger of environmental contamination and climate change. Consumption of fossil fuel in the world causes CO_2 emissions to rise to 33.1 Gt, causing serious environmental consequences, especially global warming [1]. The increasing population also posed a challenge to the global energy demand and consumption. Therefore, the average temperature will rise to 2.5–5.4 °C due to the continued use of fossil fuels, which leads to the disappearance of millions of flora and fauna species [2]. Fossil fuels continue to be the primary source of energy, accounting for more than 80% of the world's energy requirements. Therefore, it is essential to investigate alternative renewable energy instead of fossil fuel [3].

In recent years, researchers have placed a high value on lignocellulosic biomass as a raw material for production of biofuel as a fossil fuel substitute. Currently, lignocellulose biomass is a renewable energy source that is capable of meeting future energy demands. Lignocellulosic biomass is the most extensive type of biomass on the planet, with an annual production is reported around 181.5 billion tons [4]. Plants typically contain lignocellulosic biomass in their cells, which is produced by photosynthesis. Lignocellulosic biomass, commonly referred to as plant biomass, includes agricultural waste, crop leftovers, and urban garbage (Table 1). Solid fossil fuel's chemical structure is more complex than that of natural biomass. However, it is very complicated in semi-biomass combination of various non-biomass materials during biomass processing [5]. It was detected that the biomass composition is notably different from the coal. Like fossil fuels, biofuels are also made up of solid, liquid, and gaseous forms. In most cases, firewood, wood chips, wood pellets, and wood charcoal are recognized as forms of solid biofuel. The category of liquid biofuels encompasses bioethanol, biodiesel, and pyrolysis bio-oil. Biogas and syngas are considered gaseous biofuels. The fermentation process for biofuel can effectively utilize lignocellulosic materials.

Table 1: Biomass categorization based on lignocellulosic composition.

Category of Biomass	Composition (%)			Examples of Biomass
	Cellulose	Hemicellulose	Lignin	
Woody and wood biomass	30–40	24–40	0–20	Branches, leaves, bushes, chips, lumps, pellets, briquettes, sawdust, sawmills.
Agricultural and herbaceous biomass	30–45	20–50	2–12	Fibers, shells, husks, pits, flowers, straws, stalks, fruits, and grasses.
Aquatic biomass	29–38	28–42	12–16	Seaweed, lakeweed, water hyacinth.
Human and animal waste	29–40	22–35	16–20	Sponges, animal dung, poultry litter, and bone meal.
Biomass contamination and industrial waste	25–35	25–40	15–25	Paperboard, fiberboard, plywood, wood pallets and boxes, sewage sludge, demolition wood, and municipal solid waste.
Blends of biomass	30–45	25–35	15–20	Mixtures of biomass.
Algae biomass	30–50	25–35	10–15	Yellow green algae, golden algae, red algae, brown algae, green algae.
Terrestrial biomass	30–45	30–40	15–22	*Argemone mexicana*, *Galinsoga purviflora*, *Ageratum conyzoides*, *Parthenium hysterophorus* and *Lantana camara*.

*N.A. Not available

Currently, 14% of the energy in the world is covered by biomass [4]. In general, biomass is made up of hemicellulose (35–39%), cellulose (9–12%), lignin (19–24%), proteins (4–17%), and lipids (2–19%) [6]. In addition, sugars derived from biomass through hydrolysis can be utilized to produce bioenergy in various forms, including ethanol, butanol, biodiesel, biogas, and biohydrogen. Mostly due to the huge availability of biomass, there are many technologies and infrastructure present for biomass production. At present, many countries use biomass for the commercial production of electricity and transportation fuels [7]. However, several species of weeds, such as *Parthenium hysterophorus, Ageratum conyzoides, Lantana camara,* are considered hazardous biomass in the world and have reduced the growth of the agriculture sector and they have significantly reduced the yields of the agricultural sector.

Anaerobic digestion (AD) is an effective way of managing this noxious biomass. Any organic material degradation results in the form of biogas in the presence of microorganisms and the absence of oxygen [8]. Organic materials convert into biogas with the functions of microorganisms in an anaerobic condition. Biogas serves as one of the natural alternatives for renewable energy. The release of greenhouse gas (GHG) emissions can be reduced by the AD process [9].

AD plays a vital role as a renewable source of energy. Methane (CH_4) capturing from the AD has the advantages of reducing uncontrolled CH_4 emissions and has a positive impact on reducing global warming. Major composition of biogas is 50–70% CH_4, 25–40% CO_2, and trace gases (1–5%) [10]. During the anaerobic digestion process, digestate is produced, which is rich in N, P, and K, and can be applied as an organic fertilizer [11]. This scheme suggests the application of AD to integrate in the management of agricultural wastes for the production of alternative energy to meet the goals of sustainable development. This study outlines the role of biomass, AD, and various parameters such as inoculum, temperature, pH, food to microorganism (F/M) ratio, and carbon to nitrogen (C/N) ratios for AD operations. The significance of chemical, thermal, and biological pretreatment strategies for increasing anaerobic digestion efficiency is outlined. Furthermore, AD is proposed as a sustainable alternative to fossil fuels, reducing their negative effects on the environment.

2 Anaerobic Digestion of Biomass

AD is a proven biological method that changes organic substances, including lignocellulosic biomass, into biogas and digestate without the presence of oxygen. It is gaining traction as a sustainable strategy for renewable energy production and organic waste management, particularly in the context of the circular bioeconomy [12]. While AD offers a viable pathway for energy recovery from lignocellulosic biomass, its efficiency hinges on appropriate pretreatment, process optimization, and careful management of inhibitory substances. The AD process, supported by a diverse microbial consortium, consists of four major stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. In the hydrolysis stage, extracellular enzymes act to decompose complex polymers, such as cellulose and hemicellulose, into simpler monomers. Acidogenesis further converts these monomers into volatile fatty acids (VFAs), alcohols, ammonia, and hydrogen. In the acetogenesis step, VFAs are converted into acetic acid, H_2, and CO_2, which are substrates for methanogenesis, the final stage where methane (CH_4) and carbon dioxide (CO_2) are generated by methanogenic archaea (Figure 1). In addition to biogas, the AD process creates digestate, a nutrient-rich by-product that can serve as organic fertilizer, thereby contributing to nutrient recycling and enhancing the health of the soil. However, its safe application depends on adequate stabilization and pathogen reduction, which may require post-treatment processes [13].

Recent research has demonstrated that the biochemical complexity of lignocellulosic biomass and food waste requires pretreatment to enhance hydrolysis efficiency and microbial accessibility. Various pretreatment strategies—chemical (e.g., acid or alkaline hydrolysis), thermal (e.g., steam explosion), and biological (e.g., fungal delignification)—have been employed to disrupt lignin structures and improve enzymatic degradation [14]. These methods significantly improve biogas yield, with methane production ranging between 400–650 mL CH_4/g VS, depending on the substrate composition, C/N ratio, and pretreatment applied [13], [15]. However, AD performance can be affected by several inhibitory factors, including ammonia toxicity, VFAs accumulation, sulfate reduction, and pH fluctuations. For instance, excessive protein degradation can lead to ammonia accumulation, which inhibits methanogenic activity [16]. To ensure stable digestion, it is vital to uphold a balanced carbon-to-nitrogen (C/N) ratio, which usually lies between 20:1 and 30:1. The use of co-digestion by combining with high-carbon materials has shown promising results in improving process stability and methane yield [17].

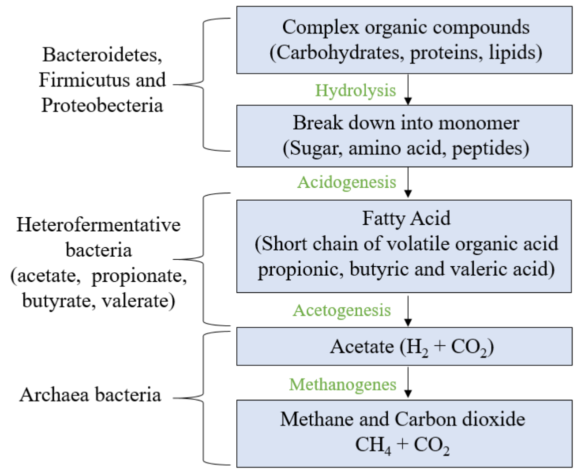

Figure 1: Sequential phases of biomass conversion by anaerobic digestion.

3 Important Parameters Affecting the Anaerobic Digestion

Significant factors that affect anaerobic digestion include the inoculum, the C/N ratio, the F/M ratio, pH, temperature, organic loading rate, hydraulic loading rate, and the presence of toxins, which can be either natural components or by-products resulting from metabolism.

3.1 *Inoculum*

Microorganism is a valuable component in anaerobic digestion. Inoculum is the primary source of microorganisms in the AD process. The composition of the inoculum in microbial consortium is a significant factor in anaerobic digestion. Generally, during hydrolysis stages, hydrolytic microorganisms are available to break down polymeric biomass to fatty acids, alcohols, and sugars. Acidogenic is the second stage, where volatile acids and alcohols break down into acetic acid and hydrogen [18]. In methanogenic stages, microorganisms, such as Archaea and acidogenic bacteria, are the main contributors. Methanogens are microorganisms that grow at a slow rate present in the inoculum and are highly sensitive to pH and environmental conditions [19]. Inoculum selection for the substrate in AD is one of the key steps for the stable operation. Inoculums choices clearly demonstrate the contribution of methane yield. Inoculum can also affect the methane output and have the ability of stress tolerance. However, for the startup or operation of the reactor quantity and the quality of inoculum play a significant role [20]. Usually, fresh inoculum is preferred for the AD. A high amount of inoculum volume increases the number of microbial populations. Inoculum also maintains the buffering capacity of AD and helps to reduce the C/N ratios. Around 10–60% of the reactor volume is filled with the inoculum [21]. Enhance biogas production and improve the quality of its final composition during the AD process by ensuring adequate microbial activity from the inoculum source.

Table 2: Application of different inoculum types for substrate varieties.

Inoculum	Substrate	Refs.
Bovine Rumen fluid	Municipal solid waste	[24]
Sludge	Swine waste	[25]
Cattle manure	Palm oil mill effluent	[26]
Poultry dung, goat dung, cow dung, piggery dung and rhinoceros dung	Food waste	[23]
Rumen, stabilized swine wastewater, sewage sludge	Swine wastewater	[20]
Fresh cow dung	Water hyacinth	[27]
Fresh cow dung	*Ageratum conyzoides*	[9]
Fresh cow dung	*Lantana camara*	[28]
Mesophilic digestate of wastewater activated sludge and digestate of agricultural sludge	Municipal Solid Waste	[29]
Unsorted organic municipal solid waste	Anaerobic sludge and cow manure	[30]

Various environmental and operational factors, including abrupt shifts in pH, temperature fluctuations, excessive organic load, and the buildup of toxic substances, can negatively impact methanogenic microorganisms and hinder the AD process [22]. Changes in inoculum from one collection to another emphasize the necessity of preserving an active anaerobic state. Inocula need to have active microorganisms that are crucial for the process of AD. This variation is dependent on the substrate, which is influenced by the amount of volatile fatty acids (VFA) and the ammonium released during the hydrolysis of carbohydrates and proteins, aiding in buffer formation [23]. The primary function of a single-stage batch reactor is to inhibit the buildup of VFAs within the 'seed' particles, ensuring they do not exceed their capacity for methanogenic assimilation. The food-to-microorganism (F/M) ratio plays a crucial role in all batch processes and the breakdown of volatile solids from organic solid particles. The degradation capacity of a substrate during AD must be upheld by its buffering capacity at a specific F/M ratio. Another important aspect is the ratio of waste to inoculum in a high solid anaerobic digestion process performed in batch mode [23]. As a mixing form of inoculum and substrate is crucial in AD, the researcher used various calculations of inoculum and substrate (such as Inoculum to substrate or Food to microorganism ratio) before settling into the reactor. The various research examining the effects of inocula on biogas production (Table 2).

