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Biomass Pretreatment for Enhancement of Biogas Production

Tamilarasan Karuppiah and Vimala Ebenezer Azariah

Abstract

Biomass is a renewable energy source developed from living or recently living plant and animal materials, which can be used as fuel. The main components present in biomass are polymers such as carbohydrate, protein, cellulose, lignin and fat. Biogas is produced when the biomass is anaerobically degraded by microorganisms. The process of anaerobic digestion (AD) takes place in four steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. The hydrolysis step is rate limiting due to the presence of complex polymers in biomass. Pretreatment is a process in which the biomass is made ready for microbial attack. This pretreatment can be physical operations such as communition, irradiation etc.; chemical treatment with alkali, acids, wet oxidation etc.; biological pretreatment, by fungi or enzymes; or a combination of these processes. During the pretreatment process, the compact structure of biomass is disrupted and exposed which.

Keywords: biomass, biogas production, anaerobic digestion, pretreatment, technologies

1. Introduction

Rapidly increasing energy demands worldwide has resulted in tremendous depletion of fossil fuel resources. This makes it necessary to find alternative energy sources which have a minimum impact on the environment. In this context, biogas is one of the sustainable energy sources that can be produced from many types of biomass including waste. AD technology is one of the most promising technologies, having the potential to convert various biomass into methane-rich biogas, a carbon-neutral alternative to fossil fuels. In addition, AD technology has a number of benefits including solids reduction, decreased odor, reduced greenhouse gas emissions, and increased income from non-market benefits compared to conventional waste treatment systems [1, 2]. In Germany, which is the leading country in this field, greater than 50% of the biogas potential results from energy crops treated in over 7000 biogas plants [3]. AD has wide application in sludge stabilization due to its low cost, energy recovery and minimized biosolids production.

AD system utilizes anaerobic microorganisms to convert the organic matter in the biomass, into biogas in an oxygen free environment. Biogas is the main byproduct of AD and contains about 60% methane by volume. Digestate is produced as a byproduct, which after an appropriate treatment can have agricultural

applications as fertilizer [4]. It reduces organic matter to more stable solids by complex biochemical reactions. There are three consecutive steps of biological process in AD. The first step involves hydrolysis of complex organic matter into simpler compounds. The second step is the acidogenesis, which involves conversion of these organics to form organic acids and hydrogen. The final step is methane and carbon dioxide production from organic acids and hydrogen, by *methanogens*. The high methane content makes biogas a useful fuel that can displace natural gas in pipelines or be converted to electricity and heat. AD typically require long residence times, as certain anaerobic microorganisms have slow rate of growth. Long residence times lead to large volumes of tanks. Therefore, to improve digestion efficiency, the most efficient approach is to disrupt the chemical bonds in the material prone to hydrolysis [5]. Other factors limiting its performance are slow hydrolysis, low biodegradability, inhibition due to toxic compounds and toxic intermediates formed and poor methanogenesis. To overcome this recalcitrant property and to improve the degradation rate, a pretreatment prior to the AD process is introduced. Thus the goal of a pretreatment is to open up the structure of the substrate, making it more accessible for enzymatic attack [6] which aids in increasing biogas yield. The effects of various pretreatment methods highly differ, depending on the characteristics of the substrates and the pretreatment type. Recently, a lot of interest has been devoted to biomass disintegration and solubilization techniques in order to overcome the biological limitations of anaerobic digestion. The pretreatment techniques include mechanical treatment [7], ultrasonic treatment [8, 9] and biological hydrolysis with enzymes [10–12], alkaline treatment [13], oxidative treatments using ozone [14, 15], microwave irradiation [5, 16, 17], thermal treatment [18] thermochemical [19], sono-thermal [20–22] etc.
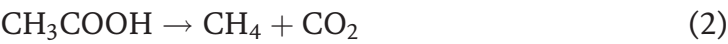
2. Microbiology of anaerobic digestion

AD process is mediated through four main steps—hydrolysis, acidogenesis, acetogenesis and methanogenesis. These are carried out by a consortium of microorganisms: acidogenic bacteria, acetogenic bacteria and methanogenic bacteria [23]. The microbial community of the anaerobic process is very complex. There are two prokaryotic kingdoms that closely interact with each other: *Bacteria* and *Archaea*. The first step involves hydrolysis of complex organic matter into simpler compounds. In the second step, the acidogenesis of these organics take place to form organic acids and hydrogen. In the final step, methane and carbon dioxide are produced from organic acids and hydrogen by *archael* methanogens.

