EVALUATION OF MECHANICAL, IRRADIATION AND CHEMICAL PRETREATMENT OF LIGNOCELULOSIC SUBSTRATE FOR ENHANCED METHANE PRODUCTION

By

Zeid Amin, BEng, Dalhousie University, 2011

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ABSTRACT

Title: Evaluation of Mechanical, Irradiation, and Chemical Pretreatment of Lignocellulosic substrate for Enhanced Methane Production
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Student name: Zeid Amin
Institution: Ryerson University

Lignocellulosic substrate is a resource that contains a locked energy reserve that is normally lost during anaerobic digestion. Lignocellulosic substrate is one of the most abundant sources of organic matter available and yet its energy recovery has much room for improvement. Lignocellulosic substrate has cellular properties that are deemed extremely difficult to degrade due to complexity which is why this energy reserve is never unlocked during anaerobic digestion. There ae several successful pretreatment methods that are used to degrade this lignocellulosic substrate and unlock this energy reserve. This paper will focus on the methods that include mechanical, irradiation, chemical and combined pretreatment processes. Analysis is conducted on all the studies that are obtained to compare the successes of the different types of pretreatment processes used. Each of the different listed pretreatment processes have different energy requirements, treatment times, and solvent requirement and are acting to enhancing methane production. The improvement in methane production varies from process to process and study to study creating a need to compile all of this valuable data into this research report. This will help future researchers in navigating the available studies of pretreatment of lignocellulosic substrate for improving methane production.

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1. INTRODUCTION

Fossil fuels are currently the major sources of energy being used and are leading to a rapid consumption of the world's natural resources. This is causing many environmental concerns all over the planet that can not be left unignored. Scientists have explored several renewable energy sources and production methods over the last decade.[1] Anaerobic digestion will be the primary energy production method to be used in this report. Lignocellulosic compounds from plants will be the main substrate to be assessed.

Improving the efficiency of harvesting energy from anaerobic digestion can be conducted by adding processes such as pretreatment. Lignocellulosic substrate is considered one of the most abundant, yet most challenging organic substances to degrade in anaerobic digestion.[2] For decades, studies have been created to improve the efficiency of methane production by adding a pretreatment process prior to anaerobic digestion. By adding this pretreatment, the energy source from the lignocellulosic substrate that is usually lost can be captured and used.

This report will focus on the improvement of methane production with the use of different pretreatment methods. The pretreatment methods to be focused are mechanical, irradiation, chemical and combined. Many of available studies online will be tabulated and analyzed in this report. This in turn will be a good roadmap for all those researching these pretreatment methods for enhancing biogas production.

2. BIOGAS PRODUCTION FROM ANAEROBIC DIGESTION

Anaerobic Digestion is an oxygen-excluded biological process used to degrade and cure organic substrates for several different environmentally sustainable applications[3]. Anaerobic digestion occurs naturally in bogs, landfills, and lakes but also can be engineered and used in man made applications that can be very beneficial.[4] These applications include:

- Biogas Production
- Biofuel Production
- Efficient degradation of organic waste

They can be used for many different types of organic waste sources. For the purpose of this report, the waste source to be applied and reviewed is lignocellulos substrate and the anaerobic digestion will target the production of methane.

Methane production from anaerobic digestion is an old established process.[5] During the processes of anaerobic digestion, bacteria degrade and convert organic matter into biogas.[6] This process produces methane and carbon dioxide (our biogas) while also producing a nutrient rich slurry.[7] The figure blow demonstrates a very basic anaerobic biogas collection technique.