Inocula are vital components in the biogas generation process. The amount of methane generated was directly related to the initial inoculum levels, with a greater proportion of inoculum resulting in increased biogas production [31]. The ratios of food for the inocula had a considerable effect on biogas production. Selecting the correct inoculum can optimize the degradation rate, augment biogas production, decrease the initial time, and promote a more stable digestion process. The higher inoculum ratio had the effect of enhancing the biogas production rate; thus, optimizing the inoculum /substrate ratio is necessary. Dhamodharan *et al.*, [23] employed five types of livestock inoculum, including poultry dung (PD), goat dung (GD), cow dung (CD), piggery dung (PGD), and rhinoceros dung (RD) in their AD study. They found that reactors using CD as an inoculum had a shorter startup time and produced more methane compared to those using other types of inoculum. Methane production remained inconsistent and at low levels in reactors treated with GD and RD over a 30-day anaerobic digestion period. Methane production was inconsistent and remained at a low level in reactors inoculated with GD and RD over a 30-day period. CD and PGD recorded the highest levels [23]. An increased inoculation volume can enhance microbial populations, particularly methanogens, improve the buffering capacity of AD, and maintain a balanced carbon-to-nitrogen (C/N) ratio. Typically, substantial amounts of fresh inoculants are not immediately accessible. The inoculum is often stored for the initiation of a new reactor and the bioaugmentation of an underperforming AD system. Moreover, inoculants possessing superior buffering capacity and substantial nutrient content can markedly enhance CH_4 production [31].

The ideal F/M ratio was established by evaluating the biochemical methane potential (BMP) of untreated and hot air oven-pretreated whole water hyacinth plants, including leaves, stems, and roots, in conjunction with fresh cow dung [27]. The untreated water hyacinth with an F/M ratio of 2 exhibited the peak methane yield of 143 ± 14 mL CH_4/g VS on day 32, whereas the hot air oven pretreated water hyacinth with an F/M ratio of 1.5 achieved a maximum methane yield of 193 ± 22 mL CH_4/g VS on day 14, as per a comparative analysis of the two types of water hyacinths [27]. Weeds were mechanically clipped to a size of 1 cm, as smaller sizes were not practical. The cow dung was combined with *Ageratum conyzoides* in varied ratios based on VS [16]. The results from the BMP assay show that using cow manure as the microorganism source for the AD of *A.conyzoides* resulted in the greatest biogas output at an F/M ratio of 2 [9]. *Lantana camara* and cow dung are mixed with varying F/M ratios [32]. In conjunction with a control that comprised solely cow dung, different F/M ratios of 1, 1.5, 2, and 2.5 were investigated. The F/M ratio of 1.5 yielded the highest methane production at 4801 mL, with a concentration of 195.5 mL CH_4/g VS, while ratios of 2 and 2.5 yielded less biogas, respectively [32]. Various inoculants significantly influence the composition of VFA and methane yields. Bovine rumen fluid was utilized as an inoculum for the anaerobic digestion of the organic fraction of municipal solid waste (MSW) [24]. The MSW/inoculum ratios in the reactors were as follows: Reactor A (100%/0%), Reactor B (95%/5%), Reactor C (90%/10%), and Reactor D (85%/15%). The level of methane in the biogas generated by Reactors A, B, C, and D was 3.6%, 13.0%, 25.0%, and 42.6%, respectively [24].

3.2 *Temperature and pH*

The effectiveness of AD is dependent on temperature. The process of anaerobic digestion can happen at psychrophilic temperatures (less than 25 °C), mesophilic temperatures (25–40 °C), and thermophilic temperatures (greater than 45 °C) [33]. Thermophilic digestion is marked by elevated metabolic rates and specific growth rates, but it frequently leads to increased mortality rates in comparison to mesophilic digestion. A major strength of the thermophilic process lies in its effectiveness in eradicating pathogens and weed seeds [34] demonstrated that fermenting livestock manure at thermophilic temperatures offers a kinetic advantage over fermentation at mesophilic temperatures. The kinetic benefits of carrying out digestion at a temperature of 60 °C as opposed to 50 °C are negligible. The study by Larsen *et al*., [35] demonstrated that both thermophilic and mesophilic digestion, when coupled with thermophilic pretreatment, can effectively lower the levels of vegetative pathogenic bacteria, including *E. coli* and *Enterococci*, as well as intestinal parasites associated with animal waste. Conversely, thermophilic treatment presents certain limitations, including reduced stability when compared to mesophilic treatment. Thermophilic system tends to produce effluent of somewhat lower quality. Furthermore, the interplay between the reduced growth yield and elevated growth rates of thermophilic organisms leads to prolonged start-up periods. This also renders these processes more susceptible to toxicity and variations in operational and environmental factors.

The impact of non-optimal pH on methane fermentation arises from the shifts in multiple reaction equilibria rather than a single specific reaction [36]. Numerous studies have determined that the optimal pH range for methane-forming bacteria, whether using mixed or pure cultures, is typically around pH 7. Within the pH range of 6.0 to 7.5, the pH levels in anaerobic processes are regulated via the interaction of the carbonic system with a strong base. The acid-base equilibrium of a digester can be effectively monitored by measuring only the pH and the partial pressure of CO_2 [37]. The starting pH and duration of fermentation significantly influenced metabolite production. In the process of AD, only the hydrolytic and acidogenic phases were noted, with bacteria from the Firmicutes, Bacteroidetes, Actinobacteria, and Spirochaetes phyla being identified. AD of kitchen waste was most effective at pH 7, solubilizing 86% of total organic carbon and 82% of chemical oxygen demand. The highest VFA concentration, 36 g/L, was reached on the fourth day [38]. At a pH of 7, most of the protein was broken down into ammonia nitrogen (NH_4^+), which contributed additional buffering capacity to the acidified solution [38]. Bicarbonate is commonly employed to regulate the reactor's pH during AD. Acidogens predominate at low pH levels (pH < 6.0), while methanogens exhibit an 88% reduction at a pH of 5.5 compared to neutral pH. In addition to the loss in methanogenic and hydrolytic capacity, the need for acid dosing to maintain low pH conditions and other adverse effects of chemical dosing have been identified as significant limitations [39]. Low pH resulted in a change in the microbial community, favoring acetogens over methanogens. This research investigated the impact of specific pH levels on the degradation rate, gas composition, and methane production of maize silage, indicating that optimal digestion of fiber-rich substrates is likely to occur within a pH range of about 6.5 to 7.5 [40].

3.3 *Food to microorganism ratio (F/M ratio) and Carbon to Nitrogen ratio (C/N ratio)*

Biogas production via the process of anaerobic digestion F/M ratio represents the relationship linking the mass of food found in the waste substrate to the mass of microorganisms that serve as decomposers. If the F/M ratio is lower than the optimal level, the microbes cannot metabolize effectively. Conversely, if the F/M ratio is excessively high, it results in metabolic imbalance [41]. Operational parameters, including the F/M ratio, significantly impact methane generation. Methane production may decline or cease altogether if the F/M ratios in AD systems are not maintained appropriately. At low F/M ratios, the primary challenge is the reduced reaction rates caused by a low reaction driving force (concentration), thereby limiting energy recovery efficiency [42]. At high F/M ratios, an overabundance of VFA is particularly problematic when processing degradable organic wastes. Additionally, a higher F/M ratio may lead to an overload, resulting in VFA accumulation. It is essential that the volume of inoculum is greater than that of the substrate on a volatile solids basis. Consequently, the F/M ratio must be regarded as a crucial factor influencing the outcomes of the AD [43].

Multiple analyses have pointed out that the optimal carbon-to-nitrogen (C/N) ratios for methane fermentation are between 25 and 30. However, the decline in carbon and nitrogen availability can be influenced and shaped by the operational conditions, particularly temperature, which may lead to inhibitory effects. Higher C/N ratios lessened ammonia's negative impact, with peak methane production at C/N ratios of 25 and 30 for 35 °C and 55 °C, respectively [44]. Carbon and nitrogen influence various digestive characteristics during AD, however, there is limited research on their interactions. In general, the preferred C/N ratio is believed to lie within the range of 15 to 30. In the past, the determination of the C/N ratio in solid samples has involved evaluating the total organic fractions, specifically through the quantification of total organic carbon (TOC) and total nitrogen (TN). This approach assumes that in cooperation, nutrient sources are entirely decomposable. Biological treatments often fail on some organic materials due to unsuitable C/N ratios or pH levels for AD. However, simply knowing or adjusting the C/N ratio at the start approach to the process does not ensure optimal performance. This is because the initial C/N ratio often does not reflect the biodegradable or bioavailable carbon-nitrogen ratio [45]. By comparing a digestion system's output to its intake or volumetric digester capacity, AD performance is evaluated. In anaerobic digestion, performance is often gauged by methane yield, which indicates the volume of methane produced per unit of material under standard conditions. This can be reported as wet weight, total solids (TS), volatile solids (VS), or chemical oxygen demand (COD). In addition, methane productivity (m^3-CH_4/m^3-reactor, per day) and the reduction percentage of total solids (TS) or volatile solids (VS) from the incoming feed are considered as alternatives. Thus, to get as near to the substrate's true potential methane production at the maximum practical digestion rate as possible, enhanced AD performance depends on raising the yield of methane during operations [46].

3.4 *Rate of organic loading and time of hydraulic retention*

The rate of organic loading (OLR) and time of hydraulic retention (HRT) are important parameters that strongly influence the biogas yield. Optimizing the conditions of these parameters could significantly maximize methane yield and ensure process stability. In recent studies, several studies have demonstrated the impact of OLR and HRT on biogas yield across different substrate and reactor configurations [47]. The effect of OLR and HRT on methane yield during thermophilic co-digestion of sewage sludge alongside food waste was investigated. It was reported that a peak methane generation of 328 CH_4/g-COD was achieved for OLR and HRT of 5.8 gVS/L.d and 15 d, respectively. The growth in methane yield was connected to the enhanced microbial interactions and metabolic activities during the AD process [48]. In a similar study, methane production of 273 L/kg-VS was obtained for OLR and HRT of 5 kg-VS/m^3.d and 2 d, respectively. The parameters OLR and HRT were investigated to advance the two-phase AD for food waste. It was further reported that valeric acid was the predominant VFA among the different VFAs produced during the AD process.

The impacts of OLR and sludge composition on biogas generation from sewage sludge using a lab-scale anaerobic membrane bioreactor (AnDMR) were investigated. Kwon *et al*., [49] reported the maximum average methane output rate of 0.7 L/L·d when the AD process was performed with an OLR of 5.0 g COD/L·d and HRT of 12 d. Though several studies have been reported for AD of sludge and food waste, [50] performed the AD of cheese whey along with glycerol in a thermophilic anaerobic fluidized bed reactor. It was determined that the yield of methane and production improved in the initial stages. Maximum methane yield, methane yield, methane production rate, and organic matter removal were achieved for an OLR of 10 g COD/L.d. However, a further increase in the OLR led to a buildup of VFAs and a decline in methane production. Overall, the studies indicate that moderate OLRs (5–10 g COD/L.d) with HRT (12–15 d) could enhance the microbial activity and methane production. Therefore, to improve the AD process efficiency, OLR and HRT have to be studied considering the degradation kinetics, VFA and pH to prevent acidification. Advanced reactor configurations, such as staged digestion, can further optimize these parameters by separating hydrolysis/acidogenesis from methanogenesis, thereby enhancing overall process efficiency and methane recovery.