Figure 1 summarizes the overall process of AD. Organic matter consists of particulate, water-insoluble polymers such as carbohydrates, lipids and proteins. Insoluble polymers cannot penetrate cellular membranes and are therefore not directly available to the microorganisms. During hydrolysis, appropriate strains of hydrolytic bacteria excrete hydrolytic enzymes [23] which break up the insoluble polymers to soluble mono and oligomers. Carbohydrates are converted to sugars, lipids are broken down to long-chain fatty acids and proteins are split into amino acids [24]. These soluble molecules are converted by acidogens to acetic acid and other longer volatile fatty acids, alcohols, carbon dioxide and hydrogen on acidogenesis. The foremost acids produced are acetic acid (CH_3COOH), propionic acid (CH_3CH_2COOH), butyric acid ($CH_3CH_2CH_2COOH$), and ethanol (C_2H_5OH). Other acid formers are *Clostridium, Peptococcusanerobus, Lactobacillus*, and *Actinomyces*. The next process is acetogenesis during which, the longer volatile fatty acids and alcohols are oxidized by proton-reducing acetogens to acetic acid and hydrogen. An acetogenesis reaction is shown below:

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 \qquad (1)$$

In the last step of the process, methanogens use acetic acid or carbon dioxide and hydrogen, to produce methane and carbon dioxide. For mesophilic bacteria, the optimal methane production rate is mostly reached at 35–37°C. The thermophilic methanogens differ from the mesophilic one and their maximum methanogenic activity is reached at about 55°C. A thermophilic digestion process can sustain a higher organic loading compared to a mesophilic one. But the thermophilic process produces gas with a lower methane concentration [25] and is more sensitive to toxicants [26]. Methanogens are also sensitive towards changes in temperature than the other species, because of their slower growth rate in the reactor environment. Methanogenesis occurs at neutral pH- in the range of 6.5–7.5, although optimum lies at pH 7.0–7.2 [26]. If, for example, a temperature shift affects the methanogens negatively, there can be a build-up of volatile fatty acids (VFAs). This lowers the pH which further affects the methanogens in a negative way which leads to a vicious circle of negative feedback. The methanogenesis reactions can be expressed as follows [27] in Eqs. (2)–(4):

$$CH_3COOH \rightarrow CH_4 + CO_2 \qquad (2)$$
$$2C_2H_5OH + CO_2 \rightarrow CH_4 + 2CH_3COOH \qquad (3)$$
$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \qquad (4)$$

The digestion efficiency and its stability can vary significantly depending upon the wastewater characteristics and type and design of the treatment system. The longer a substrate is kept under proper reaction conditions, the more complete its degradation will become. Longer retention time demands the provision of reactor with large volume for a given amount of substrate to be treated. On the other hand,
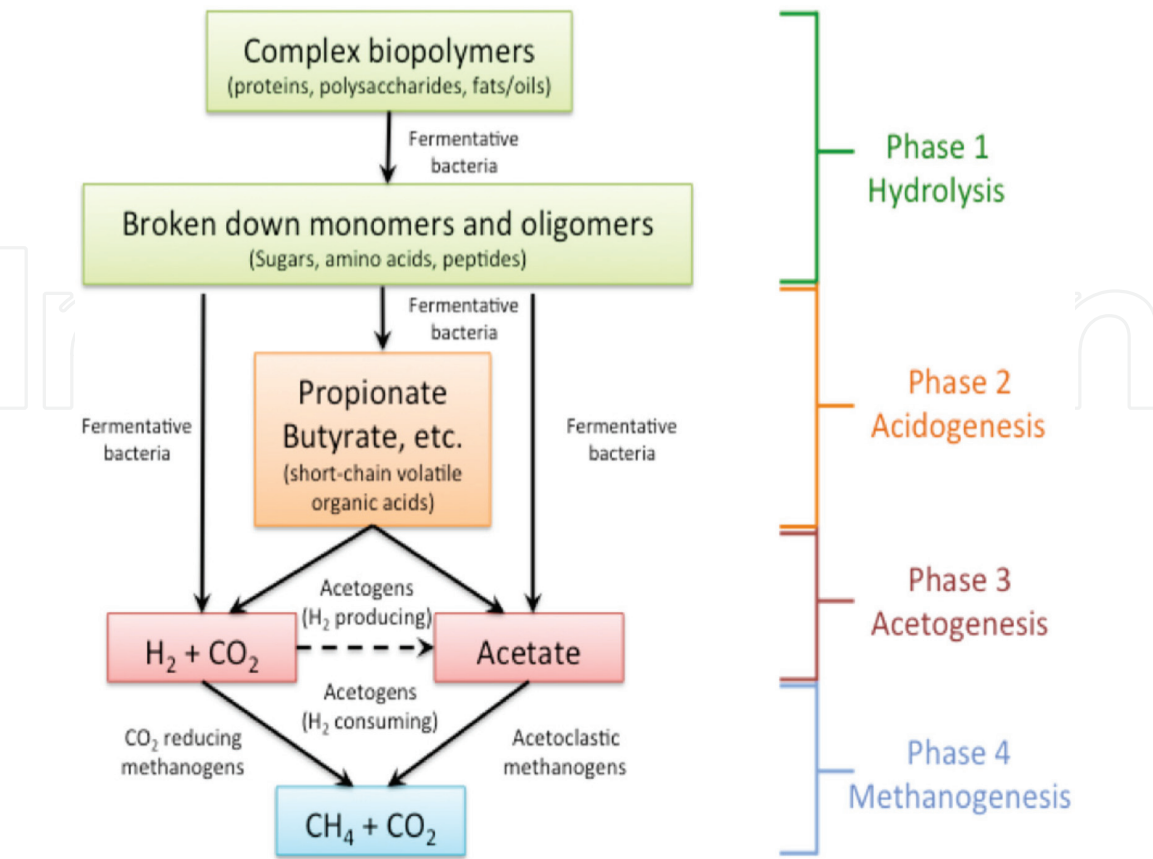

