BIOLOGICAL AND THERMAL PRETREATMENT OF LIGNOCELLULOSIC MATERIALS FOR ENHANCED BIOGAS PRODUCTION

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ABSTRACT

The rapid depletion of natural resources and the environmental concerns associated with the use of fossil fuels as the main source of global energy is leading to an increased interest in alternative and renewable energy sources. Lignocellulosic biomass is the most abundant source of organic materials that can be utilized as an energy source. Anaerobic digestion has been proven to be an effective technology for converting organic material into energy products such as biogas. However, the nature of lignocellulosic materials hinders the ability of microorganisms in an anaerobic digestion process to degrade and convert organic material to biogas. Therefore, a pretreatment step is necessary to improve the degradability of lignocellulosic materials and achieve higher biogas yield. Several pretreatment methods have been studied over the past few years including physical, thermal, chemical and biological pretreatment. This paper reviews biological and thermal pretreatment as two main promising methods used to improve biogas production from lignocelluloses. A greater focus is given on enzymatic pretreatment which is one of the promising yet under-researched biological pretreatment method. The paper addresses challenges in degrading lignocellulosic materials and the current status of research to improve biogas yield from lignocelluloses through biological and thermal pretreatment.

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1. INTRODUCTION

The past decade has witnessed a surge in challenges arising from the increased consumption of fossil fuels and non-renewable energy sources such as the depletion of natural resources and the environmental impact of the use of fossil fuel that contributes to climate change and global warming. It is estimated that more than 84% of the world's energy demand is supplied through non-renewable sources such as fossil fuels, coal and natural gas [1]. However, such challenges paved a wider way for considering and further researching the use of renewable energy sources and managing natural resources in a sustainable manner. A great amount of research has been dedicated to the use of renewable organic biomass as a more sustainable alternative to fossil fuels and a suitable mean of waste reduction.

A particular interest has been given to lignocellulosic biomasses which are the most abundant source of organic matter on biosphere. Lignocellulosic materials (LCM) are organic materials usually found in plant cell wall [2,3]. Lignocelluloses are composed of a mixture of three main polymers: cellulose, hemicellulose, and lignin that bound together to form the rigid and protective layer of the plant cell wall [1,4]. It can be collected as a waste material from forest, agricultural, industrial, and municipal areas [5]. In addition, Lignocellulosic biomass could be grown as an energy crop that does not compete with food crops and can be planted in areas not suitable for food crops as several ethical concerns have arisen from using food crops such as sugarcane for biofuels production [2,6].

Although lignocellulosic biomass is abundant, there are still many challenges hindering it as an attractive energy source due to the nature and complexity of its components. Lignocellulosic

materials are often insoluble in water at low temperature and are not easily digestible by most of living organisms including bacteria. This is mainly due to the interaction of the cellulose, hemicellulose, and lignin which form a highly resistant and recalcitrant structure [1,2]. Recent studies have shown that lignocellulosic material makes up about 14 to 44% of raw excess sludge produced in different wastewater treatment processes indicating the difficulty in digesting it through microorganisms [4].

Anaerobic digestion (AD) of organic matter has proven to be one of the most cost effective and efficient biological processes in treating and converting organic matter to energy in the form of electricity, heat and natural gas [7]. AD is a natural process that relies on microorganisms in digesting organic matter in the absence of oxygen [1]. However, lignocellulosic materials have shown great resistant to anaerobic digestion resulting in low energy yield and digestibility level if introduced to AD without any pretreatment [4,7]. Hydrolysis is often believed to be the rate limiting step in the AD of lignocelluloses [1,7]. Therefore, to increase the efficiency of AD in treating lignocellulosic materials and improve energy yield, an efficient pretreatment process is required to enhance the digestibility of lignocellulosic biomass by microorganisms.

Several pretreatment processes have been developed and researched over the past years which could be categorized as physical, thermal, chemical, biological, and combined pretreatment processes [4]. The pretreatment process is essential when handling lignocellulosic material through AD for a cost efficient and economical conversion. However, this pretreatment step is the most expensive and accounts for about 20% of the total energy cost yielded from lignocellulosic biomass [8]. Hence, it is important to improve the available pretreatment processes and find new affordable and efficient techniques.

This paper presents a review of biological and thermal pretreatment of lignocellulosic materials for enhanced biogas production, with focus on enzymatic pretreatment as an emerging pretreatment method that is gaining wider attention in this field. The review includes an overview of lignocelluloses composition and sources, the anaerobic digestion process, a summary of studies available in the literature and the economical aspect of pretreatment. The paper also compares different pretreatment methods and outlines literature limitations and recommended future areas of research.

2. LIGNOCELLULOSIC BIOMASS OVERVIEW

2.1. Composition

Lignocellulosic materials are mainly characterized by the presence of three main polymers: lignin, cellulose and hemicellulose in addition to other components found in smaller amounts such as ash, pectin and proteins. Lignocellulose is the main component of plant cell wall which makes LCM as the most abundant organic sources on earth [7,9]. A typical composition of a lignocellulosic material is presented in Figure 1 below.

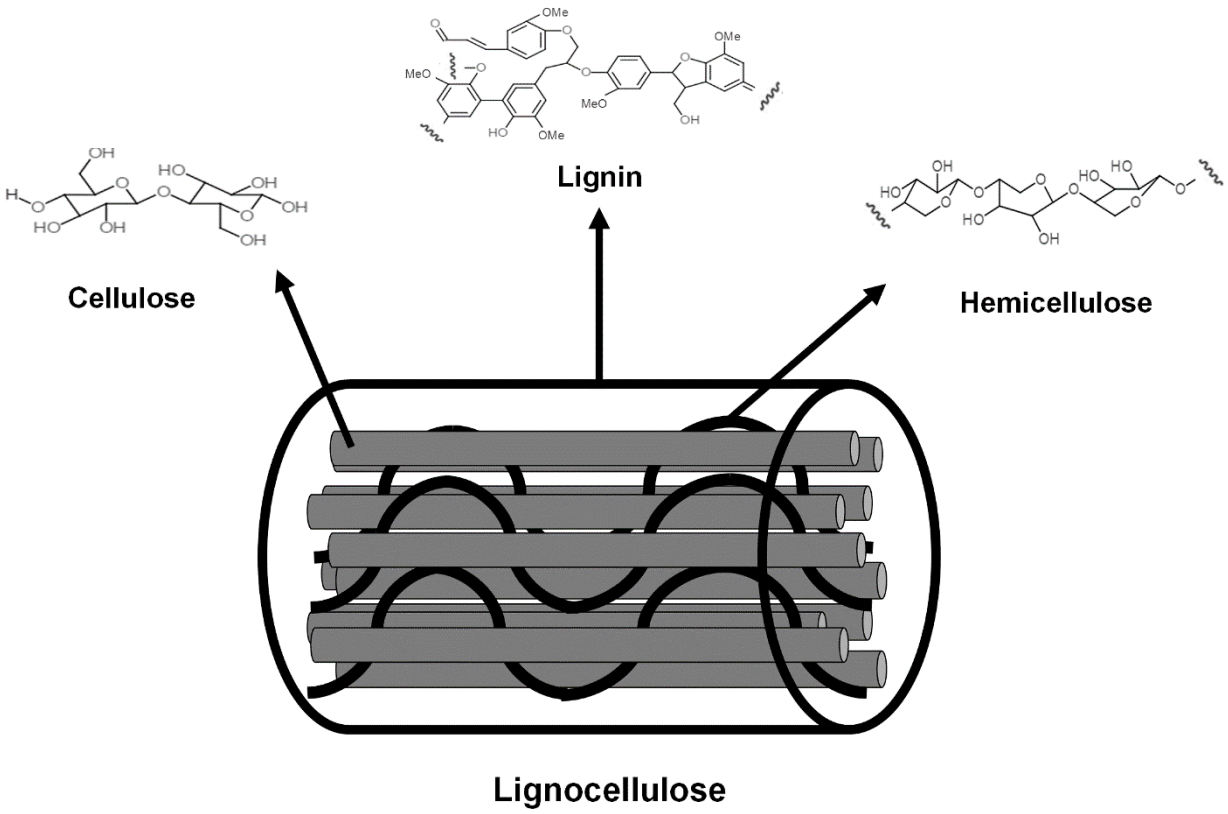

Figure 1. Typical composition of lignocellulosic material (Adapted from ref 10).

In general, the content of cellulose, hemicellulose, and lignin in lignocellulosic materials is about 30-60%, 20-40% and 15-25%, respectively as shown in Figure 2 [1,11,12].

