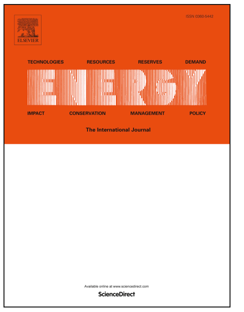

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Unlocking biogas potential: A comprehensive study on pretreatment techniques of organic substrate for enhanced anaerobic digestion

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Abstract

As the worldwide demand for green energy intensifies, the generation of biogas through the anaerobic digestion (AD) process serves as a potential and promising sustainable solution. It also addresses the problem of solid waste management through the biodegradation of solid organic waste. Despite its huge potential, the conventional AD process often encounters significant operational challenges, such as the complexity of feedstock characteristics and structures, which make the breakdown process ineffective and inefficient in biodegradability. Such issues collectively contribute to reduced biogas yields, limiting the efficiency of the AD process and the viability of biogas as a reliable energy source. To address these obstacles, process intensification of the feedstock emerges as a necessary strategy. This paper presents a review of primary pretreatment methods and hybrid methods that have been developed to optimize the AD process. The affecting parameters of the conventional pretreatment techniques, along with the working principle, methodology, and their limitations, are discussed. Hybrid methods combine multiple pretreatment methods for maximum effectiveness and overcome the limitations of individual techniques. Chemo-sonication and mecho-sonication have achieved increases in biogas generation ranging from 67% to nearly 94% compared to untreated feedstock. The review concludes with a comparative analysis of these pretreatment techniques, which provides valuable insights into the relative productivity of each technique. This study proposes a framework of future research to optimize the AD process in a more efficient way and as well as yielding a stable generation of methane. This paper offers a clear pathway to enhancing biogas production, contributing to the broader goal of renewable, sustainable, and green energy development.

Keywords: Anaerobic digestion, biogas, feedstock, pretreatment processes

Highlights

- Study of the scientific background of anaerobic digestion (AD)
- Analysis of the parameters influencing biogas generation efficiency
- Exploration of primary pretreatment and hybrid techniques for enhancing biogas generation
- Provision of remarks and observations pertaining to existing pretreatment techniques

Nomenclature and Abbreviations

AD	Anaerobic digestion	H_2	Hydrogen
GHGs	Greenhouse gases	CO_2	Carbon dioxide
MW	Megawatt	CH_4	Methane
GW	Gigawatt	H_2S	Hydrogen sulfide
HRT	High hydraulic retention time	CO	Carbon monoxide
VFAs	Volatile fatty acids	NH_3	Ammonia
N_2	Nitrogen	$H_2O_{(g)}$	Water vapor
O_2	Oxygen	$H(OSiH_2)_nOH$ & $(OSiH_2)_n$	Siloxanes
C:N	Carbon nitrogen ratio	OLR	Organic loading rate
RT	Retention time (days)	S	Feedstock concentration in total volatile solid
V	Volume of the digester (m^3)	Q	Flow rate of the slurry (m^3/day)
BMP	Biomethane potential	MSW	Municipal solid waste
TS	Total solid	VS	Volatile solid
Rpm	Rotation per minute	t	Duration time
P	Power	V_s	Slurry Volume
WAS	Waste-activated or sewage sludge	COD	Chemical oxygen demand
HPH	High-pressure homogenizer	N	cycle number
TCOD	Total chemical oxygen demand	R_O	Severity factor

T	Temperature (°C)	LPMOs	Lytic polysaccharide mono-oxygenases

Greek Letters

α_c	Moles of carbon
α_h	Moles of hydrogen
α_o	Moles of oxygen
α_n	Moles of nitrogen
α_s	Moles of sulfur
E_s	Sonication input energy
σ	Homogenization pressure (Pa)

Chapter 1: Introduction

Our environment is being significantly impacted by global warming and climate change. Hence promoting renewable energy sources such as biomass and converting waste to bioenergy efficiently is a sustainable solution for addressing the ecological, social, and financial challenges associated with conventional energy sources [1]. The worldwide demand for bioenergy will keep rising because of current renewable energy and climate change policies [2]. Bioenergy is obtained from biomass, which includes organic substances like animals, plants, and microorganisms, through biochemical processes such as combustion, fermentation, or gasification [3]. At present, the commercial transportation of fuels and the production of electricity from biomass substrates exist in most countries [4]. Biomass is turned into useful bioenergy using three primary process technologies namely, biochemical, thermochemical, and physiochemical as shown in Figure 1.1 [5].

Anaerobic digestion (biogas production) and fermentation (ethanol production) are examples of biochemical conversions. Combustion, pyrolysis, gasification, and liquefaction are examples of thermochemical conversion processes. Physiochemical conversion primarily involves extraction, such as crushing oilseeds to produce oil [6].

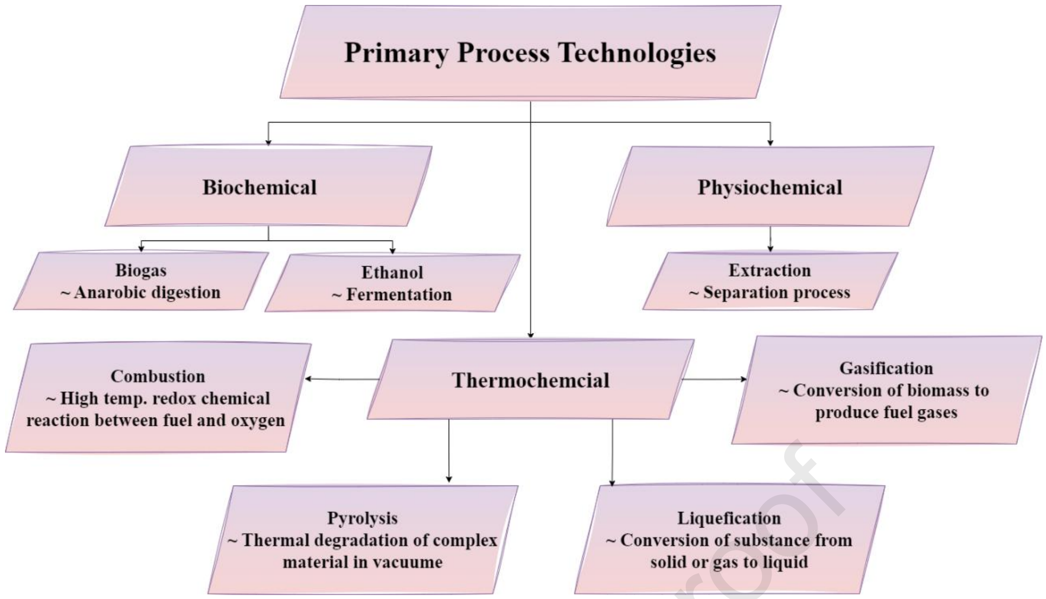

Figure 1.1:Primary Bioenergy Process Techniques

Biomass-based energy offers the dual benefit of sustainable energy generation and waste management. Within biomass technologies, AD has gained significant attention for producing biogas [7]. AD is the least energy-intensive and most environmentally friendly primary process compared to the highly energy-demanding and less eco-friendly thermochemical and physiochemical processes [6], [7] shown in Figure 1.1. One significant advantage of AD is its straightforward initiation using biodegradable waste, which is readily available in rural areas. In India, a large portion of biodegradable waste is disposed of in open spaces, water bodies, or landfills, leading to the generation of greenhouse gases (GHGs) and various environmental issues. Implementing a waste-to-energy concept, particularly through biogas generation, can address these challenges and provide a sustainable solution [8]. However, AD often suffers from challenges such as feedstock complexity, low biodegradability, and long retention times, which limit methane yield. Addressing these challenges requires effective pretreatment strategies to intensify the AD process and enhance biogas production. In supporting emerging countries like India in addressing their energy needs, this approach can also reduce their reliance on fossil fuels. India's waste-to-energy market currently stands at 750 MW, with expectations of reaching 3 GW by 2050 [8]. The current study concentrates specifically on AD, for its substantial potential to meet green energy demands that lead to a sustainable future. Agricultural waste, crop residues, animal waste, household waste, wastewater, municipal biodegradable waste, and algae are the primary substrates for generating raw biogas [9].

AD is a conventional process for generating biogas or methane. To enhance the production of biogas and the biomethane yield, it is crucial to control performance factors. Controlling factors within appropriate and acceptable ranges allows the AD process to operate optimally and efficiently, preventing failures. However, challenges such as low productivity, high hydraulic retention time (HRT), substrate complexity, and inefficient biodegradability can diminish biogas or biomethane output [10], [11].

This review paper presents an analysis of recent developments in enhancing raw biogas generation. While several recent reviews have discussed pretreatment methods for anaerobic digestion [12], [13] many remain general in scope or focus on individual approaches, without providing integrated quantitative comparisons. Hybrid pretreatments and process-intensification strategies are particularly underexplored in existing literature. To address this gap, the present review emphasizes key influencing parameters and pretreatment techniques, offering a detailed evaluation of their advantages, limitations, and quantitative impact on methane yield and process efficiency. In doing so, it highlights pretreatment strategies that can significantly improve biogas generation rates and support the intensification of AD processes.

The literature reviewed in this study was identified through a structured search strategy to ensure both transparency and reproducibility. Relevant publications were retrieved from Scopus, Web of Science, ScienceDirect, and Google Scholar, employing combinations of keywords such as "anaerobic digestion," "biogas production," "pretreatment techniques," "process intensification," "physical pretreatment," "chemical pretreatment," "biological pretreatment," and "hybrid pretreatment." To maintain quality and relevance, only peer-reviewed articles published between 2000 and 2024 were considered. Eligible studies specifically addressed pretreatment strategies for anaerobic digestion and reported measurable outcomes such as methane yield, hydrolysis rate, or improvements in biodegradability. Publications in languages other than English, conference abstracts lacking sufficient experimental detail, and studies not directly related to AD pretreatment were excluded from the review.

This paper is structured to give a comprehensive review of recent developments in the enhancement of raw biogas generation through the pretreatment method. Chapter 2 provides a scientific foundation by discussing the composition of biogas and the factors influencing its production. Chapter 3 examines the importance of various pretreatment techniques, divided into physical, chemical, biological, and hybrid categories. A comparative analysis of these techniques is presented in Chapter 4 to determine the most effective methods for improving biogas yield. The review concludes in Chapter 5 with findings and recommendations for future research directions.

Chapter 2: Anaerobic digestion (AD) process – scientific background

The anaerobic digestion process is a renewable energy technology that can generate bioenergy from biomass. AD is a complex, multistep procedure where microorganisms break down organic matter in the absence of oxygen, and it produces biogas and biofertilizers as byproducts. At the end of the AD process, biofertilizer in the form of liquid and solid digestate is a useful soil conditioner. It is rich in organic matter and nutrients, making it beneficial for improving soil structure and fertility. The breakdown of complex organic matter involves four stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis [14], [15].