Figure 1.
Schematic representation of anaerobic digestion (source: https://www.e-education.psu.edu/egee439).

with shorter retention time washout of microorganism takes place with a lower overall degradation [25]. Therefore, these two effects have to be balanced in the design of AD for the efficient and proper operation of the full scale reactor. This needs operation of AD through skilled supervision for optimal performance.

3. Need for pretreatment

Several renewable matters have been tried for biogas production which are classified into crop biomass such as maize, wheat, barley, sweet sorghum, etc.; organic wastes such as municipal solid waste, municipal and industrial wastewater sludge, animal manure, and residues from various processing; energy crops like sunflower, rape, jatropha, etc.; crop residues which include banana stem, barley straw, rice straw, softwood spruce, etc.; and non-conventional biomass like glycerol, microalgae, etc. [28–34]. **Figures 2–4** show the effect of pretreatment of lignocellulosic, sludge and macroalgal biomass respectively.

The diverse composition of lignocellulose biomass and the interactions between fractions make its structure very complex and resistant to deconstruction. Cellulose and hemicellulose are polysaccharides that can be hydrolyzed to simple sugars. Lignin which acts as a support to the cell structure, embedding cellulose and hemicellulose, hinders the susceptibility to microbial attack during hydrolysis process [35]. The aim of pretreatment is to break the lignin layer that protects the cellulose and hemicellulose, in order to make the biomass more accessible for digestion [6]. Pretreatment also helps to decrease the crystallinity of cellulose and to increase the porosity. Furthermore, biomass such as fruit wastes is easily degraded but result in low yield due to the presence of inhibitors.