4 Cellulose, Hemicellulose and Lignin

Cellulose, hemicellulose, and lignin serve as the fundamental constituents of lignocellulosic materials (Figure 2). The primary determinants of these materials' composition are the plants' origin, variety, and topographical properties.

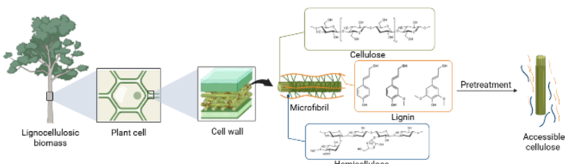

Figure 2: Core structural components of lignocellulosic biomass and the effects of pretreatment on their accessibility.

4.1 *Cellulose*

The most prevalent polymer is cellulose, which makes up over 50% of the mass in secondary cell walls and 20% to 30% of the anhydrous mass of primary cell walls [51]. Cellulose is characterized by its unbranched fibrils that are made up of glucose monomers joined by β-1, 4-glycosidic bonds. The general formula for cellulose is $(C_6H_{10}O_5)_n$. Cellulose has a degree of polymerization (DP) of 250–500 found in the fundamental cell wall and 103–104 present in the secondary cell wall [52]. Hydrogen bonds and Van der Waals interactions hold the glucose chains together. Three-dimensional microfibrils are created by the molecule's bonds. The cellulose structure becomes stable and densely packed as a result of these interactions. Every microfibril is integrated within the other biopolymers, specifically lignin and hemicellulose, that are made up of around 30 to 36 parallel chains of cellulose [53]. Both crystalline and amorphous regions can be seen in cellulose. The crystalline portion of cellulose makes it more resistant to heat breakdown than hemicellulose [54]. Compared to the crystalline portions of cellulose, the amorphous fraction is less structurally dense and is more readily broken down by enzymes.

4.2 *Hemicellulose*

Hemicellulose has a lesser degree of polymerization in relation to cellulose (100–200) [53]. Hemicellulose makes up 20–40% of the weight of lignocellulosic materials. Pentoses, hexoses, deoxyhexoses, and hexuronic acid are all found in hemicellulose, despite xylose being the most prevalent sugar type found in hemicellulose [55]. Hemicellulose has two most prevalent forms: xylans and glucomannans. By adding diluted bases or acids and hemicellulase enzymes, hemicellulose can be hydrolyzed more readily than cellulose [56]. Hardwood and softwood have different hemicellulose compositions and contents. The amount and the makeup of hemicellulose in the bark, stem, root, and branches of a single tree can differ significantly [53].

4.3 *Lignin*

Lignin is made up of phenyl propane units and has a complicated, three-dimensional structure. It is the most plentiful source of aromatic compounds that is both renewable and natural [53]. Most plants' support tissues are made up of lignin because it provides stiffness and resists rotting. Lignin is crucial for the development of cell walls, particularly in wood and bark. Energy content up to 40% and weight up to 30% of lignocellulosic materials are attributed to lignin, making it a crucial component. It gives the cell wall structural support and guards against microbial assaults and cell wall breakdown. Additionally, lignin is in charge of the transfer of water by preventing its passage through the cell wall. The utilization of this aromatic substance for bio-derived products has been hampered by its recalcitrance. Nevertheless, lignindegrading solutions will enable new industrial outputs, enhancing the profitability of lignocellulosic bio-refineries. In lignocellulosic materials, lignin is physically and chemically linked to cellulose and hemicellulose [53]. The lignin and carbohydrate association is created when lignin, as a polymer, forms a covalent link with carbohydrates, particularly hemicellulose, to bind with cellulose and hemicellulose [57]. The structural component of lignin is the cross-linking of three monomers of hydroxycinnamyl alcohol. The numbers of methoxy groups distinguishes these monomers, comprising coniferyl alcohol (G-type), sinapyl alcohol (S-type), and coumaryl alcohol (H-type) [57]. These methoxy groups protect these molecules from radical coupling events that create new bondings, and their placement has an impact on the lignin structure.

5 Pretreatment

Raw biomass mainly consists of cellulose, hemicelluloses, lignin, and carbohydrates, and proteins. Lignocellulosic material often requires pretreatment to release the sugars within cellulose fibers incorporated within the diverse structure of plant cell walls [58]. New biotechnological results for the breakdown of lignocellulosic biomass are needed to enhance the production efficiency and reduce the cost of cellulosic biofuel production. If the substrate includes lignin and lignocellulose, the anaerobic digestion process cannot be completed. The hydrolysis phase is typically regarded as the rate-limiting step, since microorganisms generate several types of hydrolytic enzymes that are not enough to break down the substrate's highly complex structure [59]. The structure of biomass includes hemicellulose as a matrix that surrounds the cellulose skeleton, while lignin provides an encasing protective layer. The covalent cross-linkages that exist between the polysaccharides and lignin contribute to the overall toughness of the material [60]. Moreover, substrates can be degraded rapidly in the hydrolysis, which might induce pH imbalances in the anaerobic reactor throughout the acidogenesis process. Consequently, methanogenic bacteria are very sensitive to low pH, which have the chances to affect the anaerobic process [61]. Hence, the selection of a pretreatment method is crucial to mitigate these negative impacts of the substrate.

Pretreatment is an essential step in the conversion of cellulose, as it modifies the structure of cellulosic biomass, thereby enhancing the accessibility of cellulose to enzymes that transform carbohydrate polymers into fermentable sugars [62]. Pretreatment methods to enhance AD have been developed over the last three decades, resulting in a significant increase in the number of scientific studies. AD enhancement in relation to the augmented output of methane and the decreases in solid wastes are well-recognized benefits of these pretreatments [46]. The pretreatment of lignocellulose biomass has demonstrated considerable advantages to improve the properties of biomass that is converted to valuable green energy (Figure 3).

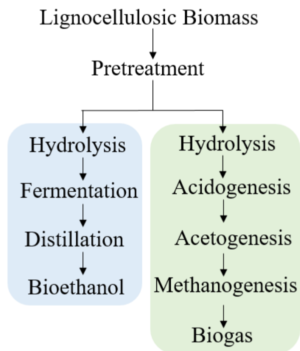

Figure 3: Valorization of lignocellulosic biomass into bioethanol and biogas.

Pretreatment culminates in reducing the interplay of toxicity and biodegradation, enhancing cascade fermentation, resulting in sustained and efficient methane yield. Effective pretreatment is considered to delignify, enhance sugar solubility, and cause a decline in cellulose crystallization in lignocellulose. Multiple pretreatment options, involving physical, chemical, and biological processes, have been identified to increase the digestibility of lignocellulosic feedstocks (Figure 4). Mainly, pretreatment in AD is chemical, thermal, biological, and ultrasound. Residues from wastewater treatment plants (WWTPs) and lignocellulose derived from plants and vegetables are present in energy crops, agricultural byproducts, manure, and, to a lesser degree, household waste. Residues from wastewater treatment facilities are the most extensively investigated in the literature on pretreatment for increasing anaerobic digestion, followed by lignocellulosic substrates [46].

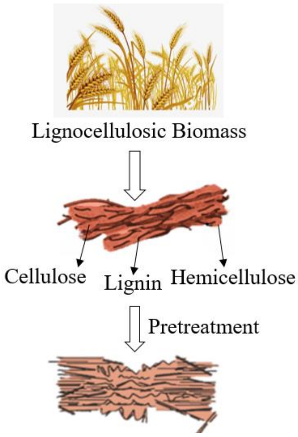

Figure 4: Comparative structural representation of lignocellulosic components before and after pretreatment.

5.1 *Chemical pretreatment*

The most popular technique for pretreating lignocellulose to eliminate the components entirely or in part is chemical pretreatment (Table 3). Over the past few years, several chemical pretreatments, such as lime, acid, steam explosion, sulfur dioxide explosion, ammonia fiber explosion, and ionic liquids, have been recognized as effective approaches for biomass pretreatment [63]. In chemical pretreatment, chemicals are added to advance the operational capabilities of AD. Chemical pretreatments reduce the amount of lignin by reactions using different chemicals and solvents. Chemical pretreatment processes that solubilize polymers enhance their degradation by microbes. Alkaline and acidic reagents are commonly utilized to soften the hemicellulose and lignin, making them more susceptible to enzymatic breakdown. Alkaline pretreatment is particularly effective for lignin removal, whereas acid pretreatment is suggested for hemicellulose [64]. Lime ($Ca(OH)_2$) is utilized to improve the solubilization of chemical oxygen demand (COD), which is subsequently followed by the AD of the organic fraction of municipal solid waste (OFMSW) [65]. Acidic reagents, including H_2SO_4, HCl, H_2O_2, and CH_3COOH were utilized at concentrations of 1–3% to assess their impact on the anaerobic digestibility of agricultural straw [66]. The investigation on dried grass silage (GS) was conducted by treating it with various concentrations of NaOH (1%–7.5 %VS) at a range of temperatures (20 °C–150 °C) to evaluate its biodegradability, yield from hydrolysis, degradation rate of lignocellulose and biogas production. The results indicated that at a temperature of 100 °C and all NaOH concentrations, a maximum of 45% of the total COD was made soluble. Additionally, the content of cellulose was reduced by 21.2%, hemicellulose by 36.1% and lignin by 65.6%. [67].

The author investigated two types of chemical pretreatments using NaOH and HNO_3 at 150 °C with a reaction time of 20 min. Using HNO_3 for pretreatment resulted in the highest degradation of hemicellulose, while NaOH pretreatment caused a considerable decline in lignin content. Rice straw underwent AD at a concentration of 20 g/L, resulting in the production rate of 6.00 g VFAs/L with 0.5% HNO_3 and 7.09 g VFA/L with 2% NaOH. The yield of VFAs with 2% NaOH was 0.35 g/g [68]. The biomethane potential was increased by 11% and both organosolv and alkaline by 15% by the pretreatments with N-methylmorpholine N-oxide (NMMO) and NaOH [69]. After pretreatment with 2%w/v NaOH and 2%w/v $Ca(OH)_2$ at room temperature for one day, the bagasse is combined with cow dung in a 1:2 ratio, and the solid to water ratio is set at 1:3 [70]. According to the results, at 35 °C, biogas production from bagasse treated with NaOH is the greatest, followed by treatment with $Ca(OH)_2$ and untreated sample. Additionally, it has been noted that biogas generation is higher at 55 °C than it is at 35 °C [70]. Utilizing solid state processes for the chemical treatment of rice husk for biogas generation was performed employing HCl, NaOH, and ethanol across varying concentrations (1%–5%) at 100 °C and 120 °C for 60 minutes [71]. Biogas purity was increased by alkaline and organosolv pretreatments, which included 50.27% and 50.68% of methane, respectively. This verifies that the solid-state chemical pretreatment of rice husk using NaOH and ethanol significantly enhances biogas production [71].