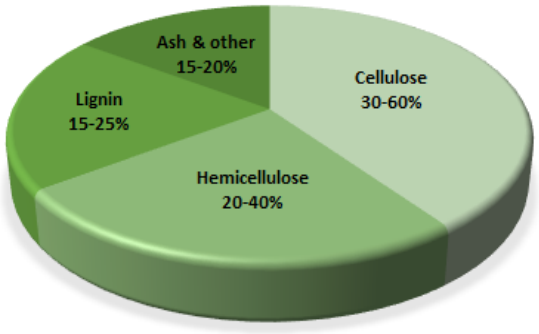

Figure 2 Typical composition of lignocellulosic biomass.

However, different types of lignocellulosic material vary in composition and the percentage content of cellulose, hemicellulose and lignin [1,13]. Table 1 summarizes the average composition of some of the common LCM. The variation in composition is not only present between different species, in fact, varying growth conditions and maturation can also impact the composition of LCM within the same species [1].

Table 1 Composition of different lignocellulosic materials.

Lignocellulosic material	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Reference
Corn stover	37.5	22.4	17.6	1, 14
Corn cobs	45	35	15	14
Cotton seed hairs	80-95	5-20	0	14
Switchgrass	31.0 - 45	20.0 - 31.0	12.0 -18.0	1,15
Bagasse	38.2	27.1	20.2	1
Sugarcane	25.0	17.0	12.0	1
Rice straw	32.0	24.0	13 - 18	1,15
Giant reed stalk	33.1	18.5	24.5	1
Giant reed leaves	20.9	17.7	25.4	1
Sunflower stalk	31.0	15.6	29.2	1
Rye straw	38.0	36.9	17.6	1
Eucalyptus	38.0-45.0	12.0-13.0	25.0-37.0	1
Hardwood stems	40.0-55.0	24.0-40.0	18-25	14,15,16
Softwood stems	45-50	25-35	25-35	14,15,16
Nut shells	25-30	25-30	30-40	14,15,16
Paper	85-99	0	0-15	14,15,16
Leaves	15-20	80-85	0	14,15,16
Newspaper	40-55	25-40	18-30	14,15,16
Grasses	25-40	35-50	10-30	2, 14,15,16
Solid cattle manure	1.6-4.7	1.4-3.3	20	14

2.1.1. Cellulose

Cellulose is composed of D-glucose subunits linked by β -1,4 glycosidic bonds and is the main component of most plant cell walls making it as one of the most abundant source of renewable polymers available [5,7]. Cellulose is insoluble in water and many organic solvents. However, it can be dissolved in water at extremely low or high pH levels as well as other solvents such as ionic liquids (ILSs) and N-methylmorphloine N-oxide (NMMO). The insolubility of cellulose is believed to be a result of the hydrogen bonds holding the crystalline structure [17,18]. The characteristics of cellulose make it difficult to be biodegraded or digested by most animals [5,7,19].

2.1.2. Hemicellulose

Hemicellulose refers to a family of heteropolymers or polysaccharides that are amorphous and random, and have highly branched structures. There are variable structures of hemicellulose that vary depending on the source of material and extractions method. Hemicellulose is part of the supporting materials in plants cell walls. The composition of hemicellulose is highly variable between different plants and materials. For example, hemicellulose found in hardwood is mainly composed of xylans, while the main component in hemicellulose in softwood is glucomannans [2,7,16]. Hemicellulose requires elevated temperatures to become soluble in water with its solubilisation starting at 150 to 180 ^{o}C [19].

2.1.3. Lignin

Lignin is the most abundant organic compound after cellulose [7]. It is a complex and large compound made up of phenylpropane units linked in a three-dimensional structure. The main monomers of lignin are p-hydroxyphenyl alcohol, coniferyl alcohol, and sinapyl alcohol [7,14, 21]. Lignin acts as cementing material that links cellulose and hemicellulose to form the rigid three-dimensional structure of plant cell wall [7]. In addition, lignin is optically inactive and

requires elevated temperatures starting at 180 ^{o}C to dissolve in water [19]. Such properties of lignin makes it the most component in LCM resistant to microbial attacks and biodegradation. Several studies have shown that higher lignin content in LCM increases resistance to biological and chemical degradation [7,14, 20].

2.2. Sources of Lignocellulosic Biomass

The abundant supply of lignocellulosic biomass could be attributed to its high variety of sources. Lignocellulosic biomass sources can be divided into two main categories which are waste sources and energy crops. Waste sources are those sources where lignocellulosic biomass is produced as a by-product and waste due to different natural and human activities such as forestry and agricultural residues, in addition to municipal solid wastes. Energy crops are those specifically grown as organic feedstocks to produce bioenergy products such as fast-growing trees and switchgrass [2,22,23].

Table 2 Typical sources and examples of lignocellulosic materials (Adapted from Refs 2, 11 and 22).

Category	Source	Example
Waste	Forestry	Residues resulting from forest logging, harvesting and other operations.
	Agricultural	Corn stover, sugarcane bagasse, wheat straw, rice husk, pinewood
	Municipal	Paper waste, paper mill sludge
Energy crops	Energy crops	Switchgrass, giant reed and miscanthus

The wide range of lignocellulosic biomass contributing to a massive amount of lignocellulosic biomass being produced annually ranks it on top of the list for alternative energy sources despite the several challenges faced in making it an economically viable source for producing energy products [19]. Although there is a contradiction in the literature on the exact amount of global biomass belonging to lignocellulosic biomass some have claimed that annual LCM production is

estimated at 10 to 50 billion tons annually [24], while others claimed that it is close to 200 billion tons annually [25]. On a smaller scale, lignocellulosic biomass production in the United States has been estimated at 1.4 billion dry tons annually [2].

2.3. Lignocellulosic Biomass Conversion

Lignocellulosic material can be utilized to produce various energy products and other potential products. There are many processes that could be applied to convert lignocelluloses to different energy products such as biofuels and biogases. Such processes include anaerobic digestion, fermentation, incineration, pyrolysis, gasification and others [26-28]. Figure 3 presents some of the potential products that could be produced through different processes using lignocellulosic materials as feedstock.

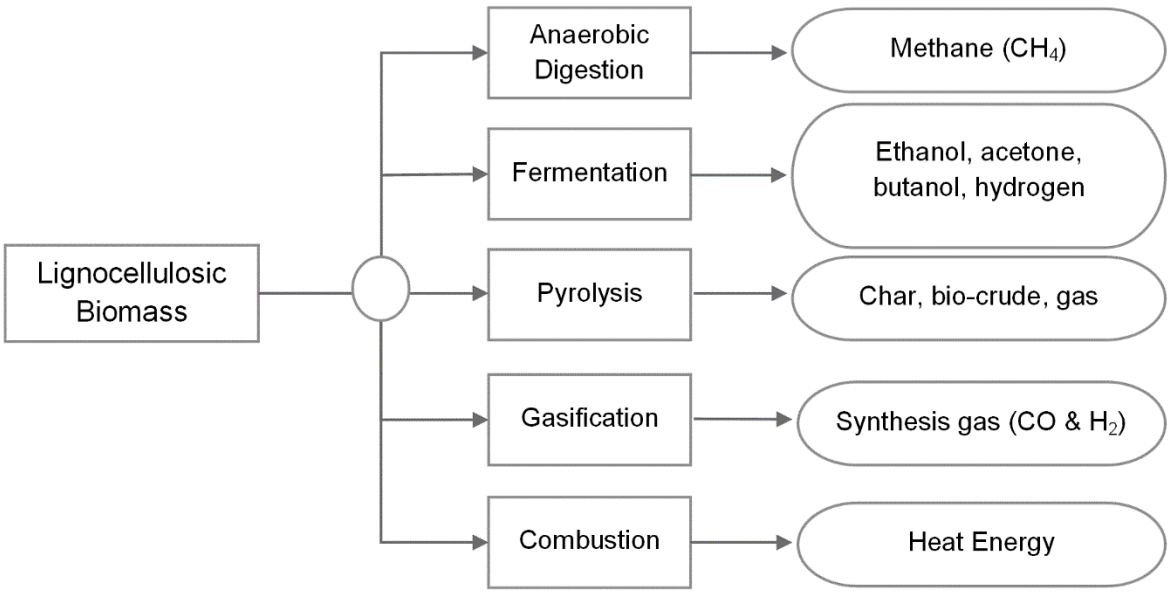

Figure 3. Potential products obtained from lignocellulosic materials through various processes.