Keratin, which is present in horns and feathers, is an insoluble protein in which the polypeptides chain is tightly packed and highly cross-linked with disulfide bonds, hydrogen bonds, and hydrophobic interactions [36]. This insoluble protein is extremely resistive to the proteolytic enzyme action, which is a major hindrance in the biological processing of these wastes. For such biomass, if the crosslinking between the polypeptides chain breaks, the keratin becomes more accessible and easier to digest. Contrarily, while keratin-rich waste is pretreated using a strong
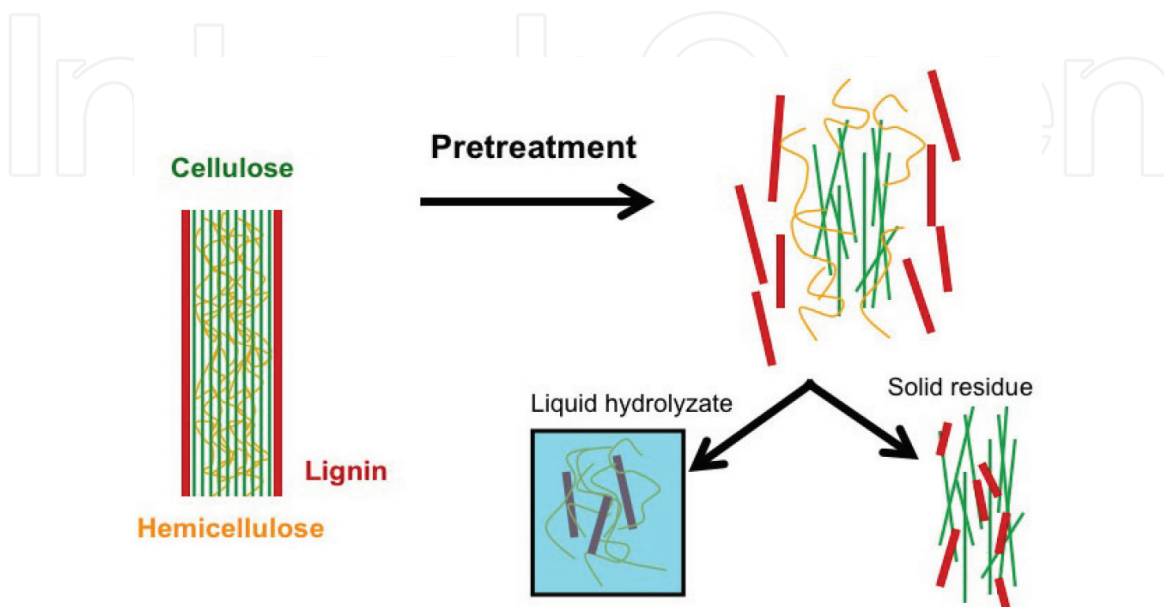

Figure 2.
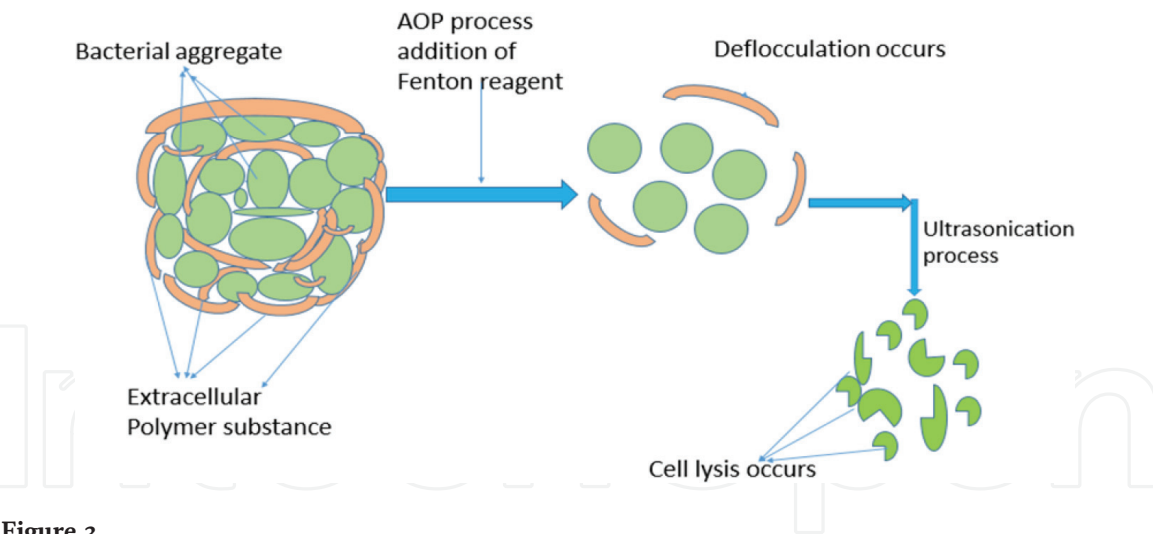*Effect of pretreatment on lignocellulosic biomass (source: https://www.e-education.psu.edu/egee439/node/653).*