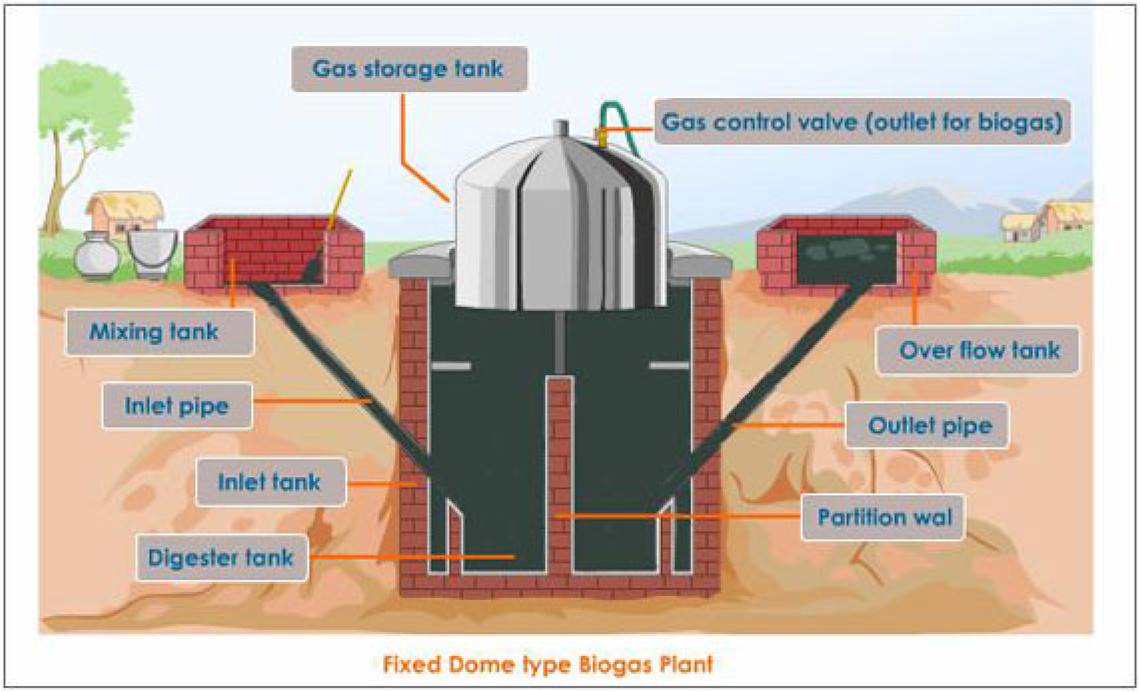

Figure 1. Diagram illustrating the process of methane collection from anaerobic digestion [8]

The waste source is fed into the digester chamber which requires a steady 35 degrees Celsius temperature for a period of approximately 2 months. This allows the bacteria to degrade the organic waste and produce biogas as a biproduct. This gas is collected in the dome and transferred for further use.

There are many different biogas utilization applications. The gas can be used as a raw material or it can be refined and made into a finished upgraded gas. The Biogas has to be cooled and filtered of all of its unwanted toxins such as H2S. The most common biogas utilization applications include:

- Production of raw heat or steam.
- Electricity source
- Vehicle fuel
- Production of chemicals and proteins[9]

3. LIGNOCELLULOSIC SUBSTRATE OVERVIEW

Lignocellulosic substrate is comprised of cellulose, hemicellulose, and lignin, in addition to other organic matter. The figure below demonstrates the common molecular structure of lignocellulosic substrate. This substrate is known to be very tough to break down during anaerobic digestion and must therefore be "softened up" using one of the pretreatment methods.

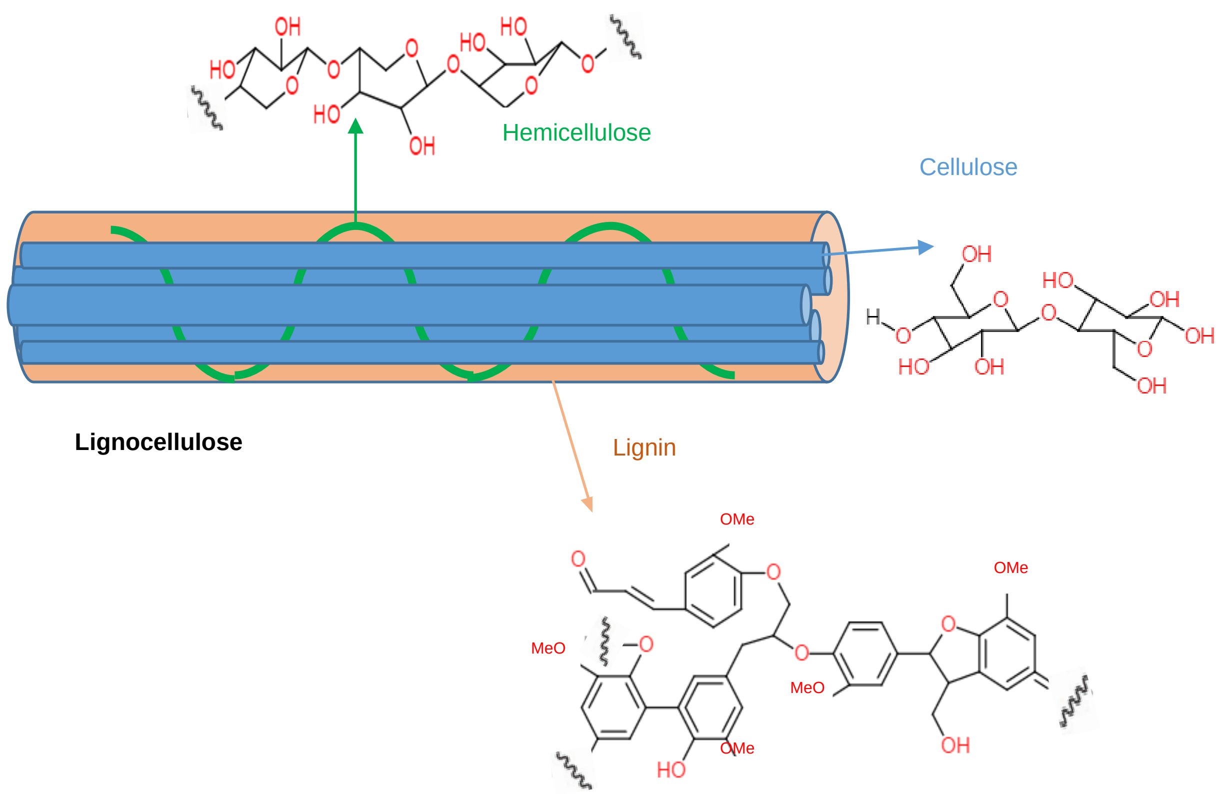

Figure 2. Typical composition of lignocellulosic material (Adapted from ref 10).