However, there remain some obstacles impeding the production of energy from the abundant supply of biomass worldwide. The main challenge is the lack of low-cost technology that would make energy production from biomass an economically feasible process [29]. For this reason, it

is important to consider the economical aspect of new technologies and methods developed to improve the energy production from lignocellulosic biomass.

The focus of this paper is the AD as a means of producing biogas (biomethane and carbon dioxide) from lignocellulosic biomass. AD is very common as it relies on the use of microorganisms as an economical energy recovery process. However, as mentioned earlier, the nature of lignocellulosic material hinders the ability of such microorganism to efficiently recover energy from lignocelluloses and thus a process enhancement in the form of pretreatment is necessary to achieve economical feasibility and process sustainability.

3. ANAEROBIC DIGESTION OF LIGNOCELLULOSIC BIOMASS

Anaerobic digestion is a technology proven for its effectiveness and sustainability in treating and converting organic materials into energy products [30,31]. AD relies on microorganism in degrading organic matter and in converting the matter mainly into biogas in the form of methane and carbon dioxide (CO2). The biological degradation of LC material is mainly dependent on enzymes such as hemicellulases and cellulases produced by microorganisms [30].

AD process consists of four main steps (Figure 4): hydrolysis, acidogenesis, acetogenesis and methanogenesis. In the hydrolysis step, extracellular enzymes excreted by hydrolytic microorganisms break down complex organic polymers into simple soluble monomers [7]. Acidogenic or fermentative bacteria then convert the monomers produces to volatile fatty acids and other compounds (e.g alcohol). The fatty acids are then converted by acetogenic bacteria into acetate, carbon dioxide and hydrogen. Finally, methanogenic bacteria in the last step (methanogenesis) converts the products to biogas as a mixture of methane, carbon dioxide and other traces of gas. The percentage of methane in biogas has been reported to fall in the range of 50 to 75% [7,32,33].

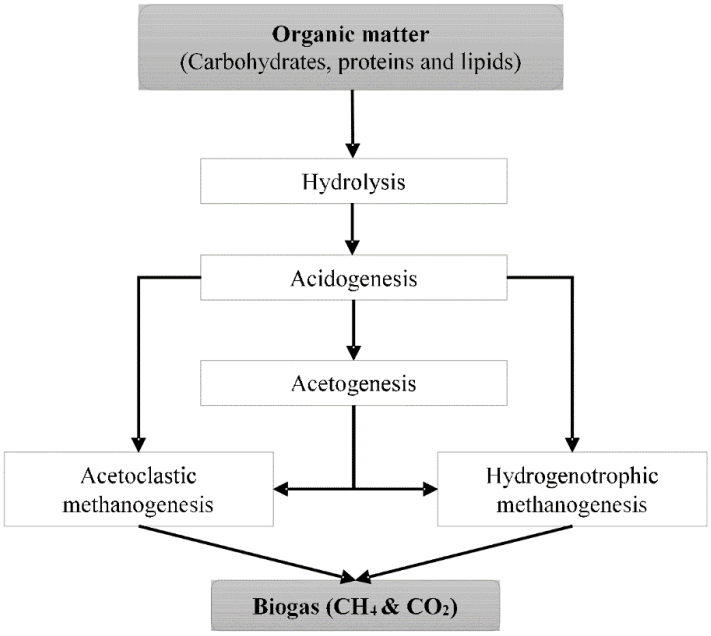

Figure 4. Anaerobic digestion process flow (Adapted from ref 7).

There are several types of reactors and mechanisms used for biogas production through anaerobic digestion. The different reactor design options and conditions have different impacts on the final process yield [34]. The main designs considered for this paper are batch, continuous and semi-continuous configurations. In addition, there are two main temperature intervals known as mesophilic and thermophilic.

In a batch AD process, all the substrate is placed in the reactor at the beginning and biogas production is initiated once the reactor is closed. The biogas is collected during this process until production ends. In the continuous or semi-continuous AD process, the substrate is fed in lesser amounts into the reactor at a steady flow rate while digestion and biogas production are ongoing [34].

As for temperature conditions, mesophilic conditions are usually those in the range of 30-40 ^{o}C, while thermophilic are in the range of 50-60 ^{o}C. The temperature range affects the types and

activity of microorganism in an AD reactor. Elevated temperatures might reduce pathogens level as well as the diversity of microorganisms. However, microorganisms and enzyme have different temperature requirements for optimum activity [34,35].

Although anaerobic digestion has proven its effectiveness in treating organic matter, this has not been the case with lignocellulosic materials. This is because the composition and properties of lignocellulosic substrates hinder the ability of microorganisms and enzymes in an anaerobic digestion process to digest the organic matter. A main reason for this is due to the presence of lignin which acts as a shield for cellulose and hemicellulose [13,31,36]. Several studies have indicated that higher lignin contents in organic matter results in lower biogas and methane yield [13,36]. In addition, the crystallinity and available surface area of lignocellulosic matter hinders the ability of enzymes to reach a significant amount of areas along the surfaces of the material and thus limiting degradation abilities [36,37]. Such obstacles make the hydrolysis of cellulose and hemicellulose the rate limiting step in the anaerobic digestion of lignocellulosic biomass [7,38].

Therefore, a pretreatment step is necessary to improve the hydrolysis of lignocellulosic material and thus biogas yield.

4. PRETREATMENT OF LIGNOCELLULOSIC BIOMASS

Lignocellulosic substrates are organic compounds that hold an enormous potential for anaerobic digestion and methane production. However, the chemical and physical composition of lignocelluloses hinders the ability of microorganisms to breakdown these organic compounds and release biogas. However, with the right pretreatment process, the biodegradation of lignocelluloses can be improved to enhance biogas and methane production.

Several pretreatment processes have been developed over the past years to improve lignocellulosic biomass amenity to microorganism and enzymes, and enhance biogas and methane production. Pretreatment methods work in different ways to achieve desired goals. However, such methods have been observed to alter some common physical and chemical characteristics of lignocellulosic biomass such as reducing lignin and hemicelluloses contents, reducing cellulose crystallinity, reducing the degree of polymerisation and increasing accessible surface area and porosity [14,21,39]. Lignin modification and removal is particularly essential for improving methane yield as several studies have suggested that lignin concentration in a substrate is negatively proportional to methane yield [13,40].

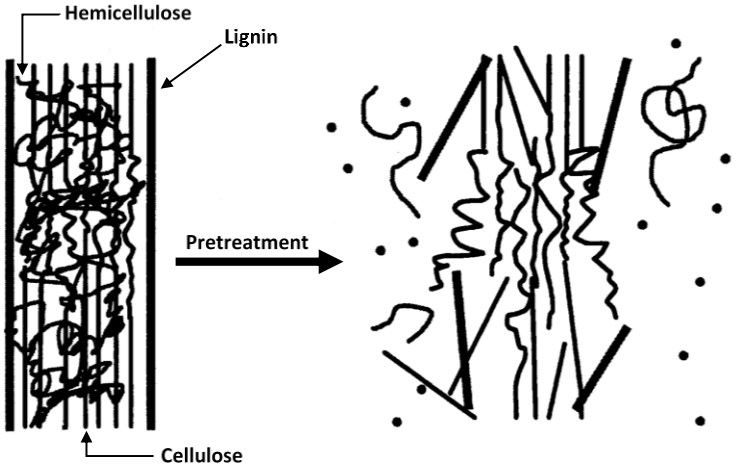

Figure 5 Schematic of pretreating lignocellulosic biomass (Adapted from Ref 41)

Available pretreatment processes can be categorized into: physical (mechanical and irradiation), thermal, chemical, biological and combined pretreatment. Mechanical, irradiation and thermal pretreatment mainly rely on applying physical, radiation and heat energy on the lignocellulosic substrates prior to hydrolysis and AD. Biological pretreatment relies on the action of added microorganisms, fungi, and enzymes to enhance the biodegradability of lignocelluloses. Chemical pretreatment involves treatment with chemicals to enhance the anaerobic digestion process. The use of two or more different pretreatment methods is considered as a combined pretreatment method.

However, the choice of a suitable pretreatment method depends on the composition and type of the lignocellulosic materials considered as the amount of cellulose, hemicellulose and lignin varies from one type to another as presented in Table 1. In addition, a variation in the physical characteristics such as accessible surface area among different LCM might require distinct types of pretreatment processes [7]. This section provides a review of the latest and most commonly used pretreatment processes of lignocellulosic wastes for enhanced methane production.