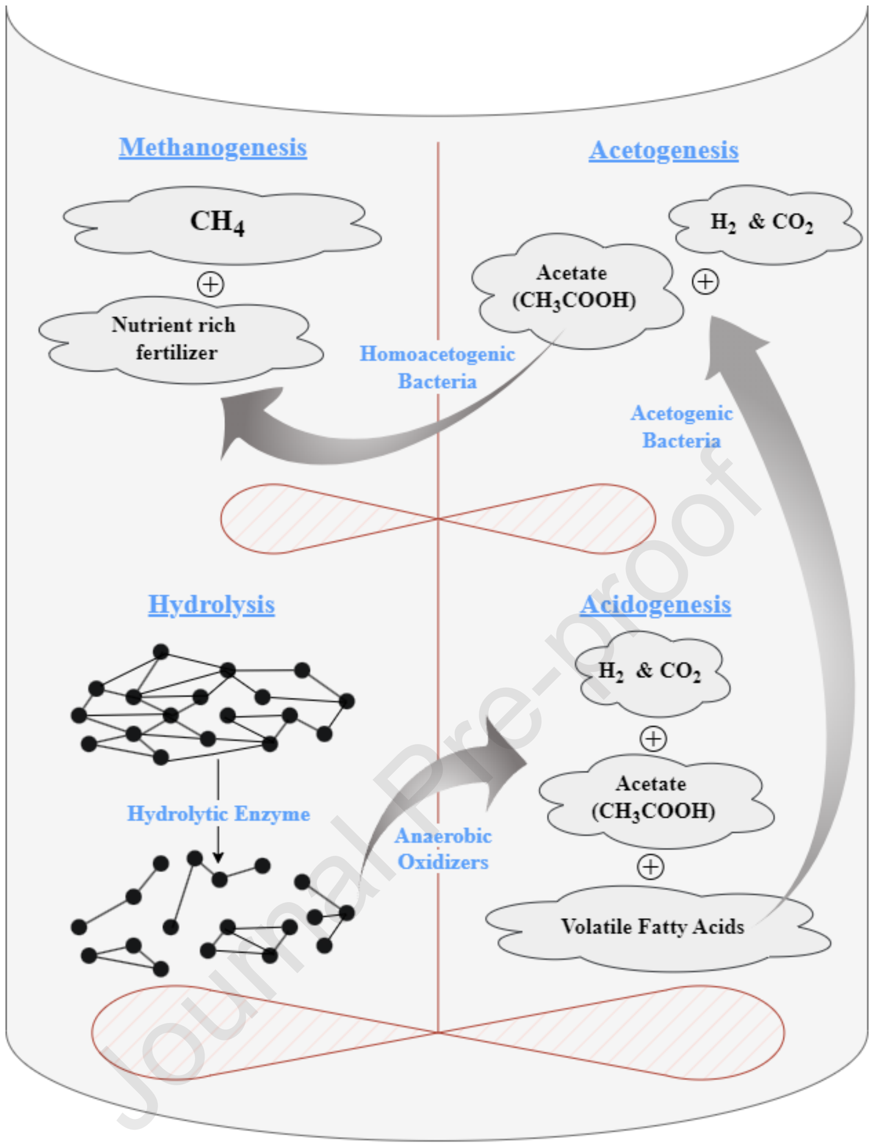

Figure 2.1: Schematic biodegradation steps of complex organic matter

As shown in Figure 2.1, the first stage of AD is hydrolysis. In this stage, bacteria from the slurry generate hydrolytic enzymes that break down long-chain polymers such as proteins ($C_{13}H_{25}O_7N_3S$), carbohydrates ($C_6H_{12}O_6$), lipids ($C_{12}H_{24}O_6$), and all insoluble polymers into shorter chains like glucose, fatty acids, glycerol, and amino acids. Although these new molecules are soluble and in high concentration, they are not yet fermentable. Therefore, the AD process moves to the second stage, called acidogenesis. In the acidogenesis stage, the monomers or oligomers (e.g., glucose, fatty acids, glycerol, and amino acids) are further broken down into volatile fatty acids (VFAs), acetate, hydrogen (H_2), and carbon dioxide (CO_2) by anaerobic oxidizers or fermentative bacteria. The process then proceeds to the acetogenesis stage, where acetogenic bacteria convert the VFAs into acetate, H_2, and CO_2. Finally, in the methanogenesis stage, acetate is converted into methane and CO_2 by acetate-oxidizing bacteria/homo acetogenic bacteria. This last step, known as methanogenesis, produces methane as a valuable end product of the AD process [1], [14], [16], [17]. The digested substrates are full of nutrient-rich biomass, which can be further directly used as a liquid fertilizer for organic farming [18], [19].

2.1 Biogas and its Composition

This biogas primarily consists of methane (CH_4) and carbon dioxide (CO_2), but also includes minor traces of hydrogen sulfide (H_2S), carbon monoxide (CO), ammonia (NH_3), water vapor ($H_2O_{(g)}$), nitrogen (N_2), oxygen (O_2), hydrogen (H_2), siloxanes, and hydrocarbons [1], [20], [21]. The typical composition of biogas is presented in Table 1. The raw Biogas potential of a specific biodegradable waste can be projected by the Buswell Equation (2.1**2.2**) [22].

$$\begin{aligned} &C_{\alpha_c}H_{\alpha_h}O_{\alpha_o}N_{\alpha_n}S_{\alpha_s} + {}^{1}/_{4}(4\alpha_c - \alpha_h - 2\alpha_o + 3\alpha_n + 2\alpha_s)H_2O \\ &\xrightarrow{AD} {}^{1}/_{8}(4\alpha_c - \alpha_h - 2\alpha_o + 3\alpha_n + 2\alpha_s)CH_4 \\ &+ {}^{1}/_{8}(4\alpha_c - \alpha_h - 2\alpha_o + 3\alpha_n + 2\alpha_s)CO_2 + \alpha_n NH_3 + \alpha_s H_2S \end{aligned} \tag{2.1}$$

In the **3.1**, the Greek letter (α) denotes the moles of elements in the feedstock's chemical formula: α_c for carbon, α_h for hydrogen, α_o for oxygen, α_n for nitrogen, and α_s for sulfur.

Table 1: Typical composition of raw biogas from waste [1], [20], [21]

Component	Symbols	Concentration (Vol%)
Methane	CH_4	45 – 75
Carbon dioxide	CO_2	25 – 50
Hydrogen sulfide	H_2S	<2 (0 – 10,000 ppm)
Carbon Monoxide	CO	Negligible
Ammonia	NH_3	<1 (0 – 100 ppm)
Water Vapor	$H_2O_{(g)}$	1 – 10
Nitrogen	N_2	0 – 15
Oxygen	O_2	0 – 3
Hydrogen	H_2	Negligible
Siloxanes	$H(OSiH_2)_nOH$ & $(OSiH_2)_n$	0.02

2.2 Factors affecting biogas generation

The AD process occurs in a digester and the efficiency of digesters in generating raw biogas or the potential yield of methane is influenced by various physical, biological, and chemical factors. These factors consist of the organic waste (structure of the feedstock and particle size), hydraulic retention time (HRT), stirring speed, C:N Ratio (Carbon Nitrogen Ratio), pH, temperature, OLR (organic loading rate) (Figure 2.2) [23]. These factors will directly influence the raw biogas generation cycle and contribute to changes in the surrounding environmental conditions and the activities of the bacterial community within the digester [1]. The controlling constraints that optimize methane yield in terms of quantity and quality are as follows.

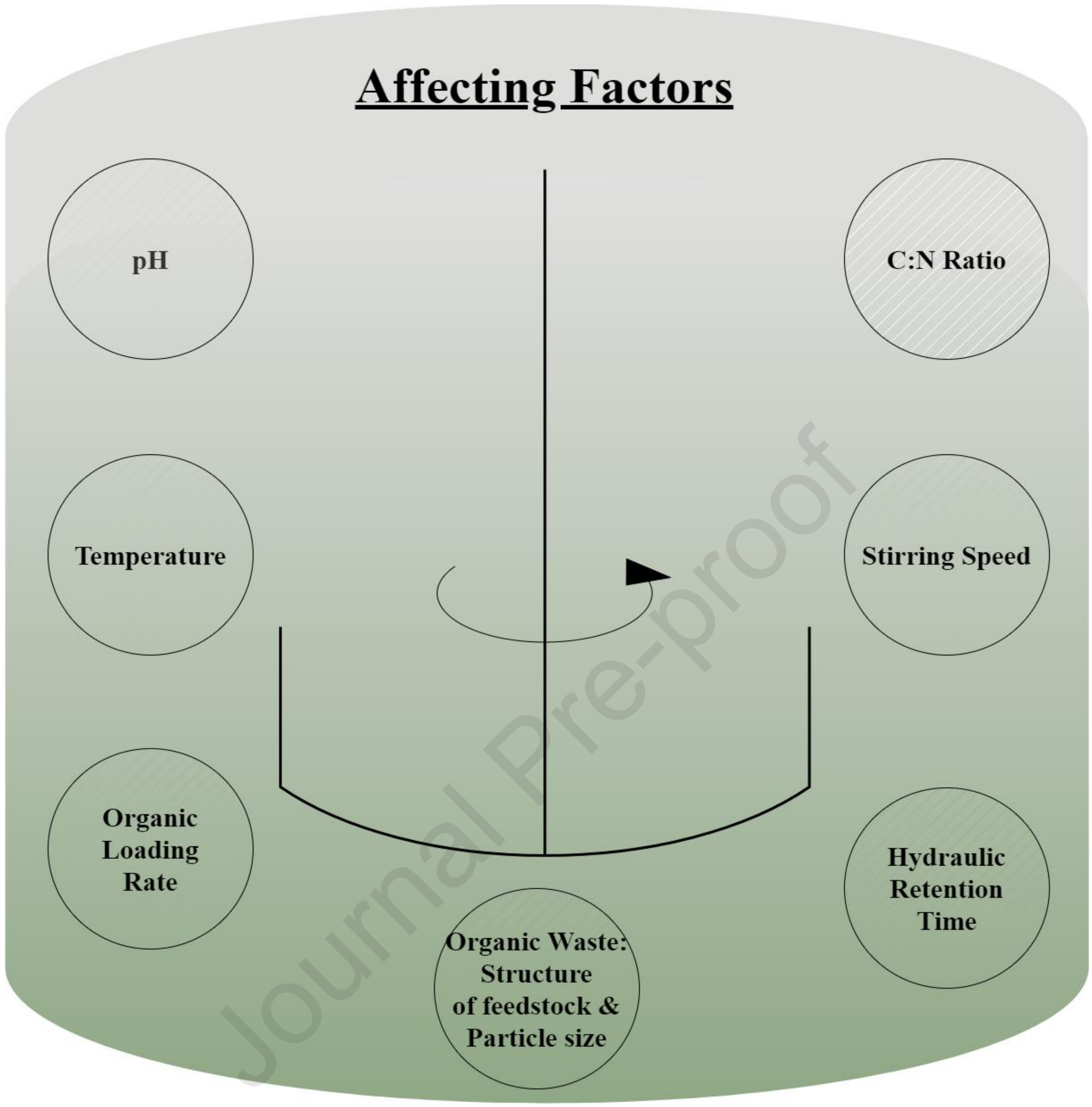

Figure 2.2: Affecting factors of the AD process

2.2.1 pH

The pH value measures the acidity or alkalinity of a sample, expressed as the logarithm of the reciprocal of the hydrogen ion concentration in gram equivalents per liter of the solution. For optimal bacterial growth during all stages of the AD process, the pH of the substrate slurry should be maintained between 6.5 and 7.5 [15], [24]. The pH of the slurry is influenced by factors such as CO_2 levels, volatile fatty acids (VFAs), and temperature [25]. According to Yadvika et al. [10], a pH range between 5 to 7.5 can result in an increased methane yield upto 75% as compared to the conventional pH range.

2.2.2 Temperature

Temperature is one of the major affecting parameters in the AD process, which significantly impacts bacterial activity from the initial hydrolysis stage [26]. The AD process typically operates within two temperature ranges: mesophilic (32°C - 42°C) and thermophilic (48°C - 55°C) [15], [27]. While higher temperatures can increase biogas production, they often result in lower methane yields and higher CO_2 percentages, reducing the calorific value of the biogas [24]. The optimal temperature for efficient and continuous biogas production is found to be between 32°C and 35°C [24]. If the temperature drops below 25°C, biogas production decreases, and at 10°C, it stops entirely. Additionally, odour problems and pathogen reduction can be solved by maintaining the temperature range of 48 °C - 55 °C [24].

2.2.3 Organic Loading Rate (OLR)

The Organic Loading Rate (OLR) represents the amount of organic substrates added to a digester per day per unit volume [15], [23]. This key factor impacts the raw biogas production rate because it is directly dependent on the volatile solids (VS) of the substrates [24]. A lower OLR generally produces more bio-methane yield [10] because of the gradual decomposition of organic matter. Optimal feeding rates of feedstock to a digester support the boost of raw biogas production, but beyond that, the raw biogas yield remains constant [28]. Generally, optimal values of OLR and yield of methane are reported in **Error! Reference source not found.**. OLR is calculated with the help of Equation 2.2 [29].

$$\mathbf{OLR} = \frac{\mathbf{S}}{\mathbf{RT}} = \frac{(\mathbf{Q}) * (\mathbf{S})}{\mathbf{V}} \quad \mathbf{2.2}$$

where OLR signifies organic loading rate (kg feedstock/m^3 digester/day), Retention time (days) is denoted by RT, S represents feedstock concentration in total volatile solid, V for a volume of the digester (m^3), Q means flow rate of the slurry (m^3/day).