Figure 3.
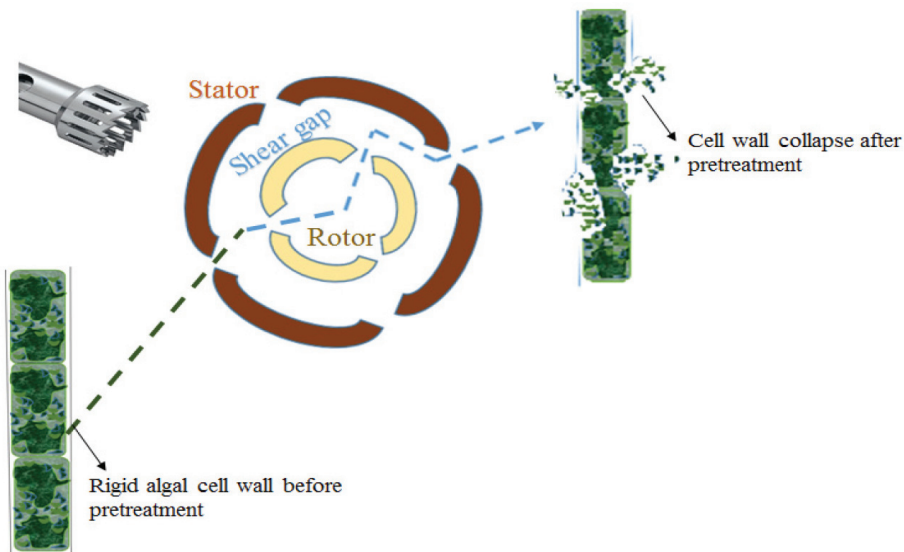*Effect of pretreatment on sludge biomass.*

Figure 4.
Effect of pretreatment of macroalgal biomass.

acid, alkali, or other harsh physicochemical methods, severe degradation and destruction of the keratin occurs [37].

Activated sludge, a bio product of aerobic wastewater treatment, can be a better raw material for generating energy because of its high organic content [38]. Secondary wastewater sludge consists of numerous microbial cells, the cell walls of which act as barriers against exo-enzyme degradation. Besides microbial cells, exocellular polymeric substances (EPS) comprise a major organic fraction in activated sludge floc structure and binding mechanisms of EPS to cations appear to be a significant factor determining the digestibility of activated sludge. Hence hydrolysis becomes the rate-limiting step and degree of degradation achieved is limited to 30–35% chemical oxygen demand (COD) reduction in conventional anaerobic sludge treatment [23]. Pretreatment of sludge is required to rupture the cell wall and to facilitate the release of intracellular matter into the aqueous phase, which improves the biodegradability thereby enhancing the AD with lower retention time and with higher biogas production [20].

The macroalgal cell envelope made of thick and hard layer composed of complex proteins and carbohydrates with more mechanical power and high chemical resistance, restricts the attack of the biopolymers by methanogenic bacteria during

AD [39]. Pretreatment leads to improvement in the liquefaction process, enhancing the biopolymer release [28]. Several pretreatment methods have been reported in detail, aiming to make these biomass viable to digestion by microorganisms, and increase the biogas yield. It is necessary to carry out the pretreatment at mild conditions to prevent excessive sugar degradation.

Several pretreatment processes such as ball mill [40], microwave irradiation [2], sodium hydroxide [13], steam explosion [41], ultrasonic [42], biological [43], ozonation [14] have been shown to enhance biodegradability of biomass by promoting the hydrolysis process. Since most available articles are addressed based on pretreatment of lignocellulosic biomass, this chapter is mainly focused towards sludge pretreatment.

4. Pretreatment technologies

The lower hydrolysis rates during conventional AD process, results in higher hydraulic retention time (HRT) in the digester and larger digester volume, constitutes the prime drawbacks of the conventional AD [6]. The non-availability of the readily biodegradable, soluble organic matters and lower digestion rate constantly necessitates the pretreatment of sludge. Pretreatment of biomass enhances the AD, with lower retention time and with higher biogas production [17]. With the advancements in various pretreatment techniques like thermal, chemical, mechanical, biological and physical and several combinations such as physicochemical, biological–physicochemical, mechanical–chemical and thermal–chemical, biodegradability of sludge can be enhanced by several orders. Extensive research has been carried throughout the world to establish the best economically feasible pretreatment technology to enhance the digestibility of biomass [12].
Tables 1 and **2** show the specific energy consumed and methane yield with various chemo-mechanical and physico-chemical pretreatment.

4.1 Physical

In physical pretreatment, the structure of the biomass gets altered and the size of the particles reduced, by the application of physical force. This leads to an increase in the surface area of the particles thereby making it susceptible to microbial and enzymatic attacks, which enhances the AD process for methane production [61]. Physical pretreatment may be done by employing microwave irradiation, sonication, mechanical beating, deflaking, dispersing, extruding, refining, milling, and cavitation etc. [62].