4.1. Biological - Enzymatic Pretreatment

Enzymatic pretreatment of LCM involves the use of oxidative and hydrolytic enzymes often produced by bacteria and fungi [9]. This pretreatment method is gaining more interest due to its lower reaction times, low consumption of nutrition by enzymes and because enzymes are not affected by most inhibitors and microbial metabolisms [37,42]. In addition, enzymatic pretreatment does not require the use of expensive equipment for processing [43], however, high enzymes cost remains a challenge to improving the economical feasibility of enzymatic pretreatment for improved biogas production [7,42,43].

The effect of enzymes on lignocellulosic substrates depends on the type of enzymes and the composition of the substrate. This is due to enzyme specificity in terms of the reaction they catalyze [44,45]. For example, the enzyme laccase derived from phenoloxidasses that contains different copper ions works by catalyzing the oxidation of phenols, anilines and aromatic thiols in substrates. As a result, microbial growth is enhanced and fermentation capability is improved during anaerobic digestion. Manganese peroxidases produced by white rot fungi catalyze the oxidation of Mn^{2+} to Mn^{3+} and thus aiding the breaking lignin bonds [21]. A commonly used enzyme known as cellulase extracted from the fungus *Trichoderma reesei* is known for its ability to degrade insoluble cellulose [46]. Such enzymatic actions catalyze anaerobic reaction, improve the degradability of lignocelluloses, and thus enhance biogas production [47].

The activity and efficiency of enzymes is dependent on several factors including substrate type, incubation time, temperature, pH level and the AD system configuration [38].

Compared to other pretreatment methods, enzymatic pretreatment of lignocellulosic biomass to improve biogas production has not been given the same attention. However, recent studies are showing a greater attention to this pretreatment method due to the potential it holds for improving anaerobic digestion and methane production. This section presents a review of the most recent studies conducted to asses the impact of different enzymatic pretreatment methods on methane production from lignocellulosic substrates through anaerobic digestion.

Tables 3-5 present the different studies conducted to improve biogas production from lignocellulosic material through enzymatic pretreatment. The studies are categorized based on the anaerobic digestion mechanism including mesophilic batch reactors, thermophilic batch reactors and continuous or semi-continuous reactors. Mesophilic batch reactors are more commonly used in research studies compared to other AD mechanisms. This might be due to the harsher conditions of thermophilic processes not suitable for all enzymes, and the higher costs associated with operating continuous or semi-continuous reactors.

More than 20 enzymes have been tested for enzymatic pretreatment of lignocellulosic material. Cellulase is the most commonly applied enzyme followed by β-glucosidase and Xylanase. Overall, enzymatic pretreatment seems to be a promising method to improve methane production from lignocellulosic biomass. On average, total methane yield was improved by 15% to 35% among the different substrates, pretreatment conditions and AD mechanisms. However, there were clear distinctions between the performance of the different substrates and AD mechanisms as discussed in the following sections.

Mesophilic Batch Reactors

The pretreatment of lignocelluloses using enzymes is most likely to be followed by a mesophilic batch AD process as presented in the literature. Such studies are presented in Table 3, which outlines the pretreatment conditions, type of enzymes used, enzymes source and the impact of the final methane yield.

Table 3 Enzymatic pretreatment of lignocellulosic biomass for biogas production in a mesophilic batch reactor.

Substrate	Enzymes	Enzyme Source	Pretreatment Conditions	AD Mechanism	Methane Yield	Ref
Damaged wheat grains	Trizyme: cellulase α-amylase protease	/ [a]	37 °C for 24 h.	Batch 37 -40 °C	+ 7 – 14%	48
Pulp and paper sludge	Endoglucanase Laccase	*Pleurotus ostreatus*	37 °C for 4 h.	Batch 37 °C	+34%	49
Filter paper Feed concentrate Hay fibers Grass silage Corn Silage	Trichoderma-enzyme complex	*Trichoderma reesei*	/	Batch 19 °C	+ 4-35 % [b]	46
Willow Corn Stover Flax Maize Wheat Straw Hemp Miscanthus	Laccase Versatile peroxidase	*Trametes versicolor* *Bjerkandera adusta*	30 °C for 6h - 24h	Batch Mesophilic	+33% +15% +14% +6% No Change No Change No Change	13
Microalgal biomass	Cellulase Enzyme mix: Cellulase glucohydrolase, and xylanase	/ [a]	37 °C for 6h	Batch 35 °C	+8% +15%	50
Marine macroalgae	β-glucosidase, pectinase, and carboxy-methyl--cellulase	*Aspergillus niger*	50 °C for 2h 100 rpm	Batch 37 °C	+54.6% [b]	51
Corn Cob	Enzymatic cocktail with endo-1,3(4)-b-glucanase, and collateral xylanase, cellobiase, cellulase and feruloyl esterase Ferulic acid esterases	*Humicola Insolens* [S57] *Aspergillus terreus*	30 °C for 7 days	Batch Mesophilic	+14%	52
Sugar beet pulp silage Vinasse	Endoglucanase and Xylanase Pectinase	*Trichoderma longibrachiatum* / [a]	50 °C for 7 days	Batch 37 °C	+32.7%	53
Corn stover	Laccase Manganese peroxidase Versatile peroxidase	White rot fungi	30 °C for 24 h. 30 °C for 6 h.	Batch Mesophilic	+25% + 17%	54

Substrate	Enzymes	Enzyme Source	Pretreatment Conditions	AD Mechanism	Methane Yield	Ref
Macroalgae: *Ulva rigida*	B-glucosidase and CMCase β-glucosidase	*Aspergillus niger*	50 ºC for 2h 100 rpm	Batch 37 ºC	+ 33% [b] + 33% [b]	55
Corn cob Vine trimming shoots	Enzymatic cocktail with endo-1,3(4)-b-glucanase, and collateral xylanase, cellobiase, cellulase, and feruloyl esterase	*Humicola Insolens* [S57]	40 ºC for 3 hr. 100 ºC for 10 min (enzyme thermal inactivation) 10,000 rpm for 15 min	Batch 35 ºC	+14.6% +59.8%	56
Switchgrass	Lignin peroxidase (LiP)	/ [a]	22 ºC for 8 h Agitated at 2.5Hz	Batch 35 ºC	+29%	57
	Manganese peroxidase (MnP)	/ [a]	37 ºC for 8 h Agitated at 2.5Hz		+42%	
Rye grain silage Maize silage Feed residue Solid cattle manure Grass silage	Cellulase, hemi-cellulase, xylanase, pectinase, xylan esterase, pectin esterase, lipase, amylase, glucosidase, protease and other non-identified enzymes in traces.	Fungal	40 ºC for 1-3 hr	Batch 35 ºC	+16% +29.8% +54.4% +105% No Change	58
Miscanthus	Cellobiase Cellulase	*Aspergillus niger Trichoderma reesei [59]*	50 ºC for 24h		132 Ndm^3 $kg\ TS^{-1}$ CH_4 [c]	44
Sida	Cellobiase Cellulase	*Aspergillus niger Trichoderma reesei [59]*	50 ºC for 24h		135 Ndm^3 $kg\ TS^{-1}$ CH_4 [c]	

[a] Source not provided by author(s)

[b] Change in biogas

[c] Methane yield from untreated sample not provided by author(s)

Enzymatic pretreatment of LCM showed mostly positive change in methane production through mesophilic AD batch reactors. Methane production improved by as low as 6% to as high as 105% while a few pretreatment attempts showed no change at all. However, from the studies reviewed there was no negative change in biogas production. The most common net increase in methane production was in the range of 14% to 35% with an average of about 25%.

The highest improvement in methane production was observed after the enzymatic pretreatment of solid cattle manure were a 105% increase in methane yield was observed. However, the same pretreatment method achieved about less than half this improvement for other substrates. The authors suggest that the high performance of solid cattle manure was due to the presence of other pretreatment methods acting prior to enzymatic treatment. These methods include animal digestion and mechanical pretreatment by animal feet stepping on the substrate and breaking it to smaller parts [58]. Such methods may have worked to increase the available surface area in the LCM and thus provided a high surface area for enzymes to work on.