3. 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 makes it more difficult. With the right pretreatment process, the biodegradation of lignocelluloses can be improved to enhance methane production.

The pretreatment processes to be discussed in this report can be categorized into: mechanical, irradiation, chemical, and combined pretreatment. Mechanical and irradiation pretreatment mainly rely on applying physical, radiation and heat energy on the lignocellulosic substrates before AD. 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.

3.1. Mechanical Pretreatment

Mechanical pretreatment methods are some of the most basic methods that can be used to pre-treat lignocellulosic substrates. The primary function of such methods is the application of physical force to breakdown the particles and reduce the substrate particles size. This directly increases the surface area available for microbial and enzymatic attacks and thus improves the anaerobic digestion process for methane production.[11,12] Table 1 illustrates some of the methane improvement results obtained from different studies on mechanical pretreatment methods.

Table 1. Impact of mechanical pretreatment on methane production from lignocellulosic substrates

Method	Substrate	Pretreatment Conditions	AD Mechanism	CH_4 Production	Ref
Hollander beater	Macroalgae Laminaria spp	10 mins of beating	Batch Thermophilic	+ 53%	13
Grubben deflaker	Ley sillage	Most particles size grinded to less than 2 mm	Batch Mesophilic	+ 59%	14
Krima disperser	Ley sillage	Most particles size grinded to less than 8 mm	Batch Mesophilic	+ 43%	14
Heavy plates	Meadow grass	Particles size grinded to 5 mm	Batch Mesophilic	+ 25%	15

Method	**Substrate**	**Pretreatment Conditions**	**AD Mechanism**	**CH_4 Production**	**Ref**
Extrusion	Reygrass	Subject to heating, mixing and	Batch Mesophilic	+ 8.5%	16
Extrusion	Festulolium	Subject to heating, mixing and shearing	Batch Mesophilic	+ 62%	17
Hydrodyna mic cavitation	Wheat straw	2300 rpm for 2 min Dry matter content: 0.5%	Batch Mesophilic	+ 145%	18
Refiner	Japanese cedar chips	Treatment frequency: 10 times	Batch Mesophilic	+ 13 mL CH_4/g vs. 0 mL CH_4/g control	19
Size reduction by blender	Biofibers: separated from digested manure, maize silage and industrial by-product.	Particles size reduced to 2 mm	Batch Thermophili c	+ 10%	20
Size	Wheat straw	Particles size reduced to 2	Batch	+ 83.5%	21

Method	**Substrate**	**Pretreatment Conditions**	**AD Mechanism**	**CH_4 Production**	**Ref**
reduction by knife mill	Barley straw	mm Particles size reduced to 5 mm	Mesophilic	+ 54.2%	

Table 1 demonstrates the impact of different mechanical pretreatment methods on methane yield from lignocellulosic substrates. The tests differ in the types of mechanical processes used to alter the physical characteristic of LCM (mainly particle size). The mechanical processes used include beating, deflaking, dispersing, extruding, refining, knife milling, and cavitation. In addition, a variety of lignocellulosic substrates have been used. Most studies were conducted in a mesophilic anaerobic batch system, while only a few were conducted under thermophilic conditions.

The mechanical pretreatment tests demonstrated an increase in methane production in the range of 8.5 to 145% during the AD process with an average of about 50%. However, the range of improvement is relatively broad with most improvements falling in the range of 40 to 60%. Mechanical pretreatment is quite reliable but does not usually achieve the highest methane improvement results as some of the lignocellulosic molecules require more than a physical process to breakdown.[15]

The highest methane yield was achieved when hydrodynamic cavitation pretreatment was used for wheat straw substrate.[18] This pretreatment process is relatively new to pre-treating lignocelluloses. The process works by producing cavitation in the LCM through a motorized device equipped with a stator and rotor. The improvement in methane yield reached as high as 145% more than that produced from the untreated substrate.[18] This value is relatively high compared to the other mechanical pretreatment studies which are a lot more consistent. The authors of this work highlighted that the high improvement in methane yield was mainly attributed to the proper optimization of the treatment relative to the substrate used. The hydrostatic cavitation process can be easily adjusted for speed, particle size, and intensity which, after proper research, can be optimized to achieve very efficient results as demonstrated in this study.[18]

On the other hand, the lowest methane yield improvement was reported when mechanical extrusion was applied to Ryegrass. Improvement in methane yield of only 8.5% was reported which is considered relatively low compared to other mechanical pretreatment methods. However, the study considered that the results achieved are consistent with those obtained in other studies available in the literature.[16] This might imply that some types of grasses are not affected by mechanical extrusion as others. Therefore, the type and composition of a substrate plays an important role in how well a substrate responds to a certain pretreatment method.