Chemical pretreatment is widely utilized for lignocellulosic biomass due to its efficiency in breaking the ester bonds between polysaccharides and lignin [73]. Among these, alkali treatment stands out as the most used chemical pretreatment method, as it significantly lowers the crystallinity of cellulose, eliminates lignin, and enhances the surface area and porosity, thereby improving the substrate's digestibility [74]. The mechanisms at play in the reaction involving lignin, lignin complexes, and OH^- ions are outlined here. OH^- ions can partially resolve and separate lignin from hemicellulose by breaking the ester–ether bonds that link lignin to polysaccharides, while also reducing the strength of the hydrogen bonds between hemicellulose and cellulose [75].

Table 3: Evaluation of the chemical pretreatment methods for different biomass feedstocks.

Methods	Substrate	Observations	Refs.
Lime ($Ca(OH)_2$)	Municipal solid waste	Boost the organic fraction of municipal solid waste. Under anaerobic digestion, the pretreated waste produced a maximum methane yield of 0.15 m^3 CH_4/kg of VS, which is 172.0% compared to the control.	[65]
NaOH (loading rates (1%, 2.5%, 5% and 7.5% by volatile solids (VS) mass in grass silage).	Dried grass silage	At 100 °C, up to 45% of the total COD was solubilized. Also, high delignification was observed at 65.6%.	[67]
H2SO4, HCl, H_2O_2, CH_3COOH, NaOH, $Ca(OH)_2$, and NH_3H_2O	Agriculture straw	The efficiency of biogas production from the samples pretreated with acid and alkaline was 115.4% and 105.3% more than the untreated straw. The straw, which was pretreated using 3% H_2O_2 and 8% $Ca(OH)_2$, achieved the maximum methane yield of 216.7 and 206.6 mL CH_4/g-VS during the acid and alkaline pretreatments, representing increases of 115.4% and 105.3% compared to the untreated straw.	[66]
HNO_3 and NaOH	Rice straw	AD of rice straw at 20 g/L produced 6.00 and 7.09 gVFAs/L when pretreated with 0.5% HNO_3 and 2% NaOH, respectively.	[68]
N-methylmorpholine N-oxide (NMMO) and NaOH	Agricultural residue	High removal of hemicellulose was observed after organosolv pretreatment. The cumulative yield of biomethane production of 274 mL CH_4/g VS achieved with the untreated feedstock was improved by 11% through NMMO pretreatment and by 15% with both organosolv and alkaline pretreatments.	[69]
HCl, NaOH, and ethanol	Rice Husk	Biogas yield was increased by solid-state pretreatment using NaOH and ethanol. The recorded biogas yield values were 67.32, 60.89, 32.26, and 9.32 mL/gVS for the samples 3E100, 3N100, RH, and 5H100, respectively.	[71]
NaOH	Corn waste	The pentosan contents were enriched after pretreatment with 1%–3% NaOH.	[72]

Chemical pretreatment transforms carbohydrate components, resulting in quantitative and qualitative differences between the raw materials being examined [72]. The alkaline treatment resulted in lower alterations in the percentage composition of carbohydrate biomass components relative to the acid treatment. It was determined that this is a modification after biomass pretreatment, having a good impact on fermentation productivity. The biodegradability of the solid phase during the chemical pretreatment procedure makes the following solid separation simple. However, most chemical pretreatments require a lot of chemicals and water; this may result in significant capital investment in facilities, elevated treatment expenses, and potential environmental contamination. The pretreatment processes also demand chemical recycling, waste solution disposal, and at times, high-temperature conditions [76]. As reported by Solé-Bundó *et al.*, [77], a pretreatment using 10% CaO resulted in an 11.99% enhancement in methane yield. Likewise, Mancini *et al.*, [69] found that alkaline pre-treatment raises the methane yield from wheat straw by 155%. The researchers suggest that alkaline pretreatment is deemed more effective in comparison to acid pretreatment for Anaerobic digestion. Alkaline pretreatment is most often carried out using sodium hydroxide (NaOH) and lime $Ca(OH)_2$, for AD [66]. Overall, utilizing low concentrations of organic acids for pre-treatment tends to yield unsatisfactory biogas production outcomes, while employing high levels of organic acids can eliminate significant quantities of dry matter, negatively impacting anaerobic digestion [78].

5.2 *Thermal pretreatment*

Thermal pretreatment employs heat energy to increase the solubility of particulate organic fractions and polymeric organic compounds. The thermal pretreatment technique for pretreating lignocellulose is discussed (Table 4). This process can effectively aid in the breakdown of lignin and hemicellulose by facilitating hydrolysis through the acids generated during treatment. Generally, the application of heat disrupts the hydrogen bonds in crystalline cellulose and lignocellulosic structures, leading to the degradation of biomass [79]. Thermal pretreatment is predominantly carried out using hot air ovens, microwaves, autoclaves, and hot water baths. Hot air oven pretreatment process is applied to pulp and paper mill sludge at 80 °C for 90 min, resulting in the highest influence on sludge solubilization and production of methane, ranging from 264–303 mL/g-VS [80].

Table 4: Summary of thermal pretreatment methods applied to various substrates.

Methods	Substrate	Observations	Refs.
Hot air oven	Pulp and paper mill sludge	The soluble COD and VFA were boosted up to 1.71 and 1.95 times, respectively.	[80]
Microwave and autoclave	Green microalgae (*Enteromorpha*)	After microwave pretreatment, methane was increased 1.30 times. The degradation rate of cells after autoclave pretreatment was increased by 95.99% at 120 °C.	[81]
Microwave	Sewage sludge	At 20,000 J/g TS and 700 W, the OLR rose by 43% and 39%, respectively.	[82]
Autoclave	Food waste	Ammonium and hydrogen sulphide concentrations were decreased.	[83]
Liquid hot water	Wheat straw	99.07% of the hemicellulose degradation and a 62.9% increase in biogas production were observed.	[84]
Liquid hot water	Hybrid Pennisetum	CH_4 yield increased by 32.9%, which was equivalent to a conversion efficiency of 76.1%.	[85]

The advancement of microwave (MW) technology has increased the amount of attention as an alternative non-conventional heating source that can be used to treat biomass and wastes. Microwaves are a type of electromagnetic energy that is located on the electromagnetic spectrum between 300 and 300,000 MHz. Microwave pretreatment on green microalgae (*Enteromorpha*), power and time fixed at 656.92 W, 5.10 min [86]. After microwave pretreatment, methane was increased from untreated sample at 188 mL to 244 mL in the treated sample [86]. Within a pilot-scale facility for the processing of sewage sludge microwave pretreatment, it is recommended to enhance the organic matter solubilization and the methane production [82]. The researcher varied MW's power and specific energy applied, and found that, at 20,000 joules/g-TS and 700 watts, the OLR rose by 43% and 39%, respectively [82]. After autoclave pretreatment of green algae at 120 °C, the degradation rate of biomass was increased to 95.99% [81]. Autoclave pretreatment was used on food waste at 160 °C and 6.2 bar, after 473 days in mesophilic reactors with a semi-continuous feeding method, reductions of ammonium and H_2S concentrations were observed [83].

According to a comparative analysis, the untreated water hyacinth with an F/M ratio of 2 yielded the most methane on the 32nd day at 143 mL/g-VS. Conversely, the hot air oven pretreated water hyacinth at an F/M ratio of 1.5 produced a higher methane yield of 193 mL/g-VS on the 14th day [27]. The water hyacinth that had been prepared with a hot air oven was found to produce more biogas in a very short amount of time [27]. To improve methane yield from wheat straw during AD, liquid hot water (LHW) pretreatment was set up at a range of temperatures (150–225 °C) and retention times (5–60 min) [84]. Approximately 27.69%–99.07% of the hemicellulose degradation was observed. Following LHW pretreatment at a temperature of 175 °C for a duration of 30 min, the methane yield rises to 62.9% when compared with untreated straw [84]. Hybrid *Pennisetum* was treated with a pretreatment involving LHW was carried out at 175 °C for 35 min, and the generation of CH_4 experienced a 32.9% increase. The theoretical energy conversion efficiency was found to be 76.1%, while the process energy efficiency, after accounting for pretreatment heat recovery, is calculated to be 51.7% [85]. Hemicelluloses are polymers that can be hydrolyzed with ease and are sensitive to thermal pretreatment. They have a lower molecular weight than cellulose. These compounds connect lignin to cellulose fibers, providing greater rigidity to the plant matrix as a whole [78]. The conversion of hemicellulose was more significant than that of cellulose and protein under mesophilic conditions. Yet, during thermophilic digestion, hemicellulose was converted with a much lower efficiency than cellulose [87].

5.3 *Biological pretreatment*

Biological pretreatments alter the chemical and physical structure of biomass by interacting with microorganisms that generate enzymes that break down the lignocellulosic structure [88]. The key findings of various biological pretreatment strategies that were applied to different biomass substrates (Table 5). By implementing biological pretreatment, the effectiveness of AD can be increased, leading to higher methane production and a faster hydrolysis stage. In general, lignocellulose-degrading bacteria and fungi are used for AD. SPT2-1 bacteria can successfully grow in pH 5.0–8.5, with a preferred temperature of 60–70 °C. During batch testing, the introduction of these isolated bacteria to preheated sludge led to the solubilization of 25–30% of the volatile suspended solids (VSS), in contrast to the negligible solubilization observed without inoculation. Microbial consortium can reduce the amount of lignin and result in increased methane production [89]. These isolated bacteria demonstrated the ability to secrete extracellular enzymes like proteases and amylases. In trials involving continuous flow, the sludge solubilization rate (VSS removal) was roughly 40% in both aerobic and microaerobic settings [90]. While aerobic conditions prevented the buildup of VFAs in treated sludge, microaerobic conditions resulted in a significant increase in their levels. Biogas production from the AD of sludge that was microaerobically pretreated was 1.5 times greater than that from untreated sludge [90]. As a strategy for pretreatment, thermophilic microbial consortia (MC1) were used to enhance the yields of biogas and methane production [91]. This study showed a considerable increase in sCOD concentrations during the initial phases of pretreatment. The MC1 hydrolysis revealed that the most abundant volatile organic compounds were ethanol, acetic acid, propionic acid, and butyric acid. Following the MC1 pretreatment, the biogas and methane production yields from lignocellulose in municipal solid waste (LMSW) observed a significant boost. Additionally, the treated LMSW produced more methane than the untreated sample [91].

Table 5: Evaluation of biological pretreatment approaches for biomass processing.