Enzymatic pretreatment of vine trimming shoots improved methane production by about 60%. N. Perez-Rodríguez et al [56] used high temperatures (100^{o}C for 10 mins) as part of the pretreatment process for thermal inactivation of the enzymes. However, the same pretreatment conditions were less effective in pretreating corn cob, as the method used on vie trimming shoots was used on corn cob and resulted in only 15% increase in the total methane yield. Further to this result, Perez-Rodriguez et al. [52] used an enzymatic cocktail to pretreat corn cob at 30 ^{o}C for 7 days and only achieved a 14% improvement in total methane yield. The low impact of enzymatic pretreatment on corn cobs as opposed to vine trimming shoots, further indicates that different substrates react differently to enzymatic pretreatment.

In addition, the choice of enzymes is important in improving the final methane yield from anaerobically digested substrates. J. Frigon et al [57] demonstrated that switch grass pretreated with manganese peroxidase showed 13% more improvement in methane yield than switch grass pretreated with lignin peroxidase, even though both enzymes work to degrade lignin. The use of

laccase and versatile peroxidase to improve methane production from wheat straw, hemp and miscanthus did not achieve any significant change in the final methane yield after pretreatment as opposed to their success in pre-treating other substrates such as corn stover [13,54]. These results emphasise the importance of selecting the right pretreatment method to each different substrate to achieve optimum results. This requires a more detailed understanding of the substrate composition as well as the type of enzyme being used.

Many of the results obtained indicate that enzymatic pretreatment is effective in improving biogas production on a laboratory scale. However, a study by Gerhardt et al. [46] assessed the applicability and effectiveness of applying enzymatic pretreatment in agricultural biogas plants in Germany. Gerhardt et al. obtained a 4% to 35% improvement in biogas yield from different LCM including filter paper, grass silage, corn silage and hay fibers after enzymatic pretreatment using a Trichoderma enzyme complex. The average increase in biogas was around 18% which financially justifies the use of enzyme in agricultural biogas plants ranging from small farms plants to much larger power plants.

Thermophilic Batch Reactors

The complex nature of the microbial anaerobic digestion process often results from a diverse set of microbial communities acting with an AD process [33]. Such microbes have different varying performance levels depending on the surrounding conditions such as temperature. For this reason, it is important to assess the impact of pretreatment and the performance microbial communities under thermophilic AD conditions. Table 4 presents some enzymatic pretreatment studies conducted under thermophilic AD conditions.

Table 4 Enzymatic pretreatment of lignocellulosic biomass for biogas production in a thermophilic batch reactor.

Substrate	Enzymes	Enzyme Source	Pretreatment Conditions	AD Mechanism	Methane Yield	Ref
Jose Tall wheatgrass	Cellulase Hemicellulase	*Humicola Insolens*	50 °C for 7 days pH 5.0	Batch 50 °C	0 – 31%	30
	Cellulase	*Trichoderma reesei*				
	B-glucosidase	*Aspergillus niger*				
Biofibers (Digested Manure)	Laccase Cellulase, hemicellulase	/[a]	37 °C for 20h	Batch 52 °C	No Change	39
Forest residues: tree tops and branches	Laccase Versatile peroxidase	/[a]	37 °C for 20 h 120 rpm	Batch 55 °C	No Change	35
Algae: *Spirulina platensis*	Cellobiase Cellulase	*Aspergillus niger* *Trichoderma reesei*	50 °C for 72 hr	Batch 50 °C	+24.8%	59
Switchgrass	Cellobiase Cellulase	*Aspergillus niger* *Trichoderma reesei*	50 °C for 72 hr	Batch 50 °C	+39%	

[a] Source not provided by author(s)

Research on the use of thermophilic batch AD reactors to produce methane from enzymatically pretreated lignocelluloses showed a relatively lower success rate in improving methane production. However, two experiments by H. El-Mashad [59] on pretreated *Spirulina platensis* (algae) and switch grass using cellobiase and cellulose enzymes demonstrated an increase of up to 24.8% and 39% respectively. The studies were conducted under thermophilic pretreatment conditions as well. A similar study by Romano et al. [30] showed that the enzymatic pretreatment of wheat grass only impacts the final methane yield produced under thermophilic AD conditions only when enzymes are added to the first stage of a two-stage digestion system. However, there was no impact on the total methane yield if enzymatic pretreatment was applied as a separate step prior to anaerobic digestion (single stage). The study also suggested that microorganisms were already effective in carrying out digestions without the need for additional

enzymes. However, it seems that thermophilic anaerobic digestion conditions might not have been suitable for efficient enzymatic activity.

Other researchers indicated that enzymatic pretreatment was unsuccessful in improving methane yield from a thermophilic batch reactors due to lack of enzyme access to the lignocellulosic structure [35, 39]. The harsher conditions of thermophilic AD reactors impacted the enzymatic pretreatment of digested manure [39]. Enzymatically pretreated digested manure showed very high improvement levels in methane production under mesophilic AD conditions [58], however, there was no impact on methane production under thermophilic AD conditions [39].

Overall, cellulose and cellobiase seemed to be the most promising when used to pretreat lignocellulosic substrates under thermophilic anaerobic digestion conditions for methane production. However, improvements using theses enzymes were achieved under thermophilic pretreatment conditions and at a relatively longer pretreatment time.

Mesophilic Continuous and Semi-Continuous Reactors

In addition to the importance of the AD temperature levels, the configuration of the AD reactor is also critical to asses the applicability of pretreatment methods in improving biogas yield in large scale biogas plants. Such plants often operate with a continuous or semi-continuous configuration as continuous supplies of biomass are fed into the reactors. A few studies considered semi-continuous and continuous AD configurations and are presented in Table 5 below.

Table 5 Enzymatic pretreatment of lignocellulosic biomass for biogas production in a mesophilic continuous/semi-continuous reactor.

Substrate	Enzymes	Enzyme Source	Pretreatment Conditions	AD Mechanism	Methane Yield	Ref
Sugar beet pulp	Endoglucanase, xylanase Pectinase	*Trichoderma longibrachiatum* / [a]	50 °C for 24h pH 6.6	Semi-continuous 37 °C	+19%	60
Spent hops	Endoglucanase, xylanase Pectinase	*Trichoderma longibrachiatum* / [a]	50 °C for 24h pH 6.0	Semi-continuous 37 °C	+13%	60
Rye grain silage Maize silage Feed residue Solid cattle manure Grass silage	Cellulase, hemi-cellulase, xylanase, pectinase, xylan esterase, pectin esterase, lipase, amylase, glucosidase, protease and other non-identified enzymes in traces [47].	Fungal	40 °C for 3 h.	Continuous 38 °C	+ 10 -17%	61
Spent grains	Multi-enzyme produced by solid state fermentation (SSF)	/ [a]	40 °C for 3 days	Continuous 37°C	+6.7%	62

[a] Source not provided by author(s)

The use of continuous/semi-continuous reactors for biogas production from enzymatically pretreated lignocelluloses is the least studied in literature. The studies reviewed included mesophilic continuous and semi-continuous reactors. Improvements in methane production from lignocellulosic substrates were in lower range of improvements (6% to 19%) compared to other AD mechanisms.

In comparison to batch anaerobic digestion reactors, continuous and semi-continuous AD reactors showed a lower performance level in improving methane production from enzymatically pretreated lignocelluloses. Two separate studies by T. Quinines et al. [58,62] demonstrated the difference in enhanced methane production from enzymatically pretreating substrates using a batch AD reactor and a continuous AD reactor. Methane production from the batch reactor was

up 5 folds higher than that achieved from the continuous reactor for the same substrates and enzymatic pretreatment methods.

Nonetheless, continuous and semi-continuous anaerobic digestion had a high rate of success in improving total methane yield from enzymatically pretreated lignocellulosic biomass. This provides evidence that enzymatic pretreatment can be a feasible and environmentally sustainable option when adopted in large scale biogas plants.

4.2. Biological Pretreatment (Non-Enzymatic)

Biological pretreatment of lignocellulosic biomass mainly relies on the employment of microorganisms, fungi and enzymes to degrade LC and enhance biogas and methane production [20,37,63]. Lignin and hemicellulose are the most vulnerable to biological attacks as opposed to cellulose. Microorganism such as *Trichoderma reesi* and *Trichoderma viride* for example are used in pretreatment due to their capability in converting polysaccharides to monosaccharides. Fungi such as white-rot fungi has shown great effectiveness in pre-treating lignocelluloses mainly by enhancing the accessibility of cellulose and hemicellulose for enzymatic hydrolysis. [38,63]. Further to that biological pretreatment methods also include ensilage of the biomass for long periods of time prior to AD and microaeration, which is essentially an aerobic pretreatment step that relies on the addition of oxygen to initiate an aerobic reaction by microbes.