Mechanical pretreatment is an excellent and reliable technique that is easily scalable to any substrate volume size. Mechanical pretreatment tools are usually adjustable which can make it easy to modify and optimize the pretreatment conditions.[14] One of the biggest drawbacks of mechanical pretreatment is the capital costs for the motorized equipment and the inability to improve the degradability of some of the tougher lignocellulosic substrates.[15] Mechanical pre-treatment processes are much more effective when combined with other pretreatment methods as reduction in particle size can synergize very well with any of the other techniques.

3.2. Irradiation Pretreatment

The primary function of irradiation pretreatment is the use of radiation energy in the form of microwave, gamma-ray and ultrasound to increase the biodegradability of lignocelluloses.[22] The pretreatment methods used involve the loading of the substrate into a containment that endures a specific intensity (in Watts) of radiation for a specific time.[23] Irradiation energy mainly exerts a disruptive effect on lignocellulosic structures and increase the accessible surface area available for microbial attacks, and in some cases reduce the polymerization of such structures.[22] Table 2 demonstrates some of these studies utilizing irradiation pretreatment methods to improve methane production from lignocellulosic substrates.

Table 2. Impact of irradiation pretreatment on methane production from lignocellulosic substrates

Method	Substrate	Pretreatment Conditions	AD Mechanism	CH_4 Production	Ref
Microwave	Switchgrass	Closed vessel microwave, 2450 MHz Power range: 400 – 1600 W 260 °C and 33 bars pressure for 90 – 120 min	Batch Mesophilic	No change	24
Microwave	Grass (Pennisetum hybrid)	1180 W for 3 min 2450 MHz Max temp: 260 °C	Batch Mesophilic	- 13.8%	25
Microwave	Agricultural straws (4 types)	200 °C for 15 min Cool to 100 °C then placed in a desiccator for 3 h	Batch Mesophilic	- 64 to -1%	26
Microwave	Lignocellulosic fractions from MSW	1-10 minutes at 500 W Intensity	Batch Mesophilic	+ 8.5%	27
Microwave	Cattail	500 W intensity for 14 minutes at 100 °C	Batch Mesophilic	+ 19%	23

Method	Substrate	Pretreatment Conditions	AD Mechanism	CH_4 Production	Ref
Microwave	Corn stalks	680 W intensity for 24 minutes at 35 °C	Batch Mesophilic	+ 20%[a]	28
Ultrasonic	Corn cob Vine wood trimming	750 W intensity for 62.5 seconds	Batch Mesophilic	- 4.6% - 60.8%	29
Ultrasonic	Lignocellulosic materials in excess sludge	Ultrasonic cleaner P = 500 W for 2 h	Batch Mesophilic	+ 184%	30
Ultrasonic (Sonolysis)	OMSW	Low-frequency (20 kHz) bench sonicator, from 0 to 750 watts for 60 min 0.4 W/mL ultrasonic density	Continuous Mesophilic	+ 24%[a]	31

Table 2 above demonstrates the impact of different irradiation pretreatment tests on methane yield from lignocellulosic substrates. Microwave and ultrasonic irradiation methods were used for the tests each having unique pretreatment conditions. In addition, a variety of lignocellulosic substrates have been tested. The studies were conducted in a mesophilic anaerobic batch system, except for a single study that was conducted in a mesophilic continuous AD reactor.

The irradiation pretreatment tests demonstrated an impact on methane production from a range of -64 to 184% during the AD process with an average of about 12%. However, the range of improvement is relatively broad with most improvements falling below 20%. This broad range in results, with the majority being very low suggests that this type of pretreatment is not very effective or may require further research to find the optimal pretreatment conditions for different substrates.

The highest methane yield was achieved by Hu et al.[30] using ultrasonic irradiation pretreatment for lignocellulosic substrate in excess sludge with a power of 500 W for a period of 2 hours. The improvement in methane yield reached as high as 184% more than that produced from the untreated substrate.[30] This value is significantly higher relative to other irradiation methods considered and were the next best improvement result was 24%. The high improvement in methane yield was mainly attributed to the ability of ultrasonic radiation in deconstructing the lignin structure and thus improving degradation of the LCM.[30] In addition, the long pretreatment duration of 2 hours might be an important factor which resulted in this high improvement but which requires a very high energy input.