Table 2: Optimum values of OLR & yield of methane for different substrates [24]

Substrates	OLR (kgVS/m³/day)	Yield of methane (m3/kgVS$_{destroyed}$)	References
Sewage sludge	8.5	0.190	[30]
Corn silage	3.5 – 8.5	0.327 – 0.410	[31]
Swine manure	4 – 8	0.050 – 0.450	[32]
Organic fraction of municipal solid waste	11.8	0.097	[33]

2.2.4 Organic Waste: (Structure of feedstock & Particle size)

The potential yield of methane is generally calculated based on the initial characteristics of the selected organic waste feedstock [29]. Different substrates show distinct characteristics, which are highlighted in Table 3. At the initial stage of biogas production, it is essential to identify the biomethane potential (BMP) of the substrates. This is achieved through laboratory tests, such as the fermentation test GB_{21} and the BMP test [29]. This selection is critical for maximizing biogas yield and ensuring the efficiency and stability of the digestion process.

The particle size of the feedstock is not as critical as temperature or pH in the AD, but it still affects biogas production. If the substrate size is too large, it can clog the digester, making it difficult for microbes to digest the feedstock efficiently, which can lead to reduced biogas production [10]. Conversely, smaller particles provide a greater surface area for microbes, enhancing microbial activity and boosting biogas generation [28]. According to Yadvika et al. [10], most biogas was produced from feedstock with particle sizes of 0.088 mm and 0.40 mm, out of the five sizes tested:

0.40, 0.088, 1.0, 30.0, and 6.0 mm. Physical pretreatment, such as grinding, significantly reduces the size of the feedstock [34].

Table 3: Characteristics of various organic waste [29], [35]

Organic Waste	Cellulose (%)	Hemi - cellulose (%)	Lignin (%)
Corn stover	37.5	22.4	17.6
Corn fiber	14.3	16.8	8.4
Wheat straw	38.2	21.2	23.4
Leaves	15–20	80–85	NA
Bagasse	38.2	27.1	20.2
Sugarcane	25	17	12
Rice straw	32	24	13
Cattle manure	1.6–4.7	1.4–3.3	2.7–5.7
MSW	33	9	17
OF-MSW	60	20	20
Newspaper	62.1	16.1	21.1
Waste paper from pulps	60–70	10–20	5–10
Coffee pulp	35	46.3	18.8
Algae	20–40	20–50	NA
Banana waste	13.2	14.8	14
Nut shells	25–30	25–30	30–40

2.2.5 Hydraulic Retention Time (HRT)

Hydraulic retention time (HRT) is the average time for which the decomposable substrate slurry remains inside the digester before it is removed from the digester [10]. HRT is affected by the type of solid substrates and temperature inside the digester [24]. Generally, in tropical countries, HRT varies from 30 – 50 days while in colder countries it may go up to 100 days [10]. In India, the hydraulic retention time is 10 – 14 days when the temperature of the digester is in the range of mesophilic, and for the thermophilic range it is 14 days [15]. Optimizing the HRT for biogas plants is necessary because a low HRT risks washout of the active microbial bacteria population and a long HRT requires a large volume for the digester and hence a greater capital cost [10]. According to Sanchez et al. [36], increasing the HRT improved the removal of substrate slurry when treating cattle manure. Desai and Madamwar [37] reported the highest raw biogas production of 2.2 l/l/day with 62 % of methane yield at an HRT of 10 days and a loading rate of 6 gm TS/l while treating a mix of cattle manure, poultry waste, and cheese whey in a 2:1:3 ratio.

2.2.6 Stirring Speed

Stirring speed, also known as agitation, is a crucial factor in the AD process. It causes the substrate slurry to rotate inside the digester, enhancing BMP [10], [24]. Stirring speed largely depends on the design of the blade and the viscosity of the slurry. Proper agitation prevents the substrate from settling down and forming scum [24]. In a study examining various stirring speeds (30, 40, 50, 60, and 70 rpm), it was found that the highest raw biogas production was achieved at 30 rpm [38]. It has been determined that slow speeds of stirring are most effective for increasing raw biogas and methane yield [15]. According to Yadvika et al. [10], the methane yield can be enhanced by physically disrupting cellular substrates. However, optimum stirring speed also depends on the digester size and volume of the substrate in the digester.

2.2.7 C:N ratio

C:N ratio is the ratio of the carbon and nitrogen compounds in substrates. It is the key factor for the AD process because it helps to maintain a steady AD process by adding the co-substrates into the AD process [29]. Generally, the ideal C:N ratio for the AD process is in the range of 20 – 23

[39]. Some of the various organic waste C:N ratio is mentioned in Table 4. If the C:N ratio is less than 25, the raw biogas production is detected to be low and if it is greater than 25, it will lead to the generation of toxic gases such as NH_3 [15]. The nitrogen in an AD reactor primarily comes from proteins and is essential for microbial growth. If the C: N ratio is too low, as a result, ammonia (NH_3) can build which disturbs the raw biogas and yield of methane production and it helps to potentially leads to process failure [23]. Adding feedstock like agricultural waste or paper waste can significantly raise the carbon content in the substrates, which helps to prevent these issues [40]. According to Zeshan et al. [41], increasing the C:N ratio from 27 to 32 by mixing green waste, food waste, and paper waste reduces the NH_3 content by approximately 30 %.

Table 4: Various organic waste with its C:N (%) ratio [29]

Organic Waste	C:N ratio (%)	Organic Waste	C:N ratio (%)
Corn stover	60–120	MSW	40
Corn fibre	35–45	OF-MSW	14–16
Wheat straw	90	Newspaper	175
Leaves	8–20	Waste paper from pulps	90
Bagasse	150	Coffee pulp	18.5
Sugarcane	50	Algae	19
Rice straw	70	Banana waste	21–34
Cattle manure	24	Nutshells	35

Chapter 3: Pretreatment techniques - reviews and remarks

Pretreatment, commonly known as the conditioning process, involves various methods applied to the feedstock to simplify and overcome the resistance caused by its cell wall (lignin compound) and structural properties. Pretreatment helps in easier feedstock solubilization and hydrolyzation, resulting in enhanced biogas yield [42], [43]. Pretreatment is essential for various feedstocks, such as lignocellulosic biomass and municipal solid waste (MSW), which contain complex organic compounds such as cellulose, hemicellulose, and lignin. These materials are not readily biodegradable and require conditioning before being used in the AD process [44]. Lignin, especially found in plant residues and energy crops [45], forms strong bonds that protect cellulose and hemicellulose, reducing the surface area for microbial attack and making digestion more difficult. Pretreatment simplifies microbial digestion by breaking down these complex structures, thereby enhancing the AD process in terms of shortening the retention time of the first stage of the AD process (hydrolysis stage) and improving the quality of methane yield upto 120% from the biogas [14], [46], [47] as compared to untreated feedstock of biomass [48], [49]. Pretreatment enhances the solubility of feedstock, stimulates the growth of methanogenic enzymes and microbes [14], and increases the porosity and surface area of the organic matter [50]. These improvements make the feedstock more accessible to microbial activity [14], leading to additional efficient breakdown and advanced methane yield production in AD processes. Pretreatment methods can be broadly classified into four main categories as presented in Figure 3.1: Physical, Chemical, and Biological; hybrid methods which are discussed in the subsequent sections.

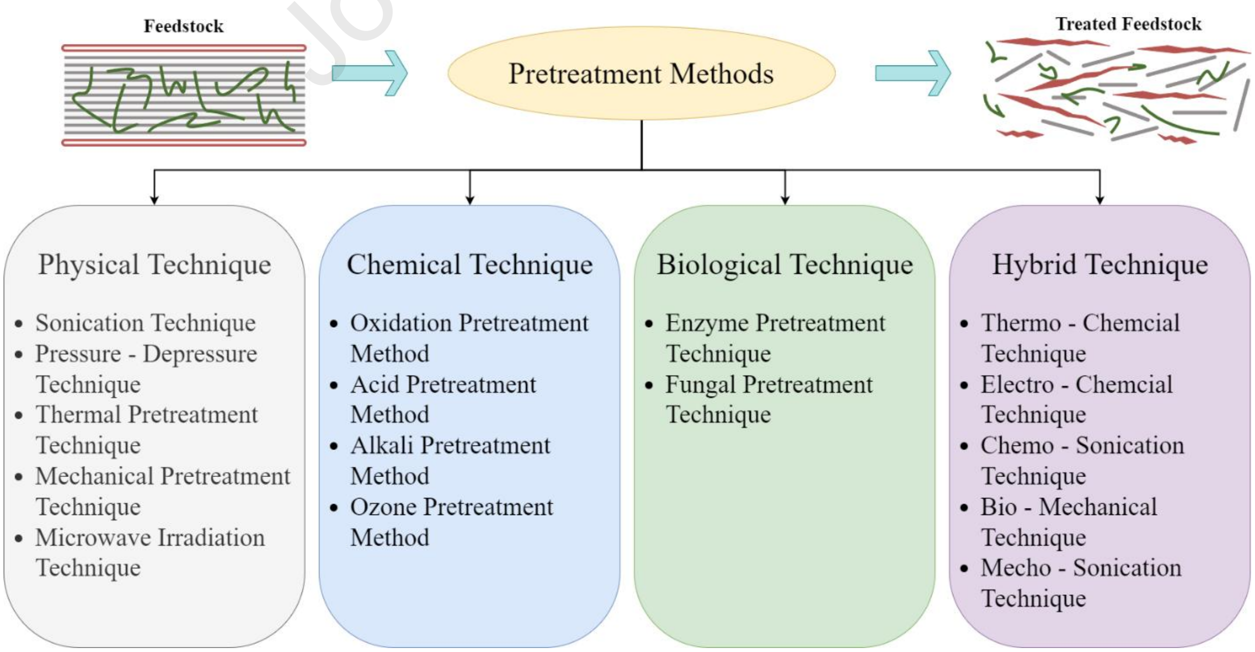

Figure 3.1: List of pretreatment methods

3.1 Physical Techniques

The physical pretreatment method significantly influences the properties of feedstock, including particle size, surface area, cellulose structure, polymerization degree (the number of monomer polymers increases), and pore size [34], [51]. This method encompasses various mechanical operations such as grinding, milling, centrifuging, high-pressure homogenization, thermal treatments, sonication, and microwave irradiation. Physical pretreatment can be broadly classified into five categories (Figure 3.1): Sonication Technique, Pressure Depressurization Technique, Thermal Pretreatment Technique, Mechanical Pretreatment Technique, and Microwave Irradiation Technique [18], [26].

3.1.1 Sonication Technique

Sonication is an effective physical pretreatment method that uses low energy input to enhance the biodegradability of substrates, leading to an increased yield of methane in biogas [52], [53], [54], [55]. The main key factors affecting the effectiveness of the sonication method, or cavitation-based pretreatment, are energy input, sonication frequency, and the type of feedstock used [55], [56]. Sonication input energy (E_s) depends on the sonication time (t), power (P), and initial TS concentration of slurry $(TS_{initial})$ as well as slurry volume (V_s). Equation 3.1 represents the sonication input energy[56].

$$\mathbf{E}_{\textbf{Sonication Technique}} = \frac{(\mathbf{P}) * (\mathbf{t})}{(\mathbf{V_s}) * (\mathbf{TS_{initial}})} \qquad \textbf{3.1}$$

Cavitation pretreatment can be performed in two ways: direct and indirect. Direct sonication involves immersing a probe into the sample, emitting ultrasonic waves that cause cavitation and generate intense shear forces to disrupt cells and macromolecules. In contrast, indirect sonication, or bath sonication, uses ultrasonic waves transmitted through a water bath to the sample container, avoiding direct probe contact and making it ideal for delicate samples. The sonication method uses high-intensity ultrasound waves, usually between 20 and 25 kHz [53], to create bubbles that break down cell surfaces, making the material easier to biodegrade than untreated feedstock. B. Deepanraj et al. [53] showed that sonication improves biogas generation and reduces volatile solids (VS) and HRT.