Biological pretreatment is viewed as an attractive pretreatment process for several reasons including its low energy requirements, mild pretreatment conditions, lack of chemical requirements and it does not result in the production of inhibitory compounds [37,63]. However, the rate of treatment and biogas yield from biological pretreatment remains relatively low and is

often economically infeasible [63]. Table 6 presents several studies on the use of biological pretreatment (non-enzymatic) methods to improve the LCM methane yield.

Table 6 Impact of biological pretreatment on methane production from lignocellulosic substrates.

Method	Substrate	Biological Source	Pretreatment Conditions	AD Mechanism	Methane Yield	Ref
Fungal	Japanese cedar chips	*Cyathus stercoreus*	Incubated for 20 days at 37 °C	Batch Mesophilic	+ 43 mL vs. 0 mL control	64
Fungal	Sweet chestnut leaves and hay	Wood-rotting fungi: *Auricularia auricula-judae*	37 °C for 4-5 weeks in the dark	Batch Mesophilic	+ 15%[a]	65
Fungal	Sisal leaf decortications residue (SLDR)	CCHT-1 strain obtained from dumps of SLDR and *Trichoderma reesei*	4 days with CCHT-1 followed by 8 days with *T. ressi* at 28 °C	Batch Mesophilic	+ 30 - 101%	66
Fungal	Albizia Chips	White-rot fungus: *Ceriporiopsis subvermispora*	Incubation at 28 °C for 48 days	SS-AD Batch Mesophilic	+ 3.7 folds	67
Fungal	Bio-waste (Organic fraction of household waste)	*Trichoderma viride*	Incubated at 25 °C for 4 days	Batch Thermophilic	+ 400%	68
Microbial consortium	Corn straw	Yeast and cellulolytic bacteria	Microbial agent dose 0.01% (w/w) 20 °C for 15 days	Batch Mesophilic	+ 75.6%	31
Microbial consortium	Pulp and paper mill sludge mixed with rice straw	Microbial consortium (OEM1) originating from spent mushroom substrate	28 °C for 9 days	Batch Mesophilic	+ 40%	69
Microbial consortium	Cotton stalk	Thermophilic microbial consortium (MC1): *Clostridium straminisolvens* CSK1, *Clostridium* sp. FG4b, *Pseudoxanthomonas* sp. strain M1-3, *Brevibacilus* sp. M1-5, and *Bordetella* sp. M1-6	50 °C for 8 days	Batch Mesophilic	+ 136%	70
Microbial consortium	Lignocellulose of municipal solid waste	Thermophilic microbial consortium (MC1)	50 °C for 4 days	Batch Mesophilic	+ 105%	71
Microbial consortium	Wheat straw	G-Proteobacteria Bacteroidetes B-Proteobacteria Firmicutes (3 types)	37 °C for 15 days	Batch Mesophilic	+ 80.3%	72

Method	Substrate	Biological Source	Pretreatment Conditions	AD Mechanism	Methane Yield	Ref
		White-rot fungi Brown-rot fungi				
Microbial consortium	Cassava residues	Thermophilic microbial consortium	55 ^{o}C for 12 h	Batch Thermophilic	+ 96.6%	73
Microbial consortium	Biofibers: separated from digested manure, maize silage and industrial by-product.	Compost from garden waste and fungi collected from straw and maize silage stored outdoor for 6 months	27 ^{o}C for 0-20 days	Batch Thermophilic	No change	39
Ensilage	Pineapple peel waste	-	Ensilaged for 6 months	Semi-continuous Mesophilic	+ 55%	74
Ensilage	Giant reed	-	Ensilaged for 60 days at room temperature	Batch Mesophilic	+ 14%	75
Microaeration	Corn straw	-	5 ml O_2/g VS at 55 ^{o}C	Batch Mesophilic	+ 16.2%	76
Microaeration	Wheat straw	-	5 ml O_2/g VS for 3 days	Batch Thermophilic	+ 7.2%	77

[a] increase in biogas

Biological pretreatment methods highlighted in Table 6 include the use of fungi, microbial consortiums, ensilage and micro aeration. Fungal and Microbial pretreatment showed the most promising level of improvement in total methane yield from lignocellulosic matter. As shown in the Table 6, fungal pretreatment showed up to 400% improvement in methane yield, while pretreatment with a microbial consortium increased methane yield by about 135%. Micro aeration and ensilage delivered an improvement of up to 16% and 55%, respectively. Overall, biological pretreatment showed a high success rate with more than 90% of studies improved methane yield by more than 13%.

Fungal pretreatment was observed to improve biodegradability of lignocelluloses and increase the available fatty acids which provided better nutrients and more substrates for microorganism

in the anaerobic digestion phase, leading to an improvement in the methane yield [65,66,68]. In addition, fungal pretreatment showed a low energy consumption compared to other non-biological pretreatments [78]. Pretreatment using microbial consortiums have shown the ability to degrade lignocelluloses and reduce the content of lignin, cellulose and hemicellulose [69-72]. Zhong et al. [31] demonstrated that biologically pretreated corn straw produced 75% more methane than untreated samples, in addition to a reduction of 5.8 to 25% in total cellulose, hemicellulose, and lignin contents. In addition, an increase in the concentration of the soluble chemical oxygen demand (sCOD) in the hydrolysates was observed after pretreatment using a microbial consortium which is directly related to increasing the biomethane yield [70]. Ensiling lignocellulosic biomass also demonstrated a potential in improving methane yield as it converts the carbohydrates to volatile fatty acids and thus enhancing methane production [74,75].

Microaeration or microaerobic pretreatment has also been applied as a biological pretreatment method, where an oxygen-induced aerobic pretreatment step is used to improve the overall AD process [77]. Fu et al. [76] detected lower crystallinity levels and higher amount of amorphous celluloses in the substrate after thermophilic microaerobic pretreatment of corn straw. This resulted in more digestible cellulose and a 16% higher methane yield after AD. The success of microaerobic pretreatment relies on the portion of oxygen supplied which was set a 5 ml O_2/g $VS_{substrate}$ in the studies reviewed. Higher amounts of oxygen have been observed to inhibit and reduce methane production [77,79].

Most biological pretreatment studies reviewed were conducted under mesophilic AD conditions. However, a few experiments were conducted under thermophilic AD conditions. Results from

the later indicate that biological pretreatment shows less consistent results under thermophilic AD conditions compared to mesophilic conditions. This is because some studies have shown almost no improvement in methane yield, while the rest have shown very high improvements such as 100% and even 400%. This inconsistency implies that more studies should be conducted to assess the impact of biological pretreatment methods under thermophilic AD conditions. In addition, it not very clear how different biological pretreatment methods perform in continuously stirred anaerobic reactors. Although positive results were obtained for semi-continuous AD reactors as in Rani et al. [74] study, however, assessing the performance of continuous reactors after biological pretreatment is necessary to determine feasibility on large-scale treatment plants [66].

4.3. Thermal Pretreatment

In thermal pretreatment, heat is often combined with pressure to improve the biodegradability of lignocellulosic biomass. There are several different thermal pretreatment methods that can be applied. Other than the conventional means of simply using an autoclave or oven to heat up the biomass, steam explosion and liquid hot water pretreatment have been also given great attention. Steam explosion pretreatment works by exposing the biomass to high temperature and pressure for short duration time. This causes the biomass to decompose explosively. Thermal pretreatment conditions may involve temperatures as high as 260 ^{o}C and pressure that may reach 4.5 MPa [7].

In liquid hot water pretreatment, water is heated and maintained at liquid state through pressure [7,80,81]. The water is then allowed to penetrate the lignocellulosic biomass, leading to the removal of some the hemicellulose and lignin and hydrating the cellulose [81]. Liquid hot water

thus improves cellulose accessibility and improves the hydrolysis and digestion of lignocelluloses [80]. However, undesirable phenolic compounds and furan derivatives such as furfural and hydroxymethylfurfal (HMF) can still form as a result of high temperature and which can inhibit microorganisms and reduce the content of fermentable sugars [81,82].

Table 7 Impact of thermal pretreatment on methane production from lignocellulosic substrates.