Methods	Substrate	Key Observations	Refs.
Bacteria SPT2-1	Organic sludge	The microaerobically prepared sludge resulted in a 1.5 increase in biogas production.	[90]
Thermophilic microbial consortia (MC1)	Lignocellulose of municipal solid waste	After pretreatment, the amount of soluble substrates in the hydrolysate increased.	[66]
Ceriporiopsis subvermispora (White rot Fungus)	Yard clippings	Pretreatment with 60% moisture content produced the maximum methane output of 44.6 L/kg-VS	[92]
Pleurotus ostreatus and *Trichoderma reesei*	Rice straw	*P. ostreatus* pretreatment was the most effective, removing 33.4% of the lignin while maintaining a high lignin selectivity.	[93]
Polyporus brumalis BRFM 985 strain (White rot Fungus)	Wheat straw	21% more methane yield was detected. Glucose addition reduces delignification.	[94]
Trichoderma longibrachiatum	Rice husk	A methane production of 438.1 mL/gVS, which was about 2.0 times greater than the control.	[95]
Pleurotus ostreatus and *Dichomitus squalens* (White rot fungi)	Corn silage	Pretreatment with *P. ostreatus* for 10 days at 28 °C increased the methane yield 1.55 times.	[96]

To increase methane generation via solid-state AD, yard clippings were treated with *Ceriporiopsis subvermispora*, a white-rot fungus that targets lignin [92]. The research focused on the effects of moisture content (45%–75%) on the degradation of holocellulose and lignin during the fungal pretreatment, as well as on methane production during digestion, comparing these outcomes to a control group (autoclaved without inoculation) and raw yard clippings [92]. At 60% MC, *C. subvermispora* degraded lignin at a rate of 20.9% but only 7.4% of cellulose and resulting in the highest methane output at 44.6 L/kg-VS. This was an increase of 106% over the raw yard clippings and 154% over the control group [92]. To enhance the biodegradability of rice straw and boost methane production via solid-state AD, *Pleurotus ostreatus* and *Trichoderma reesei* were utilized for fungal pretreatment [93]. The research assessed how different moisture contents and incubation periods influenced the degradation of lignin, cellulose, and hemicellulose, as well as methane output, compared to untreated rice straw [93]. The optimal results were observed with *P. ostreatus* at 75% moisture content and a 20-day incubation, achieving a 33.4% reduction in lignin and a lignin/cellulose removal ratio of 4.2 [93]. Another study screened 63 selected fungal strains to pretreat wheat straw. Among this fungal collection, the *Polyporus brumalis* BRFM 985 strain produced higher methane yield up to 43% than the untreated straw [94]. It also turned out that adding glucose during the pretreatment reduced the amount of methane produced from the substrate by delignification [94]. Similarly, the use of microbial consortium BYND-9 on pretreated corn stover, notable increase in *Methanosaeta* activity, climbing from 2.0% to 10.1%. The community's capacity to retain acetic acid and reduce CO_2 to produce methane. [95]. In comparison to the untreated stover, the methane output during the peak phase of pretreatment, maize stover treated with the microbial consortia BYND-9 was 62.85% [95].

Various pretreatment methods, such as physical, chemical, and biological ones, have been suggested. An effective pretreatment aims to accomplish delignification, enhance sugar solubilization, and reduce the crystallization of cellulose in the lignocellulosic feedstock [95]. Rice husk, a lignocellulosic bedding material obtained from broiler farms, was pretreated with the fungus *Trichoderma longibrachiatum* to improve the solid-state AD [97]. A series of batch experiments were performed to analyze the effectiveness of fungal pretreatment, taking into account both the pretreatment duration and the carbon-to-nitrogen (C/N) ratio. Under the optimal fungal pretreatment conditions assessed (C/N ratio of 18.9 and pretreatment duration of 7 days), disruption of the rice husk's outer layer structure was evident and a methane production of 438.1 mL/gVS was recorded, which was 2 times more than the control [97]. White rot fungi were used to treat corn silage to examine how pretreatment affected methane production [96].

6 Inhibitors in Anaerobic Digestion

During AD, the inhibition and toxicity levels of various compounds can differ greatly. This variation is largely due to the intricate nature of the anaerobic digestion process and substrate, which involves mechanisms like antagonism, synergism, acclimation, and complexing that can affect inhibition phenomena. There are several inhibitors that function in the anaerobic digestion process.

6.1 *Ammonia inhibitor*

Ammonia arises from the biological degradation of nitrogen-rich materials, predominantly proteins and urea. The production of ammonia during the anaerobic breakdown of organic substrates can be estimated using a particular stoichiometric formula. There are multiple proposed mechanisms for ammonia inhibition, such as shifts in intracellular pH, an increase in energy maintenance needs, and the inhibition of certain enzymatic processes [19]. In water, inorganic ammonia nitrogen primarily exists in two forms: ammonium ions (NH_4^+) and free ammonia (NH_3). A proton imbalance and/or a lack of potassium may occur due to the passive entry of the hydrophobic ammonia molecule into the cell. Methanogens are the least resistant and most likely to stop growing because of ammonia inhibition among the four categories of anaerobic bacteria. The granular sludge's methanogenic community saw a 56.5% drop in activity as ammonia level rose from 4.1 to 5.7 gNH_3/L, while the acidogenic populations were not impacted [19]. The ammonia inhibition can also be influenced by acclimation. Currently, methanogens are adapting to a diverse array of potentially harmful chemicals. Melbinger and Donnellon [98] explore how methanogens adapt to ammonia by subjecting them to progressively higher concentrations of Na^+, Ca^{2+}, and Mg^{2+} [99]. Gallert and Winter (1997) [100] found that free ammonia levels of 560–568 mg NH_3/L led to a 50% reduction in methanogenesis at a pH of 7.6 under thermophilic conditions. Additionally, research on cattle manure at thermophilic temperatures revealed that free ammonia concentrations exceeding 700 mg NH_3/L resulted in suboptimal treatment performance at a pH range of 7.4–7.9 [101].

In the aqueous phase, equilibria are established among ammonium ions (NH_4 (aq)$^+$), free ammonia (NH_3 (aq)) in solution, gaseous ammonia (NH_3 (g)), hydrogen ions (H^+), and hydroxyl ions (OH^-). The ratio of [ammonium] to [ammonia] is dependent on pH. A pH measurement not only reflects the concentrations of hydrogen and hydroxyl ions but also influences the overall composition of total ammonia nitrogen (TAN). At lower pH values, ammonium and hydrogen ions are the predominant species, whereas at higher pH levels, ammonia and hydroxyl ions prevail. Despite the presence of various inhibitory species, the specific composition of each can be distinctly identified at a given TAN concentration and pH value. The inhibition caused by ammonia can be eliminated by reducing the pH and subsequently lowering the concentration of free ammonia. This indicates that, while the toxicity in the digester may be indirectly associated with the concentration of free ammonia, it is directly linked to the concentration of unionized volatile acids. The process of diluting high nitrogen content waste streams with water or low nitrogen materials (co-digestion) can effectively decrease the concentration of ammoniacal nitrogen produced during anaerobic digestion. These approaches are often utilized to enhance solids loading in municipal AD or to control organic loading in food waste AD, where adjustments to the feed mixture can be made by managing sludge imports from different origins [102].

6.2 *Sulfide inhibition*

H_2S, being able to pass through the cell membrane, is considered the toxic variant of sulfide. Once it penetrates the cytoplasm, H_2S can exert inhibitory effects by disrupting various coenzyme sulfide connections, altering native proteins through the creation of sulfide and disulfide cross-links among polypeptide chains, and hindering the assimilatory metabolism of sulfur [103]. There is a notable lack of clarity in the literature about the nature of sulfide toxicity and the impact of various sulfides on microorganisms. According to Tursman and Cork [104], because H_2S may permeate into the cell membrane, it is the hazardous form of sulfide. Upon entering the cytoplasm, H_2S can inhibit functions by disrupting various coenzyme sulfide linkages [103], causing denaturation of native proteins through sulfide and disulfide cross-linking of polypeptide chains [105], and hindering sulfur's assimilatory metabolism. This perspective is supported by Speece [106] research. The inhibition of all bacterial species by sulfide was found to correlate with unionized sulfide levels within a pH range of 6.8 to 7.2, as well as total sulfide concentrations exceeding a pH of 7.2. Sulfide toxicity had less of an impact on fermentative microbes, which break down monomers into smaller compounds. There are multiple strategies available to facilitate the removal of dissolved sulfate. One option to address sulfide toxicity is to dilute the wastewater stream, although this is generally not preferred due to the consequent rise in the total volume of wastewater that needs treatment. An alternative is to add a sulfide removal phase to the overall process, which can effectively decrease sulfide concentrations in anaerobic treatment systems. Sulfide removal techniques include physicochemical approaches (like stripping), chemical methods (such as coagulation, oxidation, and precipitation), and biological processes (including partial oxidation to elemental sulfur) [107]. Sulfate is swiftly converted into toxic sulfide by sulfate-reducing bacteria (SRBs) through the dissimilatory sulfate reduction pathway in anaerobic environments, negatively impacting the AD process. In wastewater, sulfate is prevalent at concentrations between 20 to 200 mg/L in domestic settings and can be as high as 35 g/L in industrial wastewater from paper and pulp mills and chemical facilities [108]. The sulfur content varies widely across different feedstocks, and those rich in sulfur can lead to sulfide accumulation in AD processes, as sulfate from the degradation of sulfur compounds like proteins is reduced to sulfide. Within anaerobic digesters, sulfide can exist in both dissolved (S^{2-}, HS^-, and H_2Saq) and gaseous (H_2Sg) forms, depending on environmental factors such as pH and temperature.

The H_2S concentration in biogas generally falls between 0.1% and 2% (v/v), influenced by the composition of the substrate. H_2S is extremely corrosive and can inflict significant damage on the metallic components of biogas-handling systems [109]. Moreover, the distinct rotten egg smell of H_2S, detectable at very low levels (<2 ppmv), can lead to hygiene and health concerns. For commercial applications, the H_2S levels in biogas must be sufficiently low to comply with the standards for various uses [110]. Numerous *ex situ* and *in situ* technologies exist for sulfide removal to purify biogas. *ex situ* techniques have demonstrated effectiveness in extracting H_2S from biogas and are commonly implemented in full-scale operations. However, they incur high installation and operational expenses due to the necessity for intricate multi-step processes [111]. *in situ* techniques, on the other hand, either eliminate or inhibit sulfide formation during anaerobic digestion in digesters, and they do not necessitate large, separate facilities for sulfide extraction.

7 Conclusions

In an era where energy sustainability is a critical concern, AD emerges as a promising method for converting biomass into biogas. Pretreatment techniques have shown potential to enhance the AD process by increasing methane production. This enhancement can be achieved either by maintaining methane yield with reduced hydraulic retention time or by significantly increasing methane yield under comparable conditions. The growing demand for environmentally friendly waste management has driven extensive research in AD technology. Its applications span diverse sectors, including wastewater treatment, agriculture, and food waste management. Despite substantial progress in understanding the science of anaerobic digestion and the development of numerous optimization strategies, critical knowledge gaps persist. These gaps are particularly evident in batch-type digesters, where the complex biological and chemical processes are not yet fully understood. Furthermore, the wide range of AD applications highlights the continuous need for efficiency improvements, cost reduction, and time optimization. Achieving an economically viable end product requires a comprehensive understanding of small-scale batch digester design, effective pretreatment methods, and precise control of operating conditions.

Acknowledgments

This work was financially supported by Thailand Science Research and Innovation (TSRI), and National Science Research and Innovation Fund (NSRF) (Grant no. KMUTNB-FF-68-B-01).

Author Contributions

Conceptualization, B.S and M.S.; formal analysis, B.S and M.S.; investigation, B.S; data curation, N.A and M.S; writing—original draft preparation, B.S and M.S.; writing—review and editing, N.A., W.R. M.G. and M.S..; visualization, B.S, N.A, and M.S.; supervision, M.S..; project administration, M.S.; funding acquisition, M.S. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

References

[1] I. Raheem, A. Tawai, S. Amornraksa, M. Sriariyanun, A. Joshi, M. Gupta, W. Pongprayoon, D. Bharracharyya, and S. K. Maity, "A comprehensive review of approaches in carbon capture, and utilization to reduce greenhouse gases," *Applied Science and Engineering Progress*, vol. 18, no. 2, Article No. 7629, 2025, doi: 10.14416/j.asep.2024.11.004.

[2] M. Antar, D. Lyu, M. Nazari, A. Shah, X. Zhou, and D. L. Smith, "Biomass for a sustainable bioeconomy: An overview of world biomass production and utilization," *Renewable and Sustainable Energy Reviews*, vol. 139, p. 110691, 2021.