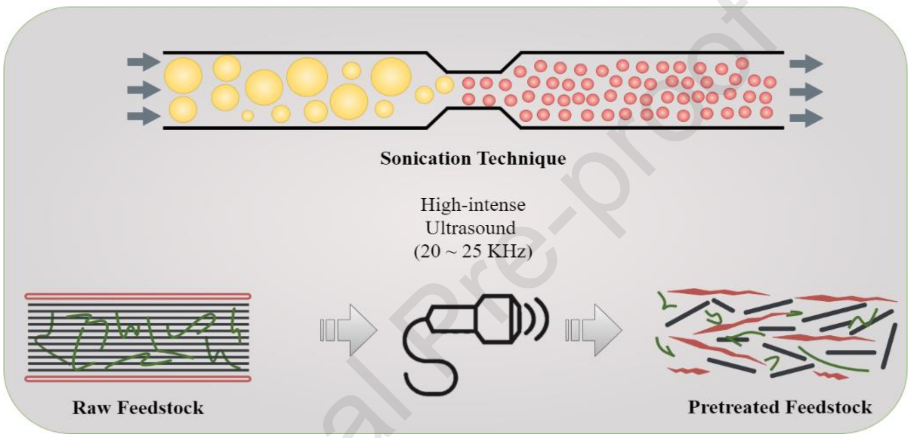

Figure 3.2: Sonication Technique

It is commonly used for waste-activated or sewage sludge (WAS) [52], which requires less sonication energy and time compared to other types of feedstocks [57], [58]. Carrère et al. [59] and Martín et al.[57] identified that the biomethane potential (BMP) of cattle manure (5.8% TS) and waste activated or sewage sludge (WAS) were respectively improved by 19% and 140%. BMP goes up when more quantity of oxygen dissolves into soluble water or in other words, when chemical oxygen demand (COD) increases [52], [59]. Mönch-Tegeder et al. [60] reported a 26.5% increase in methane production yield in comparison to the untreated substrate. However, according to Kim et al. [61] longer sonication reduces the generation of methane yield because less dissolved matter is available.

3.1.2 Pressure - Depressure Technique

This physical technique employs high-pressure homogenizer (HPH) pretreatment to break down the feedstock [62]. Its easy operation, high energy efficiency, and low investment costs make it suitable for large-scale implementation [55], [62]. The process begins by applying a high pressure of approximately 10 bar to the feedstock. Then, the pressure is rapidly reduced to around 1 bar in a process known as blasting [1], [26], [63]. This rapid pressure drop generates strong turbulence and shear forces in the slurry, which effectively break apart the structure and cell walls of the feedstock [26], [63]. These shear forces increase the surface area of the substrate, which enhances the efficiency of the initial stages of the AD process. Consequently, this improvement leads to a reduction in HRT and an increase in bio-methane potential. HPH energy consumption can be calculated using the following Equation 3.2 [62].

$$\mathbf{E_{HPH}} = \frac{(\boldsymbol{\sigma}) * (\mathbf{N})}{(\mathbf{1000}) * (\mathbf{TS_{initial}})} \qquad \mathbf{3.2}$$

where σ (Pa) is the homogenization pressure, N is the homogenization cycle number, and $TS_{initial}$ (g/L) is the initial TS concentration of slurry. According to Ma et al. [64], the application of the physical pretreatment technique, specifically the pressure-depressure method, resulted in up to a 35% increase in biogas yield from food waste. It was reported by Zhang et al. [62] that the pressure-depressure pretreatment technique became energy-intensive when the homogenization pressure (σ) was too high and the number of homogenization cycles (N) were too large. Kim et al. [65] indicated that an increase in high-pressure homogenization (HPH) led to greater degradation of organic matter, as measured by volatile solids (VS) removal and total chemical oxygen demand (TCOD).

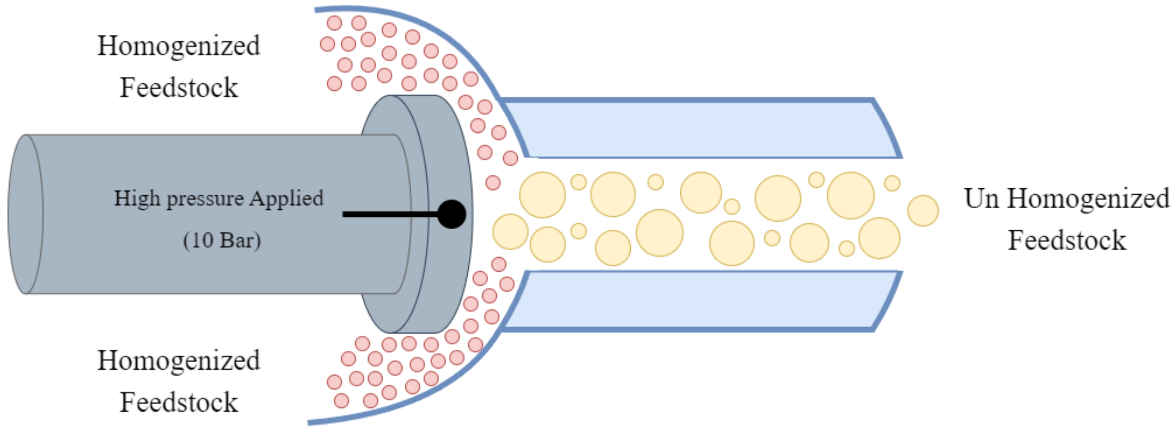

Figure 3.3: Pressure-Depressure Technique

3.1.3 Thermal Pretreatment Technique

Thermal pretreatment involves the use of thermal energy (heating) the substrate for a certain period. Types of thermal pretreatment can be divided into four pretreatment techniques which are shown in Figure 3.4: Types of thermal pretreatment techniqueFigure 3.4.

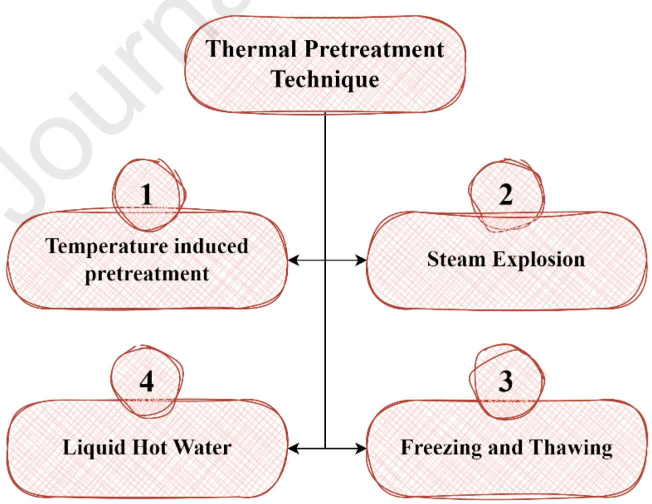

Figure 3.4: Types of thermal pretreatment technique

3.1.3.1 Thermal-induced pretreatment technique:

Thermal-induced pretreatment techniques boost the rate of AD by speeding up the initial stage of the process, which is nothing but the hydrolysis stage [1], [66]. It effectively works within the temperature range of 70 °C – 275 °C, with a reaction time of 30 to 60 minutes [53], depending on the type of substrate [1]. Temperatures over 250 °C should be avoided to prevent unwanted reactions (e.g., pyrolysis reaction) [53]. Carrère et al. [67] conducted a temperature-induced thermal pretreatment on pig manure of both liquid and solid fractions at a temperature of 190°C and observed an improvement in the BMP. Applying this thermal pretreatment technique to substrates like WAS [68], food waste [69], algae [70], MSW [71], grass and agricultural by-products [72] has been shown to enhance BMP.

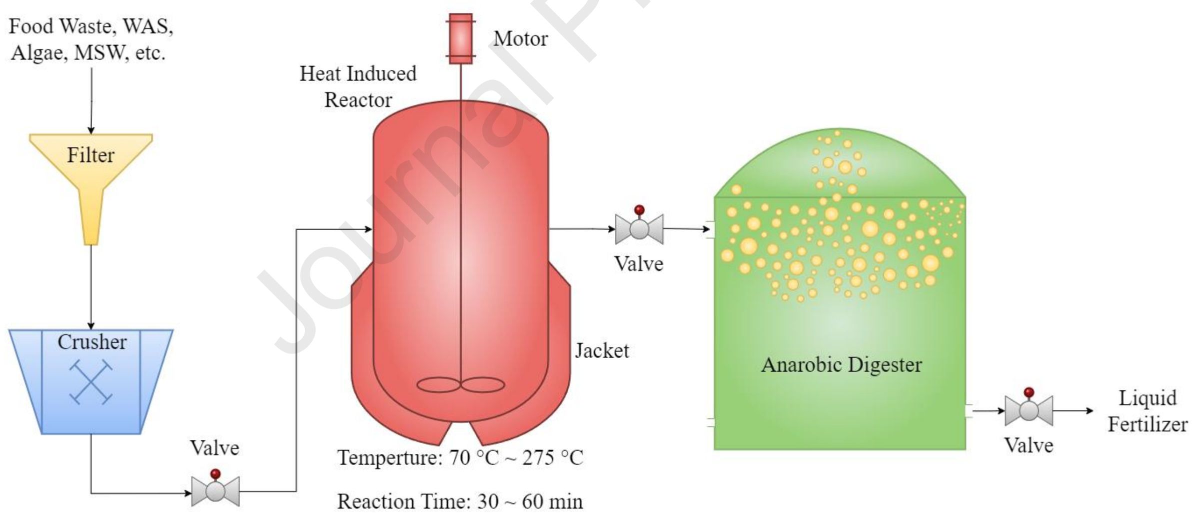

Figure 3.5: Thermal-induced technique

3.1.3.2 Steam explosion

Steam explosion is another type of pretreatment technique, which uses steam at a specified pressure (5 bar to 50 bar) and temperature (160°C – 250°C) [18], [73]. This thermal pretreatment technique is efficient for lignocellulosic biomass such as wood, grass, MSW, and agriculture waste

[19]. In this pretreatment technique, the substrate slurry is put into a sealed packed chamber, and high-temperature and pressurized steam is passed through it for a certain period [53]. This pretreatment duration depends on the moisture content of the substrate; a high moisture content in the feedstock requires a longer pretreatment duration [53]. The pretreatment time can be calculated theoretically by Equation **3.3**. In the equation, log (R_O) represents the severity factor which usually lies in the range of 3.14 to 3.56 [74]; T = temperature (°C); t = pretreatment duration (mins) [74].

$$log(R_O) = \log\left(t * e^{\left(\frac{T-100}{14.75}\right)} \right) \quad \mathbf{3.3}$$

As reported by Horn et al. [75] and Zhou et al. [76], the steam explosion pretreatment technique maximized biogas generation yield from lignocellulosic feedstock, like Salix woodchips and rice straw, at a temperature of 210 °C for 10 minutes pretreatment duration and a temperature of 200 °C for 2 minutes pretreatment duration, respectively.

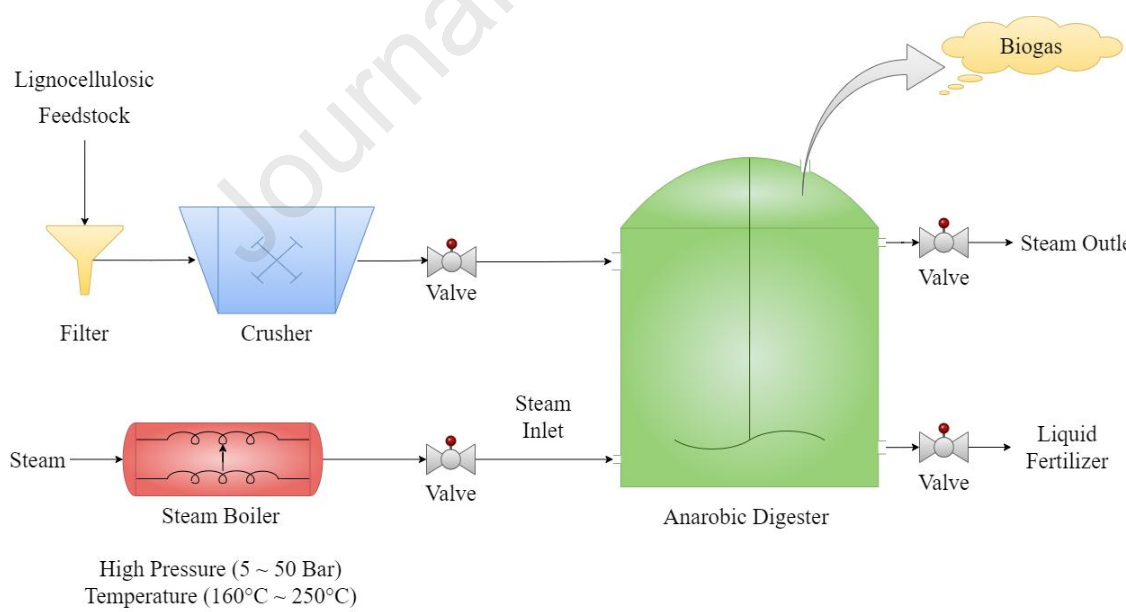

Figure 3.6: Steam Explosion

3.1.3.3 Freezing and Thawing

Freezing and thawing is a type of thermal pretreatment technique that subjects the substrate or feedstock sludge to a significant temperature change. Initially, the substrate is frozen to below-zero temperatures, causing the material to solidify. Afterwards, the substrate is gradually brought back to its normal temperature. This method leverages the stress of freezing and thawing to enhance the breakdown of the feedstock, making it more controllable for the AD process [77]. This thermal pretreatment technique comes with significant operational costs, making it less feasible and widely applicable. However, it is a particularly effective and powerful pretreatment process for treating food waste and agricultural waste [26]. The structure of the cells in the feedstock sludge will be disrupted by this thermal pretreatment technique, resulting in an enhanced generation of biogas yield. According to Elmashad et al. [77], a 30% increase in biogas yield was achieved when the freezing and thawing method was used to pretreat cattle manure.