Method	Substrate	Pretreatment Conditions	AD Mechanism	Methane Yield	Ref
Hyperthermophilic	Grass (Eleusine indica)	Oil bath at 80 °C for 3 days.	Batch Thermophilic	+ 46%	82
Conventional heating	Grass (Pennisetum hybrid)	Autoclave, water vapor for 30 min	Batch Mesophilic	+ 4.5%	83
Conventional heating	Wheat straw Sugarcane baggase	121 °C for 60 min	Batch Mesophilic	+ 29% + 11%	84
Conventional heating	Barley Straw Wheat Straw Maize stalks Rice straw	120 °C for 30 min	Batch Mesophilic	+ 40.8% + 64.3% No Change No Change	85
Conventional heating	Dewatered pig manure and digested sewage sludge	100 °C for 1 h	Batch	+ 25%	86
Conventional heating	Raw excess sludge	150 °C for 2 h	Batch Mesophilic	+ 223%	4
Steam Explosion	Wheat straw	180 °C for 15 min, 2 Mpa steam	Batch Mesophilic	+ 19.7%	87
Steam Explosion	Common reed (Phragmites australis)	200 °C for 15 min, 3.4 MPa steam	Batch Mesophilic	+ 89%	88
Steam Explosion	Miscanthus lutarioriparius (grass)	198 °C for 10 min, 1.5 MPa steam	Batch Mesophilic	+ 49.8%	89
Steam Explosion	Two-phase olive mill solid waste (OMSW) or alperujo	200 °C for 5 min, 1.57 MPa steam	Batch Mesophilic	+ 60.9%	90
Steam Explosion	Wheat straw	140 °C for 60 min	Batch Mesophilic	+ 3.6%	91
Steam Explosion	Wheat straw	200 °C for 5 min	Batch Mesophilic	+ 27%	92
Steam Explosion	Late harvested hay	175 °C for 10 min	Batch Mesophilic	+ 16%	93
Steam Explosion	Japanese cedar chips	258 °C for 5 min, 4.51 MPa	Batch	+180 mL Ch_4/g	78

Method	Substrate	Pretreatment Conditions	AD Mechanism	Methane Yield	Ref
		steam	Mesophilic	vs. 0 mL CH_4/g control	
Liquid hot water	Giant reed	190 °C for 15 min	Batch Mesophilic	+ 31%	80
Liquid hot water	Paddy straw	200 °C for 15 min	Batch Mesophilic	+ 148%	94
Liquid hot water	Sugarcane press mud	150 °C for 20 min	Batch Mesophilic	+ 63%	95
Liquid hot water	Sunflower oil cake	100 °C for 1- 6 h	Batch Mesophilic	+ 6.5%	81

Table 7 presents some of the main lignocellulosic thermal pretreatment methods including conventional heating, steam explosion, and liquid hot water. The studies considered showed an improvement in methane production from as low as 3.5% to as high as 223%. However, most of the studies showed an improvement in the range of 10-65% under mesophilic batch AD conditions.

An elevated temperature achieved through thermal pretreatment has been observed to improve methane yield among different substrates. The improvement in methane yield has been attributed to several factors including opening up the lignocellulosic structure [84], deconstruction of lignin, decrease in hemicellulose content [95], improved glucose yield [80] and improved accessibility and degradability [86].

Optimum temperature levels varied from about 100-250 ^{o}C depending on the type of substrate and method. However, it was observed that further increase in temperature levels drastically reduced methane yield [86-88,95]. This may be due to the formation of complex and toxic

compounds such as phenolic acids, furfural, HMF and in some cases melanoidins which are harmful to the microbes degrading the LCM during AD [86,95].

Similar to other pretreatment methods, different substrates are affected differently by the same thermal pretreatment process. For example, maize stalks and rice straw pretreated using a conventional thermal pretreatment method at a temperature of 120 ^{o}C for 30 min did not show any signs of improvement in methane production, as opposed to barley straw and wheat straw which showed a 40% and 64% improvement in methane yield, respectively for the same pretreatment process [85].

Looking more closely at individual thermal pretreatment methods, conventional heating had an overall improvement range of 0 to 223% which is relatively broad range. However, the improvement of 223% can be considered as a "shooting" as most improvements are almost below 60%. The pretreatment temperature range for most conventional heating studies fell in the range of 100 to 120 ^{o}C which is lower than the temperature required to solubilize cellulose and hemicellulose previously discussed. Compared to other thermal pretreatment methods, conventional heating requires a longer pretreatment duration of about 30 to 60 min long which implies that this an energy intensive pretreatment method.

Steam explosion studies had harsher pretreatment conditions than conventional heating. Applying temperatures averaging around 200 ^{o}C and pressurized steam resulted in improvements in the range of 3 to 89%. Such results can be considered more consistent than conventional heating which can be attributed to higher temperatures and steam pressure. This pretreatment

method has the shortest average pretreatment duration as it achieved successful results after exposing the LCM to heat and steam for only 5 to 15 min.

Liquid hot water pretreatment studies showed high improvement in methane yield at temperatures around 150 to 200 ^{o}C for 15 to 20 min. LHW success has been attributed to the ability of highly pressurized and heated water to penetrate the lignocellulosic biomass, hydrating the cellulose and removing hemicellulose and lignin [95]. It can also be noticed that low temperatures and long pretreatment durations have not shown success compared to higher temperatures and shorter pretreatment durations as with the case of Fernández-Cegrí et al. [81] study in which LHW pretreatment of sunflower oil cake at 100 ^{o}C for 1 to 6 hours only achieved a 6.5% improvement in total methane yield.

Overall, thermal pretreatment methods have shown promising results in improve biogas production from LCM. Although thermal pretreatment methods rely on a source of heat energy, however, the duration of the pretreatment process is relatively short with many successful methods requiring only up to 15 min of heat exposure. The exposure time is critical in thermal pretreatment as maintaining high temperatures consumes a significant amount of fuel or electricity. A few studies considered the net electricity balance for thermal pretreatment methods. For example, Menadro et al. [85] measured a positive net electricity balance from the conventional pretreatment of wheat straw at 120 ^{o}C for 30 min, highlighting the feasibility of this pretreatment process. While, Jiang et al. [80] detected a lower net energy output after pretreating giant reed using liquid hot water at 200 ^{o}C for 15 min. The decrease in energy has been considered a result of loss of dry matter during pretreatment and the high-energy requirement of

the pretreatment process [80]. Therefore, in this case thermal pretreatment is not a feasible pretreatment option.

Moreover, the possible formation of toxic and harmful compounds as a result of high heat exposure in thermal pretreatment requires a careful consideration of pretreatment conditions (mainly temperature and exposure time) to optimize the process and minimize the formation of such inhibitory compounds to ease the decomposition of lignocellulosic substrates.

4.4. Other Pretreatment Methods

Physical Pretreatment

Physical pretreatment methods do not rely on the addition of chemicals, microorganisms or any external additives. This includes grinding, chippings, milling, and use of microwave and ultrasound radiation [37]. Comminution pretreatment techniques have shown improvements of up to 30% in methane yield from pretreated lignocellulosic substrates. Extrusion pretreatment have shown up to 70% improvement in methane yield and irradiation techniques improved methane yield by up to 24% [7]. Physical pretreatment processes require high energy input and are often considered costly [54].

Chemical Pretreatment

Chemical pretreatment involves the application of chemicals such as acids, alkalis and ionic liquids. The chemicals are used to expose substrates to harsh conditions in terms of pH and temperature to break down resistive bonds. Chemical pretreatment has been widely researched for ethanol production from lignocellulosic biomass, but less so for biogas production [7,19]. Positive results have been obtained through chemical pretreatment to improve biogas production from lignocelluloses. Pretreatment using acids such as H_2SO_4, HCL, HNO_3 and others have shown an improvement of 20 to 200% in methane yield from pretreated lignocellulosic substrates. Alkaline pretreated lignocelluloses using chemicals such as $NaOH_2$, $Ca(OH)_2$ and CaO have demonstrated up to 2.3 folds increase in methane yield [7]. However, chemical pretreatment methods are often expensive and may result in the formation of inhibitory compounds such as furfural [54].

Combined Pretreatment

The combined pretreatment of LCM refers to the combination of two or more pretreatment methods in treating the same substrate. Combined pretreatment often consists of different pretreatment methods that are applied in sequence. For example, Perez-Rodriguez et al. [52] applied physical, chemical and enzymatic pretreatment on corn cob for improved biogas production. This combined pretreatment method accomplished a 22.3% increase in total methane yield after the AD of corn cob. The study also assessed the performance of individual pretreatment tests with mechanical and chemical achieving about 8% improvement in methane and enzymatic improving methane yield by about 7%. Such results show that combining more than one pretreatment method may lead to an overall result that is better than the cumulative sum of individual methods [52]. The reason for this improvement, is perhaps the effect on the physical structure and composition of LCM by an individual pretreatment method, which further improves the performance of the consecutive pretreatment. For example, physical pretreatment might open up the lignocellulosic structure and thus provide a larger surface area for microbes or enzymes to penetrate and work on.