[3] A. B. D. Nandiyanto, N. N. Azizah, and G. C. S. Girsang, "Optimal design and techno-economic analysis for corncob particles briquettes: A literature review of the utilization of agricultural waste and analysis calculation," *Applied Science and Engineering Progress*, vol. 15, no. 3, Art. no. 5508, 2022, doi: 10.14416/j.asep.2021.10.006.

[4] D. O. Hall, "Biomass energy," *Energy Policy*, vol. 19, no. 8, pp. 711–737, 1991.

[5] O. M. Abioya, M. F. Amodu, D. O. Raphael, D. A. Olasehinde, M. M. Aniobi, K. O. Yusuf, and A. I. Olosho, "An overview of the role of vermicompost in reducing green house gas emissions, improving soil health, and increasing crop yields," *Applied Science and Engineering Progress*, vol. 18, no. 2, Art. no. 7586, 2025, doi: 10.14416/j.asep.2024.09.011.

[6] P. Baranitharan, S. Dasari, S. M. Kumar, N. Kottam, K. Rattanaporn, J. Jayaprakash, and M. Sriariyanun, "Chemically pretreated biomass conversion for biorefinery: A review of current trends," *Korean Chemical Engineering Research*, vol. 63, pp. 145–164, 2025.

[7] M. Sriariyanun and B. Dharmalingam, "From waste to wealth: Challenges in producing value-added biochemicals from lignocellulose biorefinery," *Journal of Applied Science and Emerging Technologies*, vol. 22, no. 3, 2023, doi: 10.14416/JASET.KMUTNB.2023.03.001.

[8] N. Pattharaprachayakul, N. Kesonlam, P. Duangjumpa, V. Rungsardthong, W. Suvajittanont, and B. Lamsal, "Optimization of hydraulic retention time and organic loading rate in anaerobic digestion of squeezed pineapple liquid wastes for biogas production," *Applied Science and Engineering Progress*, vol. 14, no. 3, pp. 468–476, 2021, doi: 10.14416/j.asep.2021.04.004.

[9] R. J. P. Latiza, R. V. Rubi, J. Olay, and A. Soriano, "Is the future of energy rotten? Novel perspective on tri-ohase fermentation and the food waste paradox," *Applied Science and Engineering Progress*, vol. 18, no. 4, Art. no. 7735, 2025, doi: 10.14416/j.asep.2025.05.008.

[10] N. Mills, P. Pearce, J. Farrow, R. B. Thorpe, and N. F. Kirkby, "Biochemical methane potential (BMP) test for *Ageratum conyzoides* to optimize ideal food to microorganism (F/M) ratio," *Waste Management*, vol. 34, no. 1, pp. 185–195, 2014.

[11] X. Meng, Q. Wang, X. Zhao, Y. Cai, X. Ma, J. Fu, and L. Ren, "A review of the technologies used for preserving anaerobic digestion inoculum," *Renewable and Sustainable Energy Reviews*, vol. 188, p. 113804, 2023.

[12] R. J. Paul and R. V. Rubi, "Circular economy integration in 1G+2G sugarcane bioethanol production: Application of carbon capture, utilization and storage, closed-loop systems, and waste valorization for sustainability," *Applied Science and Engineering Progress*, vol. 18, no. 1, p. 7448, 2025, doi: 10.14416/j.asep.2024.07.005.

[13] F. Sher, N. Smječanin, H. Hrnjić, A. Karadža, R. Omanović, E. Šehović, and J. Sulejmanović, "Emerging technologies for biogas production: A critical review on recent progress, challenges and perspectives," *Process Safety and Environmental Protection*, vol. 188, pp. 834–859, 2024, doi: 10.1016/j.psep.2024.05.138.

[14] S. Areeya, E. J. Panakkal, M. Sriariyanun, T. Kangsadan, A. Tawai, S. Amornraksa, U. W. Hartley, and P. Yasurin, "A review on chemical pretreatment of lignocellulose biomass for the production of bioproducts: Mechanisms,

challenges and applications," *Applied Science and Engineering Progress*, vol. 16, no. 3, p. 6767, 2023, doi: 10.14416/j.asep.2023.02.008.

[15] M. K. Jameel, M. A. Mustafa, H. S. Ahmed, A. J. Mohammed, H. Ghazy, M. N. Shakir, and E. Kianfar, "Biogas: Production, properties, applications, economic and challenges: A review," *Results in Chemistry*, vol. 10, p. 101549, 2024, doi: 10.1016/j.rechem. 2024.101549.

[16] F. Piadeh, I. Offie, K. Behzadian, A. Bywater, and L. C. Campos, "Real-time operation of municipal anaerobic digestion using an ensemble data mining framework," *Bioresource Technology*, vol. 392, p. 130017, 2024, doi: 10.1016/j.biortech. 2024.130017.

[17] K. He, Y. Liu, L. Tian, W. He, and Q. Cheng, "Review in anaerobic digestion of food waste," *Heliyon*, vol. 10, no. 5, p. e25792, 2024, doi: 10.1016/j.heliyon.2024.e25792.

[18] D. Jose, A. Tawai, D. Divakaran, M. Sriariyanun, V. Phakeenuya, Y. S. Cheng, and P. Tantayotai, "Influence of acetic acid pretreatment and its residue on bioethanol and biogas production from water hyacinth," *Applied Science and Engineering Progress*, vol. 17, p. 7326, 2024, doi: 10.14416/j.asep.2024.02.014.

[19] Y. Chen, J. J. Cheng, and K. S. Creamer, "Inhibition of anaerobic digestion process: A review," *Bioresource Technology*, vol. 99, no. 10, pp. 4044–4064, 2008, doi: 10.1016/ j.biortech.2007.01.057.

[20] V. Córdoba, M. Fernández, and E. Santalla, "The effect of different inoculums on anaerobic digestion of swine wastewater," *Journal of Environmental Chemical Engineering*, vol. 4, no. 1, pp. 115–122, 2016, doi: 10.1016/j.jece. 2015.11.016.

[21] M. Wang, J. Zhou, Y. X. Yuan, Y. M. Li, D. Li, Z. D. Dai, and Z. Y. Yan, "Methane production characteristics and microbial community dynamics of mono-digestion and co-digestion using corn stalk and pig manure," *International Journal of Hydrogen Energy*, vol. 42, no. 8, pp. 4893–4901, 2017, doi: 10.1016/j.ijhydene. 2016.12.048.

[22] M. P. Gundupalli, P. Tantayotai, S. Chuetor, K. Cheenkachorn, S. Joshi, D. Bhattacharyya, and M. Sriariyanun, "Improvement of water hyacinth bioconversion by different organic and mineral acid pretreatment and the effect of post-pretreatment washing," *BioEnergy Research*, vol. 16, pp. 1718–1732, 2023, doi: 10.1007/ s12155-023-10515-5.

[23] K. Dhamodharan, V. Kumar, and A. S. Kalamdhad, "Effect of different livestock dungs as inoculum on food waste anaerobic digestion and its kinetics," *Bioresource Technology*, vol. 180, pp. 237–241, 2015, doi: 10.1016/ j.biortech.2014.12.085.

[24] W. S. Lopes, V. D. Leite, and S. Prasad, "Influence of inoculum on performance of anaerobic reactors for treating municipal solid waste," *Bioresource Technology*, vol. 94, no. 3, pp. 261–266, 2004, doi: 10.1016/j.biortech.2003. 11.005.

[25] C. González-Fernández and P. A. García-Encina, "Impact of substrate to inoculum ratio in anaerobic digestion of swine slurry," *Biomass and Bioenergy*, vol. 33, no. 8, pp. 1065–1069, 2009, doi: 10.1016/j.biombioe.2009.05.007.

[26] I. M. Nasir, T. I. M. Ghazi, R. Omar, and A. Idris, "Anaerobic digestion of cattle manure: Influence of inoculums concentration," *International Journal of Engineering and Technology*, vol. 10, no. 1, pp. 22–26, 2013.

[27] V. B. Barua and A. S. Kalamdhad, "Biochemical methane potential test of untreated and hot air oven pretreated water hyacinth: A comparative study," *Journal of Cleaner Production*, vol. 166, pp. 273–284, 2017, doi: 10.1016/j.jclepro.2017. 08.016.

[28] B. Saha, P. M. Yunus, M. Khwairakpam, and A. S. Kalamdhad, "Biochemical methane potential trial of terrestrial weeds: Evolution of mono-digestion and co-digestion on biogas production," *Materials Science for Energy Technologies*, vol. 3, pp. 748–755, 2020, doi: 10.1016/j.mset.2020.08.005.

[29] F. Demichelis, T. Tommasi, F. A. Deorsola, D. Marchisio, and D. Fino, "Effect of inoculum origin and substrate-inoculum ratio to enhance the anaerobic digestion of organic fraction municipal solid waste (OFMSW)," *Journal of Cleaner Production*, vol. 351, p. 131539, 2022, doi: 10.1016/j.jclepro.2022.131539.

[30] M. S. Hossain, T. U. Karim, M. H. Onik, D. Kumar, M. A. Rahman, A. Yousuf, and M. R. Uddin, "Impact of temperature, inoculum flow pattern, inoculum type, and their ratio on dry anaerobic digestion for biogas production," *Scientific Reports*, vol. 12, no. 1, p. 6162, 2022, doi: 10.1038/s41598-022-09810-9.

[31] T. Forster-Carneiro, M. Pérez, and L. I. Romero, "Influence of total solid and inoculum contents on performance of anaerobic reactors treating food waste," *Bioresource Technology*, vol. 99, no. 15, pp. 6994–7002, 2008, doi: 10.1016/j.biortech.2007.11.044.

[32] B. Saha, A. Sathyan, A. S. Kalamdhad, and M. Khwairakpam, "Anaerobic biodegradability test for *Lantana camara* to optimize the appropriate food to microorganism (F/M) ratio," *Environmental Technology*, vol. 41, no. 24, pp. 3191–3198, 2020, doi: 10.1080/09593330.2019.1670308.

[33] H. M. El-Mashad, G. Zeeman, W. K. Van Loon, G. P. Bot, and G. Lettinga, "Effect of temperature and temperature fluctuation on thermophilic anaerobic digestion of cattle manure," *Bioresource Technology*, vol. 95, no. 2, pp. 191–201, 2004, doi: 10.1016/j.biortech.2003.01.016.

[34] Y. R. Chen, V. H. Varel, and A. G. Hashimoto, "Effect of temperature on methane fermentation kinetics of beef-cattle manure," *Biotechnology and Bioengineering Symposium*, vol. 10, 1980.

[35] H. E. Larsen, B. Munch, and J. Schlundt, "Use of indicators for monitoring the reduction of pathogens in animal waste treated in biogas plants," *International Journal of Hygiene and Environmental Health*, vol. 195, no. 5–6, pp. 544–555, 1994, doi: 10.1016/S1438-4639(07)80090-8.

[36] R. H. Clark and R. E. Speece, "The pH tolerance of anaerobic digestion," *Advances in Water Pollution Research*, vol. 1, pp. 1–13, 1971.

[37] M. G. Capri and G. V. R. Marais, "pH adjustment in anaerobic digestion," *Water Research*, vol. 9, no. 3, pp. 307–313, 1975, doi: 10.1016/0043-1354(75)90003-7.

[38] B. Zhang, L. L. Zhang, S. C. Zhang, H. Z. Shi, and W. M. Cai, "The influence of pH on hydrolysis and acidogenesis of kitchen wastes in two-phase anaerobic digestion," *Environmental Technology*, vol. 26, no. 3, pp. 329–340, 2005, doi: 10.1080/09593330.2005.9619016.