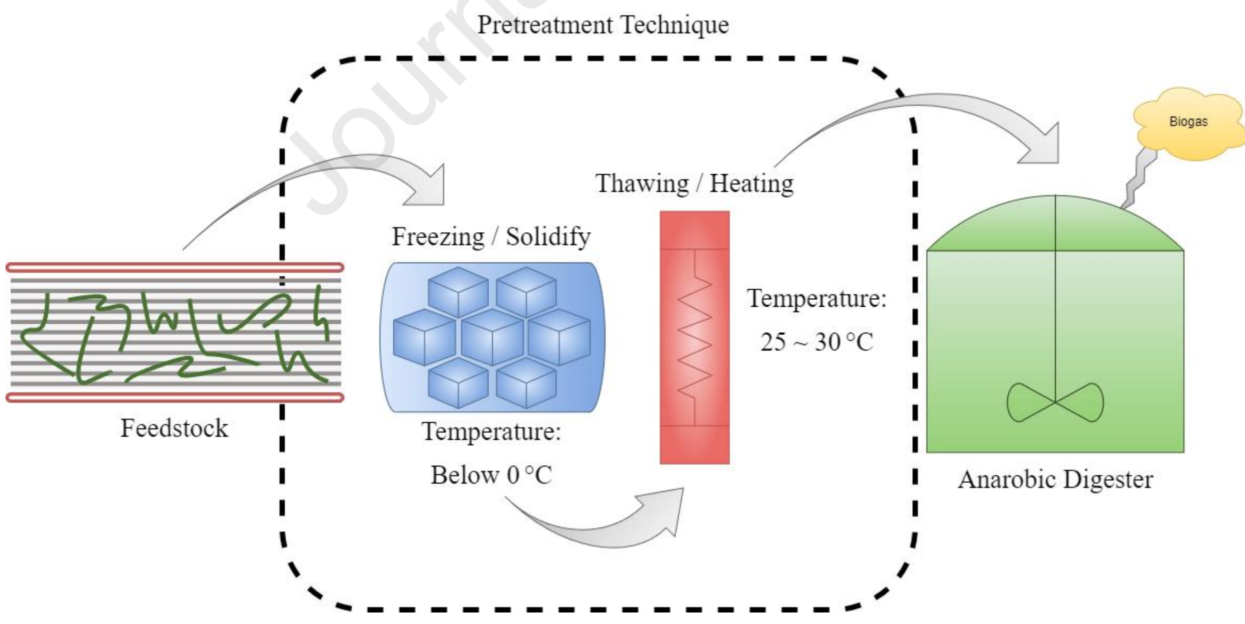

Figure 3.7: Freezing and Thawing

3.1.3.4 Liquid Hot Water

Liquid hot water is another type of thermal pretreatment technique, also known as hydrothermal pretreatment or wet torrefaction [78]. The main objective of this thermal pretreatment is to break down and degrade the hemicellulose compounds in the substrate or feedstock. One of the key benefits of using hot water is its ability to easily dissolve feedstock and prevent the formation of restrictive compounds [1]. This method is similar to the steam explosion pretreatment technique, with the key difference being that hot liquid water is used instead of high-pressure steam [1], [23], [79]. The parameters for the hot liquid water process include a temperature range of 100°C to 140°C [80] and a pressure range of 1 to 2 bar, however, some feedstock may require temperatures between 150°C and 240°C [81]. Hendrickson et al. [82] showed that keeping the pH of the substrate or feedstock sludge between 4 to 7 helps break down the cellulosic structure and reduce monosaccharide production. According to Qiao et al. [83], after wet torrefaction thermal pretreatment at 170 °C for 1-hour duration, raw biogas generation was enhanced by 68% for MSW, 195% for fruit/vegetable waste, 8% for pig manure, and 13% for cow manure while the methane yield rose by 66% for MSW, 16% for fruit/vegetable waste, 15% for pig manure, but decreased by 7% for cow manure. Similarly, Passos and Ferrer [80] reported a growth in BMP of 17% – 39% after the wet torrefaction thermal pretreatment of microalgal biomass. The study by Jiang et al. [84] presented a 31 % increase in methane yield from giant reed with liquid hot water pretreatment. Panigrahi et al. [85] demonstrated an 11 % increase from the waste of the yard, and Shang et al. [86] achieved a 63 % increase from wheat straw using the same pretreatment approach.

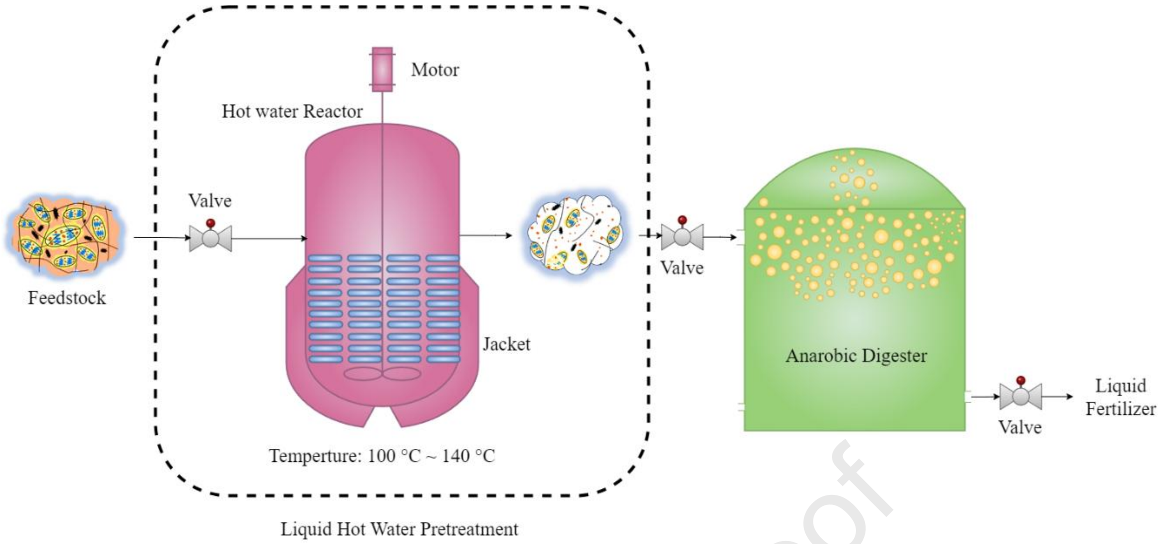

Figure 3.8: Wet torrefaction

3.1.4 Mechanical Pretreatment Technique

Mechanical pretreatment is a universal and crucial type of physical pretreatment process. This pretreatment method involves reducing the particle size of feedstock through mechanical processes such as screw presses, disc screening, milling, or grinding [26], [87], [88]. By breaking down the feedstock into small particles, the surface area available for microbial contact is significantly increased [26], [89]. This increase in surface area is essential because it allows for a more efficient and effective interaction between the substrates and the microbial community. When the particle size of the feedstock is decreased, a greater access of microbes is involved in the AD process. This pretreatment-enhanced access facilitates better microbial adhesion, colonization, and enzymatic activity on the feedstock particles. As a result, the overall rate of substrate degradation and conversion rate of methane yield is improved [26]. A mechanical milling pretreatment technique can reduce the degree of polymerization, cellulose crystallinity, and particle size of feedstocks [87]. This results in increased digestibility and surface area of the substrates. These changes improve bulk density, flow properties, bioconversion effectiveness, and porosity of the lignocellulosic biomass, leading to better overall conversion without producing toxic products [90]. Mechanical screw press extrusion pretreatment has been used on a mixer of solid waste and straw with a 30% increase in methane yield [91]. Pilarski et al. [92] found a 16.5% increase in BMP after using a single screw extrusion process to pretreat maize straw silage feedstock when compared to untreated maize straw feedstock. Mönch-Tegeder et al. [60], Pengyu et al. [93], and Dell'Omo and Froscia et al. [94] reported that mechanical pretreatment of horse manure, grass, and wheat straw led to increases in methane yield of 27 %, 45 %, and 49 %, respectively. According to Agyeman and Tao [95], mechanically pretreating food waste with the grinding method resulted in a 9 % to 34 % rise in the yield of raw biogas. However, if the particle size is reduced excessively, it can lead to hydrolysis overloading and subsequently causing VFAs accumulation [96].

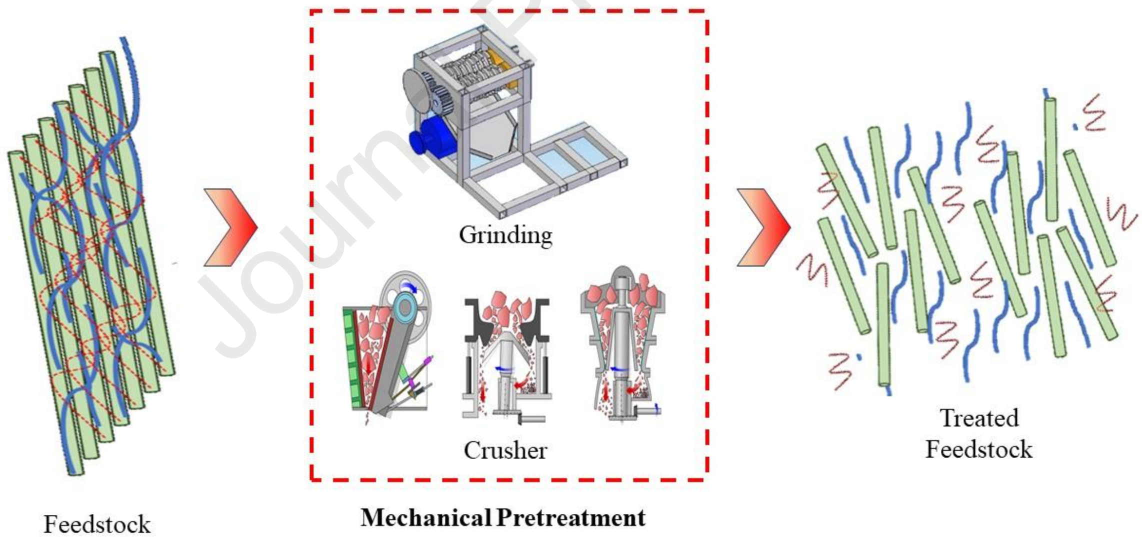

Figure 3.9: Mechanical Pretreatment

3.1.5 Microwave Irradiation Technique

The microwave irradiation pretreatment method uses magnetic and electric fields to directly interact with the molecular structure of substrate components. It causes chemical, biological, and physical reactions and therefore ion movement, heat generation, and the vibrations of polar molecules [53], [97]. Physical microwave irradiation pretreatment provides benefits such as breaking down lignin structure, increasing the substrate's surface area, reducing cellulose polymerization and crystallinity, and improving enzyme accessibility [34], [98]. Carrere et al. [59] demonstrated that the microwave irradiation pretreatment method alone enhanced BMP by approximately 60 % from microalgae (microscopic algae - invisible to the naked eye) through a continuous reactor. However, there are many technical challenges like high power consumption, high capital cost, and loud operational sound, to applying this pretreatment method on an industrial scale, and so it is mostly used in laboratory experiments [1].