On the other hand, a negative impact on methane yield was presented by Pérez-Rodríguez et al.[29] using ultrasonic irradiation pretreatment of vine wood trimmings. The pretreatment resulted in a 60.8% reduction in methane yield compared to the untreated sample. The results are in contradiction with other studies using ultrasonic pretreatment which showed positive improvements. However, there is a clear difference in the pretreatment period which was very short (62.5 s) for this study. The study suggests that ultrasonic pretreatment in this case lead to an undesired restructuring or repositioning of the lignin in the substrates which formed a shield that prevented anaerobic biodegradation.[29]

Microwave pretreatment seems to be the most common irradiation pretreatment method studied. This method works by applying thermal energy generated from electromagnetic energy to substrates. However, unlike conventional heating methods, microwave heating is selective and targets polar substances such as water molecules as opposed to non-polar substances.[23,25] This leads to the intense vibration of water molecules and inhomogeneity heating in substrates leading to the deconstruction of lignocellulose and hemicellulose solubilisation.[23] Several studies obtained a negative or zero change in methane yield from microwave pretreated LCM.[24,25,26] while a few studies obtained an improvement of only up to 20% in methane yield.[23,27] This indicates that the impact of microwave pretreatment on methane production from LCM is still not very clear and requires further research.

Irradiation pretreatment seems to have a potential to improve biogas production from LCM, particularly ultrasonic pretreatment showed very promising results. However, at this stage, improvements in methane production remain relatively low. Many studies indicated that irradiation pre-treatment might be more efficient when combined with other treatment types.[29] One of the main drawbacks of irradiation pretreatment is its constant energy requirements which may be very high for long pretreatment periods. Moving forward, irradiation treatment research should focus on improving methane yield by optimizing pretreatment conditions. 5

3.3. Chemical Pretreatment

Chemical pretreatment methods are the most commonly used methods in the literature for the degradation of lignocellulosic substrate.[31,32] The primary function of this pretreatment type is the use of chemicals such as acids, bases, and ionic liquids to alter the chemical and physical characteristics of lignocelluloses for improved anaerobic digestion.[22] The pretreatment conditions for this method involve the loading of the substrate into a solvent that contains a chemical compound for a specific time period and temperature. Table 3 below demonstrates the impact of different chemical pretreatment methods on methane yield from lignocellulosic substrates.

Table 3. Impact of chemical pretreatment on methane production from lignocellulosic substrates

Method	**Substrate**	**Pretreatment Conditions**	**AD Mechanism**	**CH_4 Production**	**Ref**
Alkaline	Giant reed	20 g/L NaOH for 24 h at 24 °C	Batch Mesophilic	+ 63%	33
Alkaline Hydrogen Peroxide (AHP) pretreatment	Sida Mithcanthus Sorghum	5% (m/m) H_2O_2 solution for 24 h at 25 °C	Batch Mesophilic	+ 44% + 85% + 83%	34
Alkali pretreatment	Rice Straw	1% NaOH for 3 h at room temp.	Batch Mesophilic	+ 34%	35

Method	Substrate	Pretreatment Conditions	AD Mechanism	CH_4 Production	Ref
Alkali pretreatment	Sugarcane bagasse and mud	1% NaOH for 45 mins at 100 °C	Batch Mesophilic	+ 81%	36
Concentrated phosphoric acid	Pine	85% phosphoric acid at 60 °C for 45 mins and 50% ethanol	Batch Mesophilic	+39%	37
Fentons oxidation reagent	Sida-Hermaphrodita	Treated with the reagent for 2 hours with a PH of 3 and mass ratio of Fe^{2+} to H_2O_2 equals 1:25	Batch Mesophilic	+ 75%	38
Calcium Hydroxide	Grass	7.5% lime loading for 20 h at 10°C	-	+ 37%	39
CaO pretreatment	Manure biofibres	8% CaO for 25 days at 15 °C	Batch Thermophilic	+ 66%	20
Combined Fe and NaOH pretreatment	Maize straw	6% NaOH dosed with Fe with and initial pH of 7 at 20 °C	Batch Mesophilic	+ 57%[a]	40

Method	**Substrate**	**Pretreatment Conditions**	**AD Mechanism**	**CH_4 Production**	**Ref**
Ethanol as organic solvent	Elmwood Pinewood Rice Straw	75% ethanol and 1% sulfuric acid at 150 to 180 °C for 0.5 to 1 h	Batch Mesophilic	+ 73% + 85% + 32%	41
Ethanol as organic solvent	Sweet sorghum stalks	50% ethanol at 160 °C for 30 min	Batch Mesophilic	+ 270%	42
Isopropanol based organosolv pretreatment	Sun flower stalks	50% isopropanol containing 1% sulfuric acid at 160 °C	Batch Mesophilic	+ 124%	43
Ionic liquid (IL) pretreatment	Water hyacinth	IL: 1-N-butyl-3-methyimidazolium chloride ([Bmim]Cl) Co-solvent: dimethyl sulfoxide (DMSO) At 120 °C for 120 min	Batch Mesophilic	+ 97.6%[a]	44
methylmorpholine-N-oxide Pretreatment	Rice Straw	85% NMMO at 120 °C for 3 h	Batch Mesophilic	+ 82%	45