[39] M. A. Latif, C. M. Mehta, and D. J. Batstone, "Influence of low pH on continuous anaerobic digestion of waste activated sludge," *Water Research*, vol. 113, pp. 42–49, 2017, doi: 10.1016/j.watres.2017.01.004.

[40] J. Lindner, S. Zielonka, H. Oechsner, and A. Lemmer, "Effect of different pH-values on process parameters in two-phase anaerobic digestion of high-solid substrates," *Environmental Technology*, vol. 36, no. 2, pp. 198–207, 2015, doi: 10.1080/09593330.2014.918030.

[41] A. Hadiyarto, B. Budiyono, S. Djohari, I. Hutama, and W. Hasyim, "The effect of F/M ratio to the anaerobic decomposition of biogas production from fish offal waste," *Waste Technology*, vol. 3, no. 2, pp. 58–61, 2015.

[42] Y. Chen, Y. Wang, H. Xie, W. Cao, and Y. Zhang, "Varied promotion effects and mechanisms of biochar on anaerobic digestion (AD) under distinct food-to-microorganism (F/M) ratios and biochar dosages," *Waste Management*, vol. 155, pp. 118–128, 2023, doi: 10.1016/j.wasman.2023.06.011.

[43] M. N. I. Siddique, M. S. A. Munaim, and A. W. Zularisam, "Effect of food to microbe ratio variation on anaerobic co-digestion of petrochemical wastewater with manure," *Journal of the Taiwan Institute of Chemical Engineers*, vol. 58, pp. 451–457, 2016, doi: 10.1016/j.jtice.2015.11.022.

[44] X. Wang, X. Lu, F. Li, and G. Yang, "Effects of temperature and carbon-nitrogen (C/N) ratio on the performance of anaerobic co-digestion of dairy manure, chicken manure and rice straw: Focusing on ammonia inhibition," *PLoS One*, vol. 9, no. 5, p. e97265, 2014, doi: 10.1371/journal.pone.0097265.

[45] R. Barrena, E. I. Pagans, A. Artola, F. Vázquez, and A. Sánchez, "Co-composting of hair waste from the tanning industry with de-inking and municipal wastewater sludges," *Biodegradation*, vol. 18, pp. 257–268, 2007, doi: 10.1007/s10532-006-9059-4.

[46] M. Carlsson, A. Lagerkvist, and F. Morgan-Sagastume, "The effects of substrate pre-treatment on anaerobic digestion systems: A review," *Waste Management*, vol. 32, no. 9, pp. 1634–1650, 2012, doi: 10.1016/j.wasman.2012.05.005.

[47] Q. Zhang, Y. Yang, L. A. Hou, H. Zhu, Y. Zhang, J. Pu, and Y. Li, "Recent advances of carbon-based additives in anaerobic digestion: A review," *Renewable and Sustainable Energy Reviews*, vol. 183, p. 113536, 2023, doi: 10.1016/j.rser.2023.114195.

[48] B. Rincón, R. Borja, J. M. González, M. C. Portillo, and C. Sáiz-Jiménez, "Influence of organic loading rate and hydraulic retention time on the performance, stability and microbial

communities of one-stage anaerobic digestion of two-phase olive mill solid residue," *Biochemical Engineering Journal*, vol. 40, pp. 253–261, 2008, doi: 10.1016/j.bej.2008.07.006.

[49] Y. Kwon, J. Park, G. B. Kim, Y. Jo, S. Park, and S. H. Kim, "Anaerobic digestion of sewage sludge using anaerobic dynamic membrane bioreactor under various sludge composition and organic loading rates," *Bioresource Technology*, vol. 384, p. 129275, 2023, doi: 10.1016/j.biortech. 2023.129275.

[50] P. de Souza Almeida, C. A. de Menezes, F. P. Camargo, I. K. Sakamoto, M. B. A. Varesche, and E. L. Silva, "Thermophilic anaerobic co-digestion of glycerol and cheese whey–Effect of increasing organic loading rate," *Process Safety and Environmental Protection*, vol. 165, pp. 895–907, 2022, doi: 10.1016/j.psep.2022.05. 049.

[51] W. Somphol, N. Chanka, T. Boonmalert, S. Loykulnart, P. Prapainainar, A. Seubsai, and P. Dittanet, "Extraction of cellulose nanocrystals and nanofibers from rubber leaves and their impacts on natural rubber properties," *Applied Science and Engineering Progress*, vol. 17, no. 2, p. 7281, 2024, doi: 10.14416/j.asep.2023.11.010.

[52] J. Waluyo, I. T. Purba, Z. A. Linanggeng, M. L. Maulana, E. Kanchanatip, M. Yanm, and D. Hantoko, "Biomass pyrolysis: A comprehensive review of production methods, derived products, and sustainable applications in advanced materials," *Applied Science and Engineering Progress*, vol. 18, no. 2, p. 7645, 2025, doi: 10.14416/j.asep.2024.11.009.

[53] M. U. Khan and B. K. Ahring, "Lignin degradation under anaerobic digestion: Influence of lignin modifications—A review," *Biomass and Bioenergy*, vol. 128, p. 105325, 2019, doi: 10.1016/j.biombioe.2019.105325.

[54] M. A. Khan, E. J. Panakkal, M. Sriariyanun, M. P. Gundupalli, S. Roddecha, K. Katam, J. Jayaprakash, and K. Cheenkachorn, "Dewaxing and post-pretreatment washing: Impact on sugar and ethanol yields from tobacco residue," *Applied Science and Engineering Progress*, vol. 17, no. 4, p. 7495, 2024, doi: 10.14416/ j.asep.2024.07.010.

[55] G. S. M. Allam, A. M. A, El-Aal, M. K. S. Morsi, and E.A. El-Salam, "Producing dietary fibers from sugarcane bagasse using various chemical treatment and evaluation of their physicalchemical, structural, and functional properties," *Applied Science and Engineering Progress*, vol. 17, no. 3, p. 7381, 2024, doi: 10.14416/j.asep.2024.06.002.

[56] N. Kongkum, V. Phakeenuya, and S. Kongruang, "Optimization of microcrystalline cellulose production from brewer's spent grain by acid hydrolysis," *Applied Science and Engineering Progress*, vol. 18, no. 2, p. 7624, 2025, doi: 10.14416/j.asep.2024.11.002.

[57] G. Brunow and K. Lundquist, "Functional groups and bonding patterns in lignin (including the lignin–carbohydrate complexes)," in *Lignin and Lignans: Advances in Chemistry*, Florida: CRC Press, pp. 267–299, 2010.

[58] K. Narayanan, P. Venkatachalam, E. J. Panakkal, P. Tantayotai, A. Tandhanskul, R. Selvasembian, S. Chuetor, and M. Sriariyanun, "Exploring ternary deep eutectic solvent pretreatment in a one-pot process with Napier grass for bioethanol production," *BioEnergy Research*, vol. 17, no. 4, pp. 2213–2225, 2024, doi: 10.1007/s12155-024-10791-y.

[59] M. Sriariyanun, M. P. Gundupalli, V. Phakeenuya, T. Phusamtisampan, Y. S. Cheng, and P. Venkatachalam, "Biorefinery approaches for production of cellulosic ethanol fuel using recombinant engineered microorganisms," *Journal of Applied Science and Engineering*, vol. 27, no. 2, pp. 1985–2005, 2023, doi: 10.6180/ jase.202402_27(2).0001.

[60] F. R. Amin, H. Khalid, H. Zhang, S. U. Rahman, R. Zhang, G. Liu, and C. Chen, "Pretreatment methods of lignocellulosic biomass for anaerobic digestion," *AMB Express*, vol. 7, p. 108, 2017, doi: 10.1186/s13568-017-0497-1.

[61] L. K. Akula, R. K. Oruganti, D. Bhattacharyya, and K. K. Kurilla, "Treatment of Marigold flower processing wastewater using a sequential biological-electrochemical process," *Applied Science and Engineering Progress*, vol. 14, no. 4, p. 5598, 2021, doi: 10.14416/j.asep.2021.11.007.

[62] S. Khaodang, N. Suriyachai, S. Imman, T. Kreetachat, S. Chuetor, S. Wongcharee, and K. Suwannahong, "Comparative study on techno-economic analysis for various organosolv fractionation of bagasse in thailand," *Applied Science and Engineering Progress,* vol. 18, no. 4, p. 7794, 2025, doi: 10.14416/j.asep.2025.06.011.

[63] D. Jose, N. Kitiborwornkul, M. Sriariyanun and K. Katam, "A review on chemical pretreatment methods of lignocellulosic biomass: Recent advances and progress," *Applied Science and*

Engineering Progress, vol. 15, no. 4, p. 6210, 2022, doi: 10.14416/j.asep.2022.08.001.
[64] T. Phusantisampan and N. Kitiborwornkul, "Progress in chemical pretreatment of lignocellulose biomass for applications in biorefinery," *The Journal of KMUTNB*, vol. 32, pp. 1087–1101, 2022, doi: 10.14416/j.kmutnb.2022.09.018.
[65] M. L. Torres and M. D. C. E. Lloréns, "Effect of alkaline pretreatment on anaerobic digestion of solid wastes," *Waste Management*, vol. 28, no. 11, pp. 2229–2234, 2008, doi: 10.1016/j.wasman.2008.05.009.
[66] Z. Song, G. Yang, X. Liu, Z. Yan, Y. Yuan, and Y. Liao, "Comparison of seven chemical pretreatments of corn straw for improving methane yield by anaerobic digestion," *PLoS One*, vol. 9, no. 4, p. e93801, 2014, doi: 10.1371/journal.pone.0093801.
[67] S. Xie, J. P. Frost, P. G. Lawlor, G. Wu, and X. Zhan, "Effects of thermo-chemical pre-treatment of grass silage on methane production by anaerobic digestion," *Bioresource Technology*, vol. 102, no. 19, pp. 8748–8755, 2011, doi: 10.1016/j.biortech.2011.07.046.
[68] G. W. Park, I. Kim, K. Jung, C. Seo, J. I. Han, H. N. Chang, and Y. C. Kim, "Enhancement of volatile fatty acids production from rice straw via anaerobic digestion with chemical pretreatment," *Bioprocess and Biosystems Engineering*, vol. 38, pp. 1623–1627, 2015, doi: 10.1007/s00449-015-1466-9.
[69] G. Mancini, S. Papirio, P. N. Lens, and G. Esposito, "Increased biogas production from wheat straw by chemical pretreatments," *Renewable Energy*, vol. 119, pp. 608–614, 2018, doi: 10.1016/j.renene.2017.11.056.
[70] M. Kaur, Y. P. Verma, and S. Chauhan, "Effect of chemical pretreatment of sugarcane bagasse on biogas production," *Materials Today: Proceedings*, vol. 21, pp. 1937–1942, 2020, doi: 10.1016/j.matpr.2020.04.345.
[71] A. D. Olugbemide, L. Lajide, A. Adebayo, and B. J. Owolabi, "Enhanced biogas production from rice husk through solid-state chemical pretreatments," *Waste and Biomass Valorization*, vol. 11, no. 6, pp. 2397–2407, 2020, doi: 10.1007/s12649-018-00567-9.
[72] E. J. Panakkal, M. Sriariyanun, J. Rattanapoompinyo, P. Yasurin, K. Cheenkachorn, W. Rodiawati, P. Tantayotai, "Influence of sulfuric acid pretreatment and inhibitor of sugarcane baggasse on the production of fermentable sugar and ethanol," *Applied Science and Engineering Progress*, vol. 15, no. 1, p. 5238, 2022, doi: 10.14416/j.asep.2021.07.006.
[73] P. Tantayotai, M. P. Gundupalli, E. J. Panakkal, M. Sriariyanun, K. Rattanaporn, D. Bhattacharyya, "Differential influence of imidazolium ionic liquid on cellulase kinetics in saccharification of cellulose and lignocellulosic biomass substrate," *Applied Science and Engineering Progress*, vol. 15, no. 3, p. 5510, 2022, doi: 10.14416/j.asep.2021.11.003.
[74] X. Li et al., "A comprehensive review of the strategies to improve anaerobic digestion: Their mechanism and digestion performance," *Methane*, vol. 3, no. 2, pp. 227–256, 2024, doi: 10.3390/methane3020015.
[75] P. P. Dell'Omo and V. A. Spena, "Mechanical pretreatment of lignocellulosic biomass to improve biogas production: Comparison of results for giant reed and wheat straw," *Energy*, vol. 203, p. 117798, 2020, doi: 10.1016/j.energy.2020.117798.
[76] S. Behera, R. Arora, N. Nandhagopal, and S. Kumar, "Importance of chemical pretreatment for bioconversion of lignocellulosic biomass," *Renewable and Sustainable Energy Reviews*, vol. 36, pp. 91–106, 2014, doi: 10.1016/j.rser.2014.04.014.
[77] M. Solé-Bundó, C. Eskicioglu, M. Garfí, H. Carrère, and I. Ferrer, "Anaerobic co-digestion of microalgal biomass and wheat straw with and without thermo-alkaline pretreatment," *Bioresource Technology*, vol. 237, pp. 89–98, 2017, doi: 10.1016/j.biortech.2017.03.053.
[78] G. Y. Chen, J. Cao, Z. Z. Chang, X. M. Ye, and J. Du, "Effect of organic acids pre-treatment on physico-chemical property and biogas production of wheat straw," *Acta Energiae Solaris Sinica*, vol. 36, pp. 2559–2564, 2015.
[79] C. Rodriguez, A. Alaswad, J. Mooney, T. Prescott, and A. G. Olabi, "Pre-treatment techniques used for anaerobic digestion of algae," *Fuel Processing Technology*, vol. 138, pp. 765–779, 2015, doi: 10.1016/j.fuproc.2015.06.010.
[80] C. Veluchamy and A. S. Kalamdhad, "Prerequisite—a hot air oven pretreatment for anaerobic digestion of lignocellulose waste material," in *CSBE/SCGAB 2017 Annual Conference*, 2017, Art. no. CSBE17043.