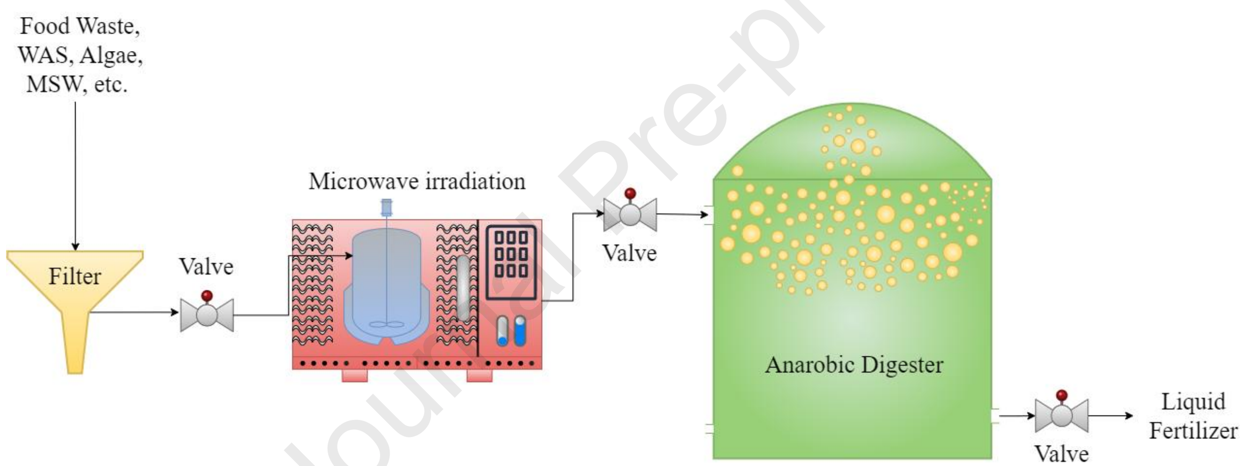

Figure 3.10: Microwave Irradiation

3.2 Chemical Technique

Chemical pretreatment significantly enhances the hydrolysis phase of the AD process. By disrupting the cell walls of the feedstock and increasing biomass accessibility, it helps dissolve organic matter and boost biogas generation as well as methane yield [26]. The key objective of the chemical pretreatment technique is to hydrolyze cellulosic materials, specifically food waste containing vegetable and lignocellulosic feedstocks [99], [100]. Chemical pretreatment can be broadly classified in four categories: Oxidation Pretreatment Method, Acid Pretreatment Method, Alkali Pretreatment Method, and Ozone Pretreatment Method.

3.2.1 Oxidation Pretreatment Method

The oxidation pretreatment process is a well-established chemical method that utilizes oxidizing agents such as peracetic acid (CH_3CO_3H) and hydrogen peroxide (H_2O_2) [53]. These chemical oxidizing agents are not utilized directly but they are applied through either wet oxidation or the advanced wet explosion pretreatment technique [18]. This pretreatment solubilizes the hemicelluloses and decomposes the lignin substrates and therefore enhances the cellulose accessibility [53], [101]. In the oxidation pretreatment process, water and substrates are combined initially and then mixed with the chemical oxidizing agent. This reaction is exothermic; thus, it releases heat during the reaction. Typically, the wet oxidation process operates at elevated temperatures between 125 °C and 300 °C and under pressures ranging from 0.5 MPa to 20 MPa. Conversely, the advanced wet explosion pretreatment method is conducted at lower temperatures, from 140 °C to 220 °C, and at pressures from 0.5 MPa to 3.5 MPa [1]. The primary difference between an advanced wet explosion and a wet oxidation pretreatment is the utilization of the decompression device; this reduces the pressure and thus physical disruption seen in the feedstock, which is not utilized in the wet oxidation pretreatment technique [102]. Thus, the key parameters influencing this chemical pretreatment process include pressure, temperature, and pretreatment duration, which collectively determine the effectiveness of the oxidation treatment [18]. According to Ahring et al.[101], a 357 % increase in BMP production was achieved when the advanced wet explosion technique was applied to feedlot manure. Biswas et al. [102] utilized the same technique on digested manure, which led to a 129% increase in biogas production. According to Lee et al., the wet oxidation pretreatment process resulted in a 43% enhancement in methane yield during the mesophilic process [103]. Additionally, a 30% enhancement in methane yield was observed during the thermophilic process [104]. A 21% rise in raw biogas generation was reported by Appels et al. [105] when peracetic acid (CH_3CO_3H) was used as a chemical oxidation agent to pretreat WAS before AD processing.

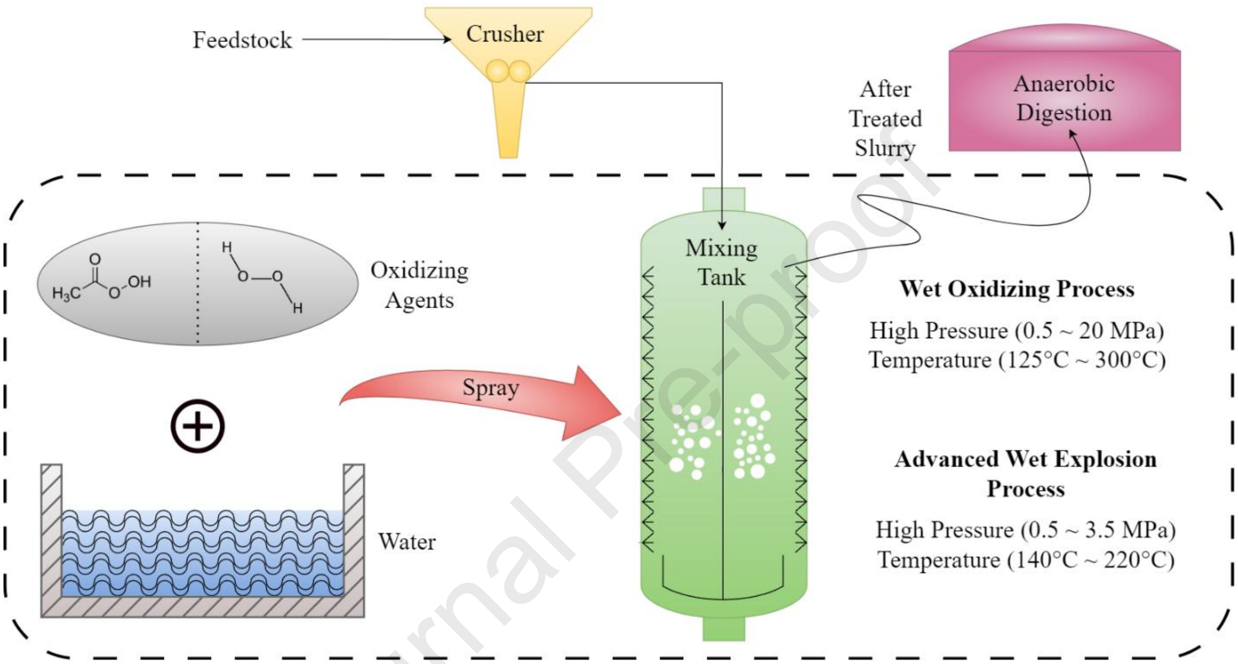

Figure 3.11: Oxidation Pretreatment Method

3.2.2 Acid Pretreatment Method

The acid pretreatment technique accelerates the solubilization of hemicellulose substrates into oligomers and decomposes lignin compounds, thereby enhancing the solubility of cellulose [106]. Typically, strong or dilute acids such as hydrochloric acid (HCl), sulfuric acid (H_2SO_4), nitric acid (HNO_3), maleic acid ($C_4H_4O_4$), acetic acid (CH_3COOH), and phosphoric acid (H_3PO_4) are utilized in this process [18]. This pretreatment can be performed in two ways. The first method involves using dilute acids, typically at concentrations of 4% w/w, at a specific temperature range of 100°C to 250°C [44]. The second method involves using strong acids, with concentrations ranging from

30% to 70%, at a lower temperature of around 100°C [18]. According to Paudel et al.[44], the use of strong acids leads to excessive degradation of the feedstock, resulting in a loss of fermentable material and the production of undesirable byproducts such as furfural and its derivatives, which can significantly inhibit anaerobic digestion. When biomass is pretreated with dilute acids at high temperatures for only 10 – 30 minutes, no increase in raw biogas or BMP is observed. However, when the dilute acid pretreatment duration is extended to 1–2 hours, a significant increase in the yield of methane has been detected [107]. Inorganic acids such as hydrochloric acid (HCl), sulfuric acid (H_2SO_4), nitric acid (HNO_3), etc. are more effective in enhancing biogas and BMP compared to organic acids [108]. For example, Zhang et al. [47] found that lignocellulosic waste pretreated with H_2SO_4 showed a 57 % increase in methane yield. However, acidic pretreatment is typically unsuitable for food waste, as it can reduce biogas generation yield due to inhibitor accumulation of lignin compounds at low pH [64], [109]. Venturin et al. [110] reported a 32% increase in methane yield from corn stalk substrate using an acid pretreatment technique. Additionally, Song et al. [111] demonstrated that H_2SO_4 and HCL acids significantly upgraded methane yield compared to CH_3COOH pretreatment, which caused a lower increment in biogas generation.

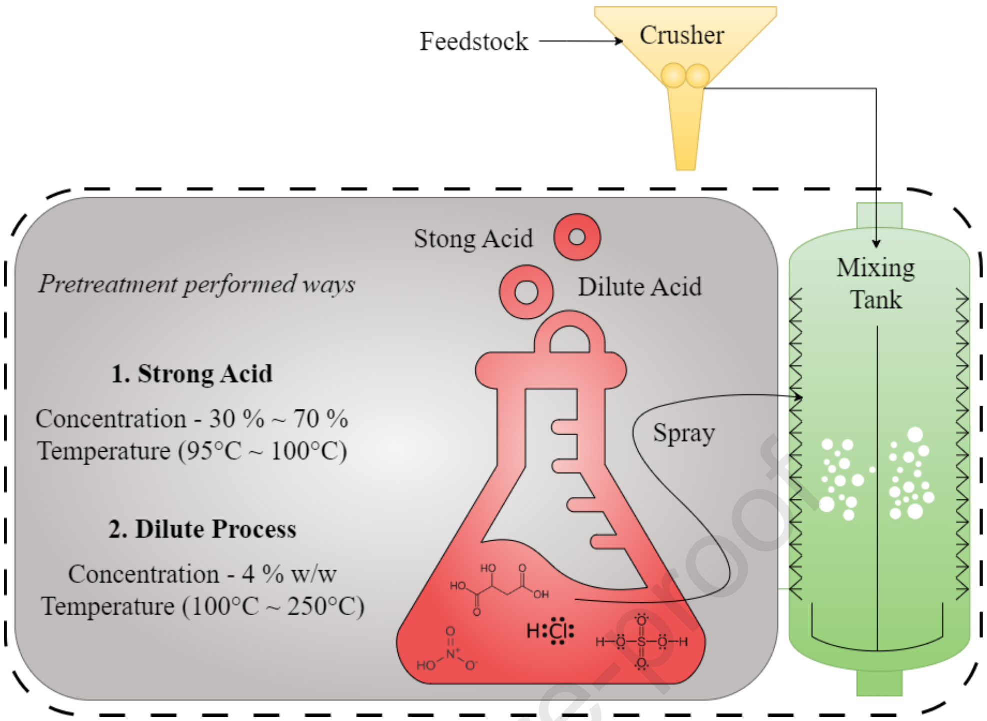

Figure 3.12: Acid Pretreatment Method

3.2.3 Alkali Pretreatment Method

Alkali pretreatment is a distinctive chemical method where alkaline agents help break down the lignin compound, a complex polymer, and enhance the solubilized hemicellulose compound of feedstock [59], [112]. This method has been shown to improve AD process efficiency more effectively than acid pretreatment because it helps to maintain the pH which is a crucial parameter for optimizing the AD process [40], resulting in a significant enhancement in biogas and methane production [59], [113]. This process is also known as the saponification process [1]. This technique employs alkali agents like sodium hydroxide (NaOH), ammonium hydroxide (NH_4OH), calcium hydroxide ($Ca(OH)_2$), and potassium hydroxide (KOH) [1]. It is particularly suitable for substrates rich in lipids [114]. Studies by Antonopoulou et al. [115] and Dasgupta and Chandel [116] demonstrated that pretreating grass lawn waste and the organic fraction of MSW with NaOH increased methane yields by 26% and 35 %, respectively, compared to untreated feedstock during the AD process. It has been stated by Rani et al. [48] that treating wheat straw with a 10% $Ca(OH)_2$ solution before AD significantly enhanced BMP compared to untreated wheat straw.