Method	Substrate	Pretreatment Conditions	AD Mechanism	CH_4 Production	Ref
methylmorpholine-N-oxide Pretreatment	Barley Straw Forest Residue	85% NMMO at 90°C for 3.5 h	Batch Thermophilic	+ 100% + 100%	46

Several chemical pretreatment methods are presented in table 3 above. The tests differ in the types of chemical reagents used and the pretreatment conditions. The main chemical reagents used include acids, alkalis, ionic liquids and other chemical compounds. In addition, a variety of lignocellulosic substrates have been used. Most of the studies were conducted in a mesophilic anaerobic reactor batch system, while only a few were conducted under thermophilic AD conditions.

The chemical pretreatment tests demonstrated an increase in methane production from a range of 32 to 270%. However, the range of improvement is relatively broad with most improvements falling in the range of 35 to 100% increase in methane yield. This support the argument that chemical pretreatment shows consistent and reliable result for improving methane production.[32]

The highest methane yield improvement was achieved by Ostovareh et al.[42] using ethanol as an organic solvent to pre-treat sweet sorghum stalks. The improvement in methane yield reached as high as 270% more than that produced from the untreated substrate. This value is significantly higher than those obtained in the researched studies. The study assessed different combinations of ethanol and sulfuric acid at varying temperatures. The optimum methane improvement was achieved by using ethanol (50%) at 140 ^{o}C without the addition of sulfuric acid. The high methane improvement was mainly attributed to lignin and hemicellulose removal, reduced cellulose crystallinity and opening up the substrate structure.[42] However, the results of this study are relatively skewed from other studies considered as the next best result obtained is 124% improvement in methane yield. The studies which achieved very promising results indicated that the modification in the structure of the LCM such as lignin removal and crystallinity reduction are the main factors leading to improved methane yield.[42,43,44]

On the other hand, the lowest methane yield improvement was achieved by Mirmohamadsadeghi et al.[41] using ethanol as organic solvent. Improvement in methane yield achieved was only 32%. The study used 75% ethanol at 150 ^{o}C with the addition of 1% sulfuric acid as a catalyst. Although this study is similar to the study by Ostovareh et al.[42] in terms of using ethanol as an organic solvent under high temperatures. Ostavareh et al. study used a lower concentration of ethanol (50% as opposed to 75%) and did not use sulfuric acid as a catalyst. In fact, the study found that the addition of acid to the pretreatment resulted in lower methane yield, even though it improved enzymatic hydrolysis. In addition, increasing the ethanol concentration at higher temperatures was found to be less effective in improving the methane yield.[42]

Chemical pretreatment is a suitable and convenient method for the treatment of lignocellulosic substrates. Chemical pretreatment methods are widely studied and quickly progressing as proven by the large number of related publications in the literature. One of the biggest drawbacks of chemical pretreatment is the requirement for the constant dosing of a chemical. This procedure is both expensive and un-environmentally friendly.[31] Reducing the overall concentration of the solvents used in the pretreatment process, while achieving a larger increase in methane yield, should be the goal for future research in this area of study.

The limitations of chemical pretreatment for AD pretreatment include:

• Reagent cost –the costs for purchasing chemicals are high.

• Equipment damage – extreme pH conditions can be problematic for equipment maintenance because of scaling and corrosion.

• Loss of energy sources - the breakdown of complex substrates can sometimes lose some of the methane production potential.

• Adverse effects on agricultural applications

3.4. Combined Pretreatment

Combined pretreatment methods are those which involve the application of two or more different pretreatment methods often conducted in series. The main purpose of such pretreatment methods is to gain better results from different methods that would not have been gained if the methods were applied individually. For example, Bruni et al.[20] demonstrated the impact of different pretreatment methods on the methane yield of biofibers from digested manure. Of such methods, enzymatic pretreatment showed no effect on the methane yield. On the other hand, chemical pretreatment combined with thermal pretreatment improved the methane yield by 26%. However, combining enzymatic, chemical and thermal pre-treatments lead to an improvement of 34% in the methane yield. This indicates that enzymatic pretreatment becomes more effective and leads to a positive contribution to the methane yield once combined with other methods.

Table 4 demonstrates the impact of some combined pretreatment methods on methane production from lignocellulosic biomass.