[81] R. Feng, Q. Li, A. A. Zaidi, H. Peng, and Y. Shi, "Effect of autoclave pretreatment on biogas production through anaerobic digestion of green algae," *Periodica Polytechnica Chemical Engineering*, vol. 65, no. 4, pp. 483–492, 2021, doi: 10.3311/PPch.16356.

[82] A. Serrano, J. A. Siles, M. A. Martín, A. F. Chica, F. S. Estévez-Pastor, and E. Toro-Baptista, "Improvement of anaerobic digestion of sewage sludge through microwave pre-treatment," *Journal of Environmental Management*, vol. 177, pp. 231–239, 2016, doi: 10.1016/j.jenvman.2016.03.019.

[83] E. Tampio, S. Ervasti, T. Paavola, S. Heaven, C. Banks, and J. Rintala, "Anaerobic digestion of autoclaved and untreated food waste," *Waste Management*, vol. 34, no. 2, pp. 370–377, 2014, doi: 10.1016/j.wasman.2013.11.015.

[84] G. Shang, C. Zhang, F. Wang, L. Qiu, X. Guo, and F. Xu, "Liquid hot water pretreatment to enhance the anaerobic digestion of wheat straw—effects of temperature and retention time," *Environmental Science and Pollution Research*, vol. 26, pp. 29424–29434, 2019, doi: 10.1007/s11356-019-06693-0.

[85] X. Kang, Y. Zhang, R. Lin, L. Li, F. Zhen, X. Kong, and Z. Yuan, "Optimization of liquid hot water pretreatment on hybrid *Pennisetum* anaerobic digestion and its effect on energy efficiency," *Energy Conversion and Management*, vol. 210, p. 112718, 2020, doi: 10.1016/j.enconman.2020.112718.

[86] R. Feng, A. A. Zaidi, K. Zhang, and Y. Shi, "Optimisation of microwave pretreatment for biogas enhancement through anaerobic digestion of microalgal biomass," *Periodica Polytechnica Chemical Engineering*, vol. 63, no. 1, pp. 65–72, 2019, doi: 10.3311/PPch.13057.

[87] S. Ghosh, M. P. Henry, and R. W. Christopher, "Hemicellulose conversion by anaerobic digestion," *Biomass*, vol. 6, no. 4, pp. 257–269, 1985.

[88] A. Ramos, E. Monteiro, and A. Rouboa, "Biomass pre-treatment techniques for the production of biofuels using thermal conversion methods–a review," *Energy Conversion and Management*, vol. 270, p. 116271, 2022, doi: 10.1016/j.enconman.2022.116271.

[89] S. S. Ali, A. E. F. Abomohra, and J. Sun, "Effective bio-pretreatment of sawdust waste with a novel microbial consortium for enhanced biomethanation," *Bioresource Technology*, vol. 238, pp. 425–432, 2017, doi: 10.1016/j.biortech.2017.04.095.

[90] S. Hasegawa, N. Shiota, K. Katsura, and A. Akashi, "Solubilization of organic sludge by thermophilic aerobic bacteria as a pretreatment for anaerobic digestion," *Water Science and Technology*, vol. 41, no. 3, pp. 163–169, 2000.

[91] X. Yuan, B. Wen, X. Ma, W. Zhu, X. Wang, S. Chen, and Z. Cui, "Enhancing the anaerobic digestion of lignocellulose of municipal solid waste using a microbial pretreatment method," *Bioresource Technology*, vol. 154, pp. 1–9, 2014, doi: 10.1016/j.biortech.2013.12.042.

[92] J. Zhao, Y. Zheng, and Y. Li, "Fungal pretreatment of yard trimmings for enhancement of methane yield from solid-state anaerobic digestion," *Bioresource Technology*, vol. 156, pp. 176–181, 2014, doi: 10.1016/j.biortech.2014.01.089.

[93] A. M. Mustafa, T. G. Poulsen, and K. Sheng, "Fungal pretreatment of rice straw with *Pleurotus ostreatus* and *Trichoderma reesei* to enhance methane production under solid-state anaerobic digestion," *Applied Energy*, vol. 180, pp. 661–671, 2016, doi: 10.1016/j.apenergy.2016.08.123.

[94] E. Rouches, S. Zhou, J. P. Steyer, and H. Carrère, "White-Rot Fungi pretreatment of lignocellulosic biomass for anaerobic digestion: Impact of glucose supplementation," *Process Biochemistry*, vol. 51, no. 11, pp. 1784–1792, 2016, doi: 10.1016/j.procbio.2016.08.020.

[95] Y. Zhao, C. Xu, S. Ai, H. Wang, Y. Gao, L. Yan, and W. Wang, "Biological pretreatment enhances the activity of functional microorganisms and the ability of methanogenesis during anaerobic digestion," *Bioresource Technology*, vol. 290, p. 121660, 2019, doi: 10.1016/j.biortech.2019.121660.

[96] P. Basinas, J. Rusín, K. Chamrádová, K. Malachová, Z. Rybková, and Č. Novotný, "Fungal pretreatment parameters for improving methane generation from anaerobic digestion of corn silage," *Bioresource Technology*, vol. 345, p. 126526, 2022, doi: 10.1016/j.biortech.2021.126526.

[97] N. Chaitanoo, P. Aggarangsi, and S. Nitayavardhana, "Improvement of solid-state anaerobic digestion of broiler farm-derived waste via fungal pretreatment," *Bioresource Technology*, vol. 332, p. 125146, 2021, doi: 10.1016/j.biortech.2021.125146.

[98] N. R. Melbinger, J. Donnellon, and H. R. Zablatzky, “Toxic effects of ammonia nitrogen in high-rate digestion [with discussion],” *Water Pollution Control Federation*, vol. 43, no. 8, pp. 1658–1670, 1971.

[99] S. W. Miller and G. F. Parkin, “Response of methane fermentation to continuous addition of selected industrial toxicants,” in *Proceedings of the 37th Purdue Industrial Waste Conference*, 1983, pp. 729–743.

[100] C. Gallert and J. Winter, “Mesophilic and thermophilic anaerobic digestion of source-sorted organic wastes: Effect of ammonia on glucose degradation and methane production,” *Applied Microbiology and Biotechnology*, vol. 48, pp. 405–410, 1997, doi: 10.1007/s002530051095.

[101] I. Angelidaki and B. K. Ahring, “Anaerobic thermophilic digestion of manure at different ammonia loads: effect of temperature,” *Water Research*, vol. 28, no. 3, pp. 727–731, 1994, doi: 10.1016/0043-1354(94)90268-0.

[102] Y. Jiang, E. McAdam, Y. Zhang, S. Heaven, C. Banks, and P. Longhurst, “Ammonia inhibition and toxicity in anaerobic digestion: A critical review,” *Journal of Water Process Engineering*, vol. 32, p. 100899, 2019, doi: 10.1016/j.jwpe.2019.100899.

[103] E. Conn and P. Stumpf, *Outlines of Biochemistry*, 5th ed., New York: John Wiley & Sons, 2009.

[104] J. F. Tursman and D. J. Cork, “Influence of sulfate and sulfate-reducing bacteria on anaerobic digestion technology,” *Advances in Biotechnological Processes*, vol. 12, pp. 273–285, 1989.

[105] G. D. Vogels, “Biochemistry of methane production,” in *Biology of Anaerobic Microorganisms*. New Jersey: Wiley, pp. 707–770, 1988.

[106] R. E. Speece, “Anaerobic biotechnology for industrial wastewater treatment,” *Environmental Science & Technology*, vol. 17, no. 9, pp. 416A–427A, 1983, doi: 10.1021/es00117a001.

[107] Z. Song, C. J. Williams, and R. G. J. Edyvean, “Coagulation and anaerobic digestion of tannery wastewater,” *Process Safety and Environmental Protection*, vol. 79, no. 1, pp. 23–28, 2001, doi: 10.1205/095758201750114648.

[108] H. Jung, D. Kim, H. Choi, and C. Lee, “A review of technologies for in-situ sulfide control in anaerobic digestion,” *Renewable and Sustainable Energy Reviews*, vol. 157, p. 112068, 2022, doi: 10.1016/j.rser.2022.112068.

[109] M. Florent and T. J. Bandosz, “Effects of surface heterogeneity of cobalt oxyhydroxide/graphite oxide composites on reactive adsorption of hydrogen sulfide,” *Microporous and Mesoporous Materials*, vol. 204, pp. 8–14, 2015, doi: 10.1016/j.micromeso.2014.12.027.

[110] A. Choudhury and S. Lansing, “Biochar addition with Fe impregnation to reduce H2S production from anaerobic digestion,” *Bioresource Technology*, vol. 306, p. 123121, 2020, doi: 10.1016/j.biortech.2020.123121.

[111] D. Kim, H. Kim, J. Kim, and C. Lee, “Co-feeding spent coffee grounds in anaerobic food waste digesters: Effects of co-substrate and stabilization strategy,” *Bioresource Technology*, vol. 288, p. 1215, 2019, doi: 10.1016/j.biortech.2019.121511.