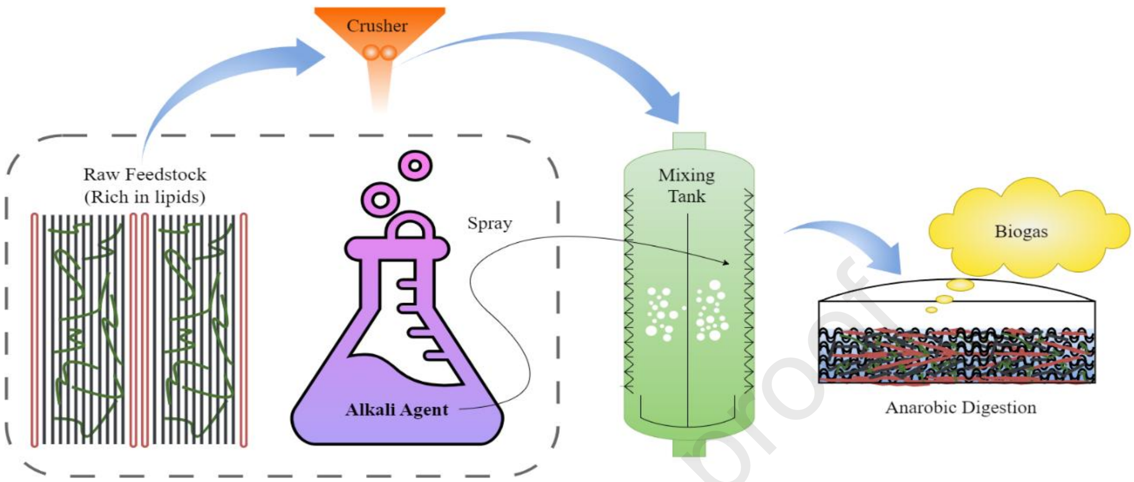

Figure 3.13: Alkali Pretreatment Method

3.2.4 Ozone Pretreatment Method

Ozone pretreatment is also known as ozonolysis or ozonation pretreatment. It is an attractive chemical pretreatment process where ozone is used at room temperature and pressure to treat various feedstocks [18], [53]. This pretreatment method is quite effective, as ozone (O_3) reacts with the substrates, breaking them into oligomers and causing the cellular wall and cell contents disruption [117]. One of the notable aspects of this Ozone pretreatment method is that it can efficiently remove lignin compounds and partially eliminate hemicellulose compounds from feedstock without leaving behind any basic, toxic, or acidic byproducts [53], [118]. Further, a sufficiently high amount of O_3 can even mineralize the released cellular compounds [117]. The success of this method hinges on several key parameters, such as the size of the feedstock, the concentration of O_3 in the gas flow, and the moisture percentages in the substrates [18]. The potential of ozonolysis to improve BMP was shown by Cesaro and Belgiorno [117], who found that using 0.16 g O_3/g TS increased BMP by 37%. This ozonolysis chemical pretreatment is a promising, environmentally friendly approach to enhancing bio - methane yield, which showcases the innovative use of ozone (O_3) in the field of waste treatment and renewable energy.

3.3 Biological Technique

The biological pretreatment technique has received significant attention due to its ecological nature and effectiveness in enhancing feedstock breakdown [43]. This pretreatment technique uses microorganisms such as fungi and enzymes to pre-degrade complex organic waste slurries before they enter the digester [1], [119]. This pretreatment technique can break down lignocellulosic substrates and it also supports the removal of lignin compounds from lignocellulosic biomass, which are resistant to microbial degradation, thereby increasing the availability of fermentable sugar compounds for methanogenic bacteria [43], [120]. It is very sensitive to inhibition and requires a highly controllable condition in AD [43]. This method is particularly crucial for feedstocks with high lignocellulosic content, such as agricultural waste, MSW, etc. [64], otherwise it is a low efficiency technique for other substrates. Biological pretreatment techniques can broadly be classified into two categories: 1. Enzyme Pretreatment Technique, and 2. Fungal pretreatment.

3.3.1 Enzyme Pretreatment Technique

The effectiveness of enzyme pretreatment on lignocellulosic substrates depends on the type of enzymes used and the composition of the substrates [121]. This pretreatment involves the application of oxidative and hydrolytic enzymes, which help to generate co-microbial bacteria cultures and complex communities like fungi [1]. Hosseini Koupaie et al. [121] and Carrerre et al. [59] tested forty different enzymes and found that some, such as α-amylases, xylanases, endoglucanase, β-glucosidase, cellulases, peroxidases, proteases, pectinases, and laccases, significantly enhance methane yield. Enzymatic pretreatment is very effective in reducing the degree of cellulose polymerization [121]. Its key advantages over other pretreatment methods include minimal chemical requirements, low capital investment, and low energy consumption, which makes it one of the most attractive and effective environmentally friendly pretreatment techniques [121], [122], [123], [124]. The efficiency of the AD pretreatment technique is decided by the activities of microbial enzyme responses on the feedstock slurry [121], [125]. These microbial enzyme responses are significantly influenced by several factors, including temperature, substrate composition, HRT, pH levels, and the configuration of the digester [121], [125]. Each of these factors plays a critical role in optimizing the conditions necessary for effective microbial activity and subsequent biogas production. According to Aworanti et al. [1], the application of this pretreatment technique to solid cattle dung, combined with microbial culture in the AD process, led to a remarkable enhancement in methane production. Their study demonstrated that this method could result in a substantial 105% increase in methane yield. Furthermore, Lin et al. [123] conducted a study on the pretreatment of pulp and paper sludge with endoglucanase and laccase microbial co-culture bacteria before subjecting it to mesophilic AD process. The findings revealed a 34% enhancement in methane yield as a result of this pretreatment approach. Likewise, Frigon et al. [126] observed increases in methane production of 29% and 42% when switchgrass was pretreated with lignin peroxidase and manganese peroxidase enzymes, respectively. These increments highlight the effectiveness of enzyme-based pretreatments in breaking down complex organic waste, in that way enabling more efficient microbial digestion and enhanced methane production.

3.3.2 Fungal pretreatment

Fungal pretreatment is an effective and optimizing biological technique used to enhance AD processes. Typically, enzymes that facilitate the AD process are produced from various fungi, such as those belonging to the Trichoderma and Aspergillus genera [1]. To eliminate the costs and complexity associated with enzyme production, these fungi can be used directly as an alternative [106]. Moreover, combining these fungi with supplementary enzymes like lytic polysaccharide mono-oxygenases (LPMOs) can significantly enhance cellulose-fiber degradation [127]. Various types of fungi, including white-rot, brown-rot, and soft-rot fungi, are commonly employed for the degradation of lignin and hemicellulose compounds in substrates [18], [53], [128]. For instance, Muller and Trosch [129] biologically pretreated wheat straw using white-rot fungi and reported a 100% rise in the yield of raw biogas compared to the untreated wheat straw substrate. Similarly, Ghosh and Bhattacharyya [130] found that using brown-rot and white-rot fungi to pretreat bamboo substrate before the AD process resulted in biogas yield increases of 32% and 46%, respectively. Additionally, Mackuľak et al. [131] reported a 15% enhancement in methane yield from the AD process of grass and leaves pretreated with Auricularia auricula-judge. Rouches et al. [132] reported that wheat straw substrates utilized with Polyporus brumalis fungal pretreatment enlarged BMP by 45%. In contrast, Paul et al. [133] observed that the fungal pretreatment of agricultural biomass did not boost BMP. These findings highlight the potential of fungal pretreatment, indicating that this biological technique is still in the development stage.

3.4 Hybrid techniques

Hybrid pretreatment techniques mean combining physical, chemical, and biological pretreatment techniques and making hybrid techniques that can enhance the yield of raw biogas and BMP, diminish energy consumption, and lower costs [18], [134], [135]. Various types of hybrid technique are listed in Figure 3.1.

3.4.1 Thermochemical pretreatment

Combining thermal and chemical techniques as a pretreatment strategy enhances the AD process and increases the productivity of raw biogas. This pretreatment method reduces the particle size of the substrate [96], boosts VS reduction [136], and improves the solubility of COD [137]. The main chemicals used in thermo-chemical pretreatment include alkalis [138], acids [139], and ozone [68]. Passos and Ferrer [80] reported that applying the thermal alkali hybrid pretreatment technique (10 % NaOH at a specific temperature 100 °C and reaction duration of 5 minutes) on dairy cow manure substrate resulted in a 24 % rise in the yield of methane production when compared with the untreated substrates. Kaur and Phutela [140] observed that when the use of paddy straw as a substrate was subjected to thermochemical pretreatment using a combination of microwave and NaOH, there was a 55% upsurge in the yield of biomethane compared to the untreated feedstock.

3.4.2 Electrochemical pretreatment

The electrochemical pretreatment technique integrates both electrical and chemical methods to enhance the breakdown of substrates for the AD process and improve the efficiency of substrate degradation. This hybrid pretreatment includes various approaches such as electro-flotation, electro-deposition, electro-oxidation, and electro-coagulation [1]. Yu et al. [141] conducted a study where electrochemical pretreatment was applied to WAS using a pair of Ti/RuO_2 mesh plates as electrodes which facilitated effective electrochemical reactions. The result: a 63% increase in raw biogas yield compared to untreated sludge. Furthermore, Kumar et al. [135] reported that the combination of electrolysis and ultrasonic pretreatment on mixed microalgae substates led to an enhanced yield of biomethane during the AD process. The initial pretreatment using electrolysis helped in disintegrating the cellular structure of the microalgae, while the ultrasonic pretreatment further disrupted the feedstock, making it more accessible for microbial digestion. This combination of techniques proved to be highly effective in increasing the overall efficiency of methane production [135], [141].

3.4.3 Chemo-sonication pretreatment

The chemo-sonication pretreatment technique is an innovative method that combines the benefits of chemical and sonication pretreatment processes. This hybrid pretreatment technique effectively addresses the limitations of each individual method, resulting in a significant enhancement in the production of raw biogas [142]. Also, this hybrid method is environmentally friendly, which makes it a green sustainable option for biogas generation [143]. According to Panigrahi et al. [143], the chemo - sonication pretreatment technique is mainly effective in reducing the resistance of substrates, which is a major hurdle in the AD process for achieving high biogas production. Wang et al. [144] demonstrated the effectiveness of this hybrid technique, reporting a remarkable increase in biogas production by 67 % – 76 % after treating rice stalks with 2 % NaOH and subjecting them to ultrasonication at a frequency of 30 kHz for an hour.

3.4.4 Thermo - sonication pretreatment

The thermo-sonication pretreatment technique is an advanced method that combines the benefits of thermal and sonication pretreatment processes. Dhar et al. [145] found that using the thermo-sonication method to pretreat WAS, with appropriate heating and sonication energy led to a 30 % increase in bio-methane yield and also, a 29 % - 38 % reduction in VS. According to Hassan et al. [146], the thermal - sonication hybrid pretreatment technique combined with organic loading management was crucial for AD of goose manure, with 45 - 60 minutes of sonication at 28 kHz and an OLR of 2.9 (gm*VS) / (l*day) resulting in optimal bio-methane yield production of 282 (ml) / (gm*VS).

3.4.5 Biomechanical pretreatment

The biomechanical pretreatment technique is a hybrid approach that combines biological and mechanical methods to enhance the AD process and significantly increase bio-methane yield. Mechanical pretreatment increases the contact surface area for microbial action, facilitating greater interaction and breakdown of the substrate [26], [87]. Meanwhile, biological methods, such as enzymatic action or fungal treatment, help in the solubilization of the substrate, making it more accessible for microbial degradation [1], [119]. This synergistic effect of combining mechanical and biological pretreatments results in an extensive boost in raw biogas production. According to Mustafa et al. [49] mechanical milling combined with fungal pretreatment can significantly enhance biomethane and biogas yield. Pérez-Rodríguez et al. [147] found that using a combination of extrusion as mechanical and enzymatic pretreatment on corn cobs feedstock resulted in much higher biomethane production compared to untreated feedstock.