Table 4. Impact of combined pretreatment on methane production from lignocellulosic substrates

Method	Substrate	Pretreatment Conditions	AD Mechanism	CH_4 Production	Ref
Thermo-chemical	Grass silage	100 °C and NaOH loading rate of 5% (w/w) for 1.9 - 3.6 h	Batch Mesophilic	+ 38.9%	47
Thermo-chemical	Dewatered pig manure and digested sewage sludge	5% (W/W) $Ca(OH)_2$ added and allowed to react for 1 h Heating at 70°C for 1 h	Batch	+ 72%	48
Thermal		Heating at 100°C for 1 h		+ 28%	
Chemical		5% (W/W) $Ca(OH)_2$ added and allowed to react for 2 h HCL added to neutralize pH after reaction		- 10%	
Thermo-chemical (NaOH + H_2O_2)	Paper tube residuals	220°C for 10 min heating, 15-20 bar 2% NaOH and 2% H_2O_2	Batch Thermophilic	+ 107%	49
Thermal		220°C for 10 min heating, 15-20 bar		+ 5%	
Thermal – NaOH		190°C for 10 min heating, 15-20 bar 2% NaOH		+ 69%	
Thermal - H_2O_2		190°C for 10 min heating, 15-20 bar 2% H_2O_2		- 15%	
Thermo-	Digested manure	55^{o}C and 6% NaOH.	Continuous	+ 26%	50

Method	Substrate	Pretreatment Conditions	AD Mechanism	CH_4 Production	Ref
chemical	biofibers	Shaking incubator (120 rpm) for 24h	Thermophilic		
Thermo-chemical	Rice straw	200°C for 10 min 5% NaOH added to maintain pH	Batch Mesophilic	+ 222%	51
Chemical		3% NaOH for 120 h at 37^{o}C		+ 123.9%	
Irradiation - chemical	Paddy straw	4% sodium carbonate for 48 hours 60 min in the microwave (720W) at 180 °C	Batch Mesophilic	+ 54.4%[a]	52
Chemical		4% sodium carbonate for 48 hours		+ 41.5% [a]	
Mechanical-chemical – enzymatic	Corn Cob	Fast Extrusion for 35 s at room temperature 0.4% NaOH (w/v) Enzyme: Ultraflo® L: Enzymatic cocktail (endo-1,3(4)- β -glucanase, collateral xylanase, cellobiase, cellulase, and feruloyl esterase activities)	Batch Mesophilic	+ 22.3%	53
Mechanical		Fast Extrusion for 35 s at room temperature		+ 7.7%	
Mechanical-chemical		Fast Extrusion for 35 s at room temperature 0.4% NaOH (w/v)		+ 7.7%	
Enzymatic		Enzyme: Ultraflo® L		+ 7.0%	
Mechanical-enzymatic		Fast Extrusion for 35 s at room temperature Enzyme: Ultraflo® L		+ 13.4%	
Thermo-chemical-enzymatic	Biofibers: separated from digested manure, maize silage and	Steam + NaOH: 160 °C for 15 min Laccase at 37 °C for 20 h	Batch Thermophilic	+ 34%	20

Method	Substrate	Pretreatment Conditions	AD Mechanism	CH_4 Production	Ref
Thermo-chemical	industrial by-product	Steam at 160°C for 15 min Catalysts: Sodium hydroxide (NaOH) Phosphoric acid (H_3PO_4)		+ 26% + 8%	

There are many studies and experiments on combined pretreatment methods available in the literature. Table 4 presents a few of those studies which highlight some of the most common pretreatment combinations. In general, an increase in the methane yield due to the combined pretreatment was in the range of 20-80% with a few exceptions. Most notably thermo-chemical methods are the most popular combined pretreatment processes.

Individual pretreatment methods similar to those discussed in earlier sections seem to have a different effect on the methane yield when combined with other methods. Rafique et al.[48] studied the impact of thermal, chemical and combined thermo-chemical pre-treatments on the methane potential of dewatered pig manure. Thermal pretreatment at 100 ^oC improved methane yield by up to 28%. On the other hand, chemical pretreatment using 5% calcium hydroxide reduced the substrate methane potential by about 10%. However, the combined thermo-chemical pretreatment at 70 ^oC with 5% calcium hydroxide showed significantly better results with up to 72% higher methane yield. The results clearly indicate that the performance of thermal and chemical pre-treatments significantly improved when combined together.

A similar study by Teghammar et al.[49] examined different thermo-chemical pretreatment combinations to improve the biogas production of paper tube residuals. Thermal, thermo-chemical (NaOH) and thermo-chemical (H_2O_2) retreatments were conducted within a temperature range of 190-220 ^{o}C and 15-20 bars of steam pressure. Thermal and thermo-chemical (NaOH) pretreatments improved methane yield by 5% and 69% respectively, while thermo-chemical (H_2O_2) reduced methane yield by -15%. Combining thermal pretreatment with both chemicals (NaOH and H_2O_2) under similar conditions and chemical concentrations the methane yield improved by 107%. This improvement was attributed to the ability of the combined pretreatment to open up the cellulose crystalline structure and delignify the LCM prior to AD. More interestingly, the achieved improvement level due to combine pretreatment is much higher than that of individual pretreatments as well as higher than the sum of all their achieved improvement levels. Similar to Rafique et al.[47] study, a pretreatment that negatively impacted methane yield such as the combined thermo-chemical (H_2O_2) had a positive impact once combined with other methods.