6. DISCUSSION AND COMPARISON

This section summarizes and presents a comparison between the different thermal and biological pretreatment methods considered in this study. The comparison is based on the pretreatment conditions such as temperature and reaction time, in addition to the impact each pretreatment method had on biomethane production. Table 8 summarizes the main pretreatment methods considered and offers a reasonable comparison that mainly focuses on average values as opposed to extremely high or low values that are less frequent.

Table 8 Comparison of different pretreatment methods.

Pretreament Category	Pretreatment Method	Pretreatment Conditions		CH_4 Improvement (%)
		Temperature (°C)	Reaction time	
Thermal	Conventional	100 – 150	0.5 – 1 h	0 – 60
	Steam Explosion	150 – 250	5 – 15 min	5 – 60
	Liquid Hot Water	100 – 200	15 – 20 min	30 – 60
Biological	Fungal	25 – 37	2 -6 weeks	15+ [a]
	Microbial	20 - 55	1 – 2 weeks	40 – 100
	Microaeration	55	Few days	7 - 16
	Ensilage	Room temp	2 – 6 months	15 – 55
	Enzymatic	30 - 50	Few hours – few days	15 – 35

[a] Funal pretreatment showed inconsistent results and so a specific range cannot be determined

The comparison presented in Table 8 shows that in terms of methane improvement, liquid hot water and microbial pretreatment are of the most consistent and reliable thermal and biological pretreatment methods respectively. On the other hand, conventional thermal and biological microaeration pretreatment methods showed the lowest performance among the methods considered in terms of the impact on methane yield. However, there are other essential factors that should be taken into consideration when comparing pretreatment methods along the impact

on biogas production. Such methods are mainly the pretreatment duration, temperature and energy requirements. As such parameters greatly affect the technical and economical feasibility of a pretreatment method.

In thermal pretreatment, steam explosion and LHW require the highest temperature levels that may exceed 200 ^{o}C, while conventional pretreatment relies on lower average temperatures. However, the average duration range for steam explosion (5-15 min) is the lowest among thermal pretreatment methods. A shorter duration is desirable when handling bulk and continuous supplies and may require less energy to maintain. Conventional pretreatment methods require significantly longer pretreatment durations averaging from 30 to 60 mins. This relatively long duration might require a high-energy input to maintain.

Biological pretreatment methods are by far the slowest among other pretreatment methods. Ensilage is a very long process that may take up to 6 months and hence require considerable storage space when applied on a large scale. Fungal and microbial may take a few weeks, while microaeration may take up to a few days. However, enzymatic pretreatment has shown the most promising results in terms of pretreatment duration with many successful pretreatment attempt requiring less than 24 hours to complete and as short as 2 hours as shown in Table 3. Therefore, with the current technologies, a trade off between short reaction times and high methane improvement is clearly evident among biological pretreatment methods. For this reason, enzymatic pretreatment is a very promising method as it has the shortest duration time but requires further process optimization to achieve higher average methane yields.

Therefore, the selection of the optimum pretreatment method when planning and designing a biogas plant requires an advanced analysis and comparison among available pretreatment methods. The analysis should first identify possible substrates and available pretreatment technologies. This includes factoring in the pretreatment conditions (reaction time and temperature), energy requirements, capital and operational costs and the improvement in methane yield. The economic feasibility will often be the main determinant as discussed in the next chapter.

5. ECONOMIC FEASIBILITY

The choice of pretreatment method will often rely on the economic feasibility of the methods considered. In pretreatment to improve biogas production, economic feasibility is often determined by measuring the net economic gain from the additional methane produced after pretreatment. If the value of this additional methane is significantly higher than the cost of pretreatment, then the pretreatment is often economically feasible. The cost of pretreatment includes the capital, operational, maintenance and material costs incurred during the pretreatment process. For thermal pretreatment, this mainly includes the cost of energy and equipment required to heat up the LCM, as well as maintenance and operational costs. However, in biological pretreatment the cost of pretreatment mainly consists of the cost of the biological additives (microbes, fungi and enzymes) or an energy supply as in the case of microaeration.

Enzymatic pretreatment is considered a relatively expensive pretreatment due to the high cost of enzymes production. However, the cost of enzymes production is expected to decrease due to technological and technical advances as well as large-scale commercial development that can be encouraged by the promising advantages of enzymatic pretreatment [38]. This is also often the case with other biological pretreatment methods. Therefore, further research on enzymatic pretreatment is encouraged to maximize the potential of this pretreatment and promote new initiatives to lower the costs of enzymes production.

The majority of studies reviewed assessed the pretreatment of LCM on a laboratory scale, while only a few considered the economical aspect of such studies. A techno-economical analyses for biogas production in the Netherlands from different waste showed that biogas production is not

economically feasible unless subsidies are provided by the government [96]. This emphasizes the importance of pretreatment to improve the economical feasibility of biogas production. From the studies reviewed, Hjorth et al. [97] assessed the economical feasibility of mechanically pretreatment lignocelluloses. The study showed that there is a 68% net electrical surplus from the additional methane yield taking into account the energy consumed during the pretreatment. Similar results were obtained by Bruni et al. [39] after assessing the combined pretreatment of biofibers. The study found that pretreatment achieved a net gain of 68 kwh from the additional methane yield.

On a broader scale, there have been a few studies that assessed the economic feasibility of lignocellulosic pretreatment in biogas plants. A study by Shafiei et al. [98] performed a techno-economical evaluation of biogas production from wheat straw and paper tube residuals pretreated using steam explosion. The process evaluated was simulated using a simulation software and based experimental data from other studies. The simulation was conducted for an assumed biogas plant with a capacity of 200,000 ton/year of raw material. The study found that steam explosion pretreatment improved methane yield resulting in a 36% decrease in the manufacturing cost of methane, while increasing the capital investment by 13%. A further sensitivity analysis demonstrated that a 5% improvement achieved through pretreatment results in a 5.5% decreases in methane manufacturing costs. In addition, the most important parameters affecting the production costs were the raw material price and the methane yield [98].

A similar study also assessed the economical feasibility of different pretreatment methods including steam explosion and combined (thermal and chemical) pretreatment. Three substrates

were considered for the study which include straw, wood and paper. The results of the study showed that the pretreatment of straw and wood is not an economically feasible option as the cost of pretreatment may equal or exceed the value of additional gas yield by up to 50%. However, pretreating paper was found to be economically feasible with the value of additional methane yield exceeding the cost of pretreatment by 15%. The study concluded that the cost of raw materials was the main reason for the lack of economical feasibility [99]. However, a better pretreatment method that further improves methane yield may offset the cost of raw materials.

Although there are some attempts to economically evaluate the feasibility of different pretreatment methods as a means of improving biogas production, there remains a significant shortage in more detailed economical reviews that address the economical feasibility of pretreatment at biogas plants. The need for such reviews is critical in encouraging more biogas plants and utilizing the potential of the abundant supply of lignocelluloses worldwide.

7. CONCLUSION

This paper presented a review of studies on improving the biogas yield of lignocellulosic biomass through biological and thermal pretreatment. Pretreatment methods work to improve the degradability of lignocelluloses mainly by altering their physical characteristics to allow the microbes in the anaerobic digestion process to better degrade LCM. In biological pretreatment, biological components or reactions such as enzymes, microbes, fungi, ensilage and microaeration are employed to improve the biodegradability of LCM. Biological pretreatment methods are less energy intensive and require less capital cost than other pretreatment methods. However, the high cost of some biological additives such as enzymes remains an economical challenge.

Thermal pretreatment relies on heat energy and in some cases pressure to break down lignocelluloses prior to AD. However, thermal pretreatment methods require a careful process optimization as very harsh conditions may lead to the formation of toxic and harmful compounds which may negatively impact biogas production. In addition, high temperatures and long reaction times may require a high-energy input and thus may not be economically feasible.

From this review, it can be seen that more research is required to assess the impact of both biological and thermal pretreatment methods on different lignocellulosic substrates. This is because LCM differ in composition and thus react differently to pretreatment methods. Most importantly, there is also a lack of economical feasibility studies and experiments which evaluate the effectiveness of pretreatment methods on a biogas plant scale. Most studies available are conducted on a laboratory scale and therefore might not truly reflect the actual pretreatment scenarios when conducted on a larger scale.

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