3.4.6 Mecho - sonication pretreatment

Mecho-sonication hybrid pretreatment combines two distinct physical techniques: mechanical pretreatment and the sonication technique. This hybrid method is also known as Mechanical – sonication pretreatment. This hybrid approach leverages the strengths of both techniques to enhance the effectiveness of the pretreatment technique. Research by Elbeshbishy and Nakhla [148] and Cesaro et al. [149] demonstrated the significant benefits of this hybrid pretreatment method. They found that subjecting food waste to a combination of grinding and sonication resulted in approximately a 94% increase in both raw biogas and biomethane yield. This significant improvement highlights the possible development of this pretreatment in the future for optimizing biogas as well as biomethane production. To provide a concise summary, **Table 5** presents an overview of the major pretreatment techniques, highlighting their mechanisms of action and reported effects on biogas yield.

Table 5 Overview of pretreatment techniques, mechanisms, and effect on biogas yield

Pretreatment Technique	**Mechanism of Action**	**Effect on Biogas Yield**
Physical (Sonication, Grinding, Thermal, Microwave)	Disrupts cell walls, reduces particle size, increases surface area for microbial attack	20–140% increase in methane yield (depending on substrate and conditions)
Chemical (Acid, Alkali, Oxidation, Ozone)	Hydrolyzes lignin/hemicellulose, enhances solubility, improves substrate accessibility	25–357% increase in methane yield; risk of inhibitory byproducts
Biological (Enzymatic, Fungal)	Uses microbial enzymes or fungi to degrade lignin and cellulose	15–105% increase in methane yield; eco-friendly but slower
Hybrid (Thermo-chemical, Chemo-sonication, Thermo-sonication, Bio-mechanical, etc.)	Combines strengths of physical, chemical, or biological methods for synergistic effect	30–94% increase in methane yield; cost and scalability vary

Chapter 4: Comparison of pretreatment techniques

Table 6 presents a comprehensive comparison of all physical, chemical, biological, and hybrid pretreatment techniques. These comparisons are based on five key criteria: hydrolysis rate, generation of inhibitory (toxic) compounds, operational cost, energy requirements, and BMP enhancement.

Table 6: Comparison of pretreatment techniques [1], [18], [26], [48], [52], [53], [54], [55], [59], [61], [74], [103], [105], [121], [123], [127], [128], [133], [142], [143], [150], [151], [152], [153], [154], [155], [156], [157], [158], [159], [160]

Pretreatment Techniques	Key Criteria				
	Hydrolysis Rate	**Inhibitory Compounds Generation**	**Operational Cost**	**Energy Requirements**	**BMP Enhancement**
Physical Technique:					
Sonication Technique	Very Fast	Yes	Very Expensive	Moderate	Effective
Pressure - Depressure Technique	Very Fast	Yes	Very Expensive	Very High	Moderate
Thermal Pretreatment Technique					
▪ Thermal – Induced	Very Fast	Yes	Very Expensive	Very High	Effective
▪ Steam Explosion	Very Fast	Yes	Very Expensive	Low	Effective
▪ Freezing And Thawing	Very Fast	Yes	Very Expensive	Very High	Moderate
▪ Liquid Hot Water	Very Fast	Yes	Very Expensive	High	Effective
Mechanical Pretreatment Technique	Very Fast	No	Very Expensive	Very High	Moderate
Microwave Irradiation Technique	Very Fast	Yes	Very Expensive	Very High	Very Effective
Chemical Technique:					
Oxidation Method	Fast	No	Very Expensive	High	Very Effective

Acid Method	Fast	Yes	Very Expensive	High	Very Effective
Alkali Method	Fast	Yes	Cost-Effective	High	Very Effective
Ozone Method	Fast	Yes	Very Expensive	High	Very Effective
Biological Technique:					
Enzyme Pretreatment Technique	Fast	No	Very Expensive	Very Low	Effective
Fungal Pretreatment Technique	Slow	No	Cost-Effective	Very Low	Less Effective
Hybrid Technique					
Thermo – Chemical	Fast	Yes	Cost-Effective	Moderate	Effective
Electro – Chemical	Fast	Yes	Cost-Effective	Moderate	Effective
Chemo – Sonication	Fast	Yes	Cost-Effective	Moderate	Effective
Thermo – Sonication	Fast	Yes	Cost-Effective	Moderate	Effective
Bio-Mechanical	Fast	Yes	Cost-Effective	Moderate	Effective
Mecho – Sonication	Fast	Yes	Cost-Effective	Moderate	Effective

Physical Pretreatment Techniques: These pretreatment techniques such as sonication and pressure-depressurization methods are very fast in hydrolysis rate and effective, but these pretreatments are associated with extreme operational costs and energy requirements. Thermal pretreatment techniques, including thermally induced, steam explosion, freezing and thawing, and liquid hot water, are also very fast in breaking down long chain polymers into shorter chains. However, these techniques tend to generate inhibitory compounds and have high operational and energy costs. Mechanical pretreatment is very fast in hydrolysis rate but at the same time, it is very expensive and energy intensive. Mechanical pretreatment of wheat straw (particle size reduced to <1 mm) under mesophilic digestion (37°C, 30-day HRT) improved methane yield by 49.3%

compared to untreated feedstock [94]. Similarly, microwave irradiation pretreatment applied to microalgae under continuous digestion resulted in ~60% higher BMP [59].

Chemical Pretreatment Techniques: These techniques include oxidation pretreatment, which is fast and does not generate inhibitory compounds, but it is very expensive, and energy concentrated. Acid, alkali, and ozone methods are rapid and effective in accomplishing the hydrolysis stage but also have high operational prices. Alkali pretreatment is somewhat more cost-effective but still has high energy requirements.

Biological Pretreatment Techniques: Biological techniques offer several important benefits. Enzyme pretreatment is fast, does not generate inhibitory compounds, and is effective, although it can be very expensive. Fungal pretreatment is slower and less effective in the generation of BMP but is cost-effective and requires very low energy.

Hybrid Techniques: Hybrid techniques combine fundamentals of physical, chemical, and biological pretreatment, which include thermo-chemical, electro-chemical, chemo-sonication, thermo-sonication, biomechanical, and mecho-sonication methods. These methods are generally fast and effective for the generation of raw biogas as well as BMP. However, their operational and energy costs vary, with some being more cost-effective and others having moderate to high energy requirements. This variation is basically due to different types of substrate composition and its characteristics.

While pretreatment methods have demonstrated substantial improvements in methane yield, their practical application requires careful consideration of limitations. High energy requirements make physical techniques such as sonication and microwave treatment less viable for large-scale biogas plants. Chemical pretreatments, though effective in enhancing biodegradability, often involve significant costs for reagents and may generate inhibitory byproducts or secondary environmental burdens. Biological approaches are environmentally friendly but generally slower and less predictable, which restricts their commercial adoption. Hybrid pretreatments can offer great benefits; however, they increase process complexity and require comprehensive techno-economic assessments before large-scale implementation. Therefore, future research should focus not only on maximizing yield but also on addressing cost-effectiveness, scalability, and environmental sustainability to ensure the practical viability of these methods.

Thermal pretreatment alone generally results in 20–60% higher methane yields, while chemical methods such as acid or alkali pretreatment achieve 25–120% improvements depending on the substrate. Hybrid thermo-chemical pretreatment of dairy cow manure (10% NaOH at 100 °C, 5 min) increased methane yield by 23.6% compared to untreated manure [80]. In contrast, microwave-assisted alkali pretreatment of paddy straw resulted in a much higher 55% methane yield increase [140]. Such wide variability highlights the strong influence of both feedstock type and process parameters on performance. Likewise, while sonication and microwave pretreatments can reduce hydrolysis time by 10–25%, chemo-sonication approaches have reported reductions in HRT of up to 35–40%. These findings confirm that hybrid approaches not only maximize yield but also accelerate digestion kinetics, although further techno-economic and environmental assessments are required for large-scale adoption. A comparative overview of the major pretreatment techniques, along with their respective advantages and disadvantages, is presented in **Table 7**.

Table 7 Comparative summary of pretreatment methods highlighting their main advantages and disadvantages in relation to methane yield and process feasibility.

Pretreatment Method	Advantages	Disadvantages
Physical (e.g., milling, sonication, microwave)	Rapid hydrolysis; improves surface area; effective methane yield increase (20–140%).	High energy demand; costly equipment; risk of inhibitory byproducts (microwave).
Chemical (acid, alkali, oxidation, ozone)	Strong lignin breakdown; effective for lignocellulosic feedstock; methane yield up to 357%.	Risk of inhibitor formation; expensive reagents; environmental concerns.
Biological (enzymes, fungi)	Eco-friendly; low energy input; good lignin removal (15–105% yield increase).	Slow process; sensitive to conditions; limited scalability.
Hybrid (chemo-sonication, thermo-chemical, mecho-sonication)	Synergistic effects; faster hydrolysis; high methane yield (30–94%, sometimes >150%).	Process complexity; moderate-to-high costs; scalability challenges.

Chapter 5: Government Policy Perspectives & Economic Considerations

The adoption of pretreatment technologies for anaerobic digestion (AD) has broader policy and economic implications that extend beyond laboratory performance. Governments can play a critical role in accelerating the integration of pretreatment-enhanced biogas into renewable energy portfolios. Policy instruments such as subsidies, tax incentives, carbon credits, and long-term offtake agreements can help mitigate the initial costs of pretreatment technologies and make biogas plants more attractive for investors. In India, for example, the Ministry of New and Renewable Energy (MNRE) has launched the National Bioenergy Programme (2021–26) and schemes such as SATAT and GOBAR-Dhan, which provide financial assistance and market support for biogas and compressed biogas projects. Several state governments, including Gujarat and Uttar Pradesh, have complemented these with additional capital subsidies, tax exemptions, and infrastructure support to encourage large-scale deployment of biogas systems [161].

While this review primarily focuses on the technical performance of pretreatment methods, we recognize that their economic feasibility is equally critical for large-scale adoption. A detailed techno-economic analysis was not within the scope of this study; however, it remains an essential area for future investigation. The cost-effectiveness of pretreatment depends strongly on factors such as energy input, chemical usage, and scalability to different feedstocks. Future research should therefore integrate techno-economic and life-cycle assessments to complement laboratory findings and guide policymakers and practitioners toward the most sustainable deployment strategies.

Chapter 6: Conclusion

This review highlights the importance of pretreatment strategies in overcoming key bottlenecks in anaerobic digestion and positioning biogas as a reliable contributor to renewable energy systems. By improving the efficiency and stability of methane generation, these methods have implications well beyond laboratory experiments—they can accelerate the transition to cleaner energy portfolios, reduce reliance on fossil fuels, and support circular waste-to-energy solutions. Pretreatment can enable the large-scale valorization of agricultural residues, municipal solid waste, and wastewater into energy, thereby addressing both energy security and waste management challenges. For emerging economies, the adoption of such strategies could help meet rising energy demands while aligning with global decarbonization and climate targets.

Physical pretreatment techniques, such as sonication and thermal pretreatment, are noted for their high effectiveness in disrupting substrates and accelerating the AD process, but they come with substantial operational costs and energy demands. Chemical methods, including oxidation and alkali treatments, provide rapid and effective enhancements to biogas production but often introduce inhibitory compounds that can hamper the overall process. Biological pretreatment methods, while generally more cost-effective and environmentally friendly, tend to be slower and less effective in breaking down complex organic substrates. The comparative analysis of these techniques reveals that there is no single, universally superior method for AD process intensification. Instead, the optimal pretreatment strategy must be selected based on the specific operational conditions, goals, and constraints of the AD system. This in-depth review should form a strong basis towards a clear pathway to enhance biogas production, contributing to the broader goal of renewable, sustainable, and green energy development.

Future research should focus on techno-economic assessments of hybrid pretreatment methods to determine their feasibility for large-scale implementation. In addition, more studies are needed to evaluate the effectiveness of these approaches across diverse feedstocks such as municipal solid waste, agricultural residues, and algal biomass. Research into optimizing process parameters for energy efficiency and minimizing inhibitory byproducts will also be crucial. These directions will help ensure that pretreatment strategies are not only effective but also sustainable, cost-efficient, and applicable under real-world operating conditions.

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Warm Regards,

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