Most studies reviewed performed a comparison between the performance of individual pretreatment methods and combined methods. In most cases combined pretreatment enhanced methane production by more than the sum of enhancements achieved by individual pretreatments or at least higher than the best individual pretreatment. It is also evident that there is sometimes a synergetic effect when combining two or more pretreatment methods as clearly demonstrated by Rafique et al.[48] and Teghammar et al.[49], where the combined pretreatment results in an enhanced methane production level significantly higher than that achieved by the summation of all individual methods. The causes of this synergetic effect are not clearly examined in the studies. However, this effect might be explained by the ability of a certain pretreatment to impact some specific parameters of the LCM that would allow the other pretreatment methods to work better. For example, in Rafique et al.[48] study, explosive thermal pretreatment was mainly responsible for breaking down the lignocellulosic structure and reducing crystallinity. This change might have exposed chemicals to a greater surface area to penetrate and thus had a stronger effect.

4. DISCUSSION

Some of the key parameters considered when selecting the best pretreatment method out of the 4 considered shown in table 5 below. The parameters of interest are temperature, reaction time, methane improvement, and cost considerations. Table 5 summarizes the average ranges as obtained from literature and presented in the previous discussion section, while omitting highly dispersed inaccurate, outlier values.

Table 5. Comparison of different pretreatment methods

	Pretreatment Conditions			
Pretreatment	Temperature (^{o}C)	Reaction time	CH_4 Improvement (%)	Cost Consideration(s)
Mechanical	Room temperature	A few minutes	10 - 60	Electrical energy input
Irradiation	35 – 260	1 -120 min	5 - 24	Electrical energy input
Chemical	25 - 120	1– 24 hr	30 - 85	Cost of chemicals and thermal energy input
Combined	55 - 200	10 min – 20 hr	10 - 80	Varies

As shown in table 5, chemical pretreatment methods are of the most reliable and efficient methods available in terms of improving methane yield from lignocellulosic substrates. Mechanical and combined pre-treatments have demonstrated very close performance in improving methane yield, while irradiation pretreatment has the lowest performance among all pretreatment methods.

However, improving methane yield alone is not enough. Other factors should be taken into consideration when selecting a suitable pretreatment method to ensure the feasibility of such methods. Pretreatment conditions are essential in terms of energy and time requirements. In addition, the cost of chemical additives is an essential factor.

In terms of energy requirements, irradiation and combined pretreatment often relies on a high and continuous energy input to achieve desirable temperature or irradiations levels. Chemical pretreatment methods may also require a heat energy input for certain processes that rely on elevated temperatures. Mechanical pretreatment also relies on an electrical power supply to operate machinery and equipment used to reduce the size of feedstock particles.

Reaction time is also essential in particular for large scale plants. Chemical and combined pretreatment processes are quite fast and may require few minutes or hours or up to a day. Irradiation pretreatment methods can be considered fast as they may take a few minutes and up to a couple of hours. However, the longer the reaction time, the higher the energy requirements are. In such cases a difference in the order of hours can make a significant difference in terms of energy input. Mechanical pretreatment can be considered as the fastest and most controllable among the different methods, as the reaction time could reach a few minutes only, depending on the type of machine used in terms of power, efficiency and set-up.

The most decisive factor in determining the optimum pretreatment methods is often the economic feasibility of the method in terms of the cost of pretreatment versus the value of added methane yield. The cost of pretreatment includes both capital and operational costs. In most cases, the operation and maintenance cost is most significant and it may include the cost of chemical, the operational and maintenance costs of operating equipment used to perform mechanical or heating activities.

5. CONCLUSION

Lignocellulosic substrate is a very hardy and tough organic material that can be difficult to degrade in anaerobic digestion. By adding pretreatment methods, the energy source that is usually lost in this anaerobic process can be captured. The pretreatment methods used in this study are mechanical, irradiation, chemical, and combined. The studies have shown much success in the pretreatment of lignocellulosic substrate but the most successful pretreatment method is chemical. Irradiation is considered the method that is least studied and with rom for improvement. Considering the studies reviewed it should be emphasized that a small improvement in methane yield that is economically feasible is better than a high improvement that is not economically feasible. This is particularly important when transforming such methods from lab-scale processes to large scale plants. Most studies in the literature are conducted as lab-scale experiments and do not represent the same output that could be achieved through large scale biogas production facilities. This research has shown that the studies available are only scratching the surface in the world of lignocellulosic substrate pretreatment. There is much more knowledge to be researched and attained in order to optimally extract energy from lignocellulosic substrate.

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