4.1.1 Milling

Milling pretreatment is carried out, especially for lignocellulose and algal biomass to reduce the size of the substrate to break open the cellular structure, and improve their bio accessibility to the cell tissues, by increasing the specific surface area of the biomass [40]. Particle size reduction not only increases the rate of enzymatic degradation, but also reduces viscosity in digesters thus making mixing easier and can reduce the problems of floating layers. For effective hydrolysis of lignocellulose, beta particle size of 1–2 mm has been recommended [63]. Using three batch reactors, Motte et al. [40] demonstrated, treating straw particle milled to different sizes 0.25 mm, 1 mm and 10 mm followed during 62 days. They achieved the highest methane production for straw with 10 mm particle size (192 ± 25 Nm L/g VS) which was associated with a straw biodegradability of 43%.

S. No.	Name of the pretreatment	Specific energy consumed (KJ/kg TS)	Solubilization achieved (%)	Biomethane yield	References
1	Disperser + alkali	4544	24	1391 ml	Rani et al. [21]
2	Thermo chemo disperser	3360.94	18.6	0.455 L/g VS	Kavitha et al. [44]
3	Chemo disperser	5013	20	0.522 L/g VS	Poornima Devi et al. [45]
4	Sono alkaline	4172	59	0.108 ml/g VS removed	Rani et al. [46]
5	Thermo chemo sonic	5290.5	27	0.413 g COD/ g COD	Kavitha et al. [47]
6	Citric acid + ultrasonic	171.9	22.7	0.435 L/g VS	Gayathri et al. [29]
7	Fenton + ultrasonic	641	34.4	0.3 g COD/g COD	Kavitha et al. [48]
8	Thermo chemo sonic	5500	35	0.60 g COD/g COD	Kavitha et al. [49]
9	Disperser + microwave	18,000	22	0.28 g COD/g COD	Kavitha et al. [50]
10	Chemo mechanical	7377	38	50 ml/g VS removed	Kavitha et al. [51]
11	Sonic mediated biological	2.45	23	0.19 d1	Kavitha et al. [52]
12	Chemo thermo disperser	174	60	0.84 g COD/g COD	Kavitha et al. [43]
13	Surfactant sonic	5120	24.7	0.24 g/g COD	Ushani et al. [53]
14	Chemo disperser	3312.6	15	0.14 g COD/g COD	Tamilarasan et al. [28]
15	Surfactant + sonic	5400	26	0.6 g/g COD	Santhi et al. [54]
16	Disperser + bacterial	9.5	22.4	0. 279 g COD/ g COD	Banu et al. [55]
17	Ultrasound + microwave	16,700	33.2	0.3 L/g COD	Kavitha et al. [56]
18	Surfactant + sonic	9600	23.9	0.239 g/g COD	Tamilarasan et al. [57]

Table 1.
Specific energy consumed and methane yield with various chemo-mechanical pretreatment.

4.1.2 Cavitation

The most frequently applied cavitation techniques include acoustic cavitation, which is produced by passing ultrasonic waves through the liquid medium and hydrodynamic cavitation produced using hydraulic systems. In acoustic cavitation, microbubbles called cavitation were developed when the ultrasound waves propagate in a liquid medium, due to a repeating pattern of compressions and

S. No.	Name of the pretreatment	Specific energy consumed (KJ/kg TS)	Solubilization achieved (%)	Biomethane yield	References
1.	Microwave	1844	18.6	0.162 ml/g VS removed	Rani et al. [33]
2.	Microwave + citric acid	14,000	31	0.615 L/g VS	Ebenezer et al. [38]
3.	Microwave + surfactant	14,000	28	0.47 L/g VS	Ebenezer et al. [58]
4.	Microwave + H_2O_2	18,600	56	0.323 L/g VS	Eswari et al. [59]
5.	H_2O_2 + microwave	18,910	46.6	250 ml/g VS	Eswari et al. [60]
6.	Thermo ozone	141.02	30.4	0.32 g COD/g COD	Kannah et al. [1]

Table 2.
Specific energy consumed with various physico-chemical pretreatment.

rarefactions. These cavitation expand to unstable size, and then rapidly collapse resulting in temperatures up to 5000 K and pressures up to 180 MPa. The rapid collapse of a numerous microbubbles generates powerful shear forces in the surrounding liquid, which damages the cell walls of microorganisms [21, 53]. However, higher sonication power level is reported to adversely affect the pretreatment process. At higher power level, bubbles are formed near the tip of the ultrasound transducer, which hinders the transfer of energy to the liquid medium [64].

In the ultrasonic pretreatment study on waste activated sludge (WAS), Apul & Sanin [7] investigated an improvement in anaerobic biodegradability at 15 min of sonication. They achieved an increase in daily biogas production and methane production by 49 and 74% respectively compared to control in semi continuous reactors at a solid retention time (SRT) of 15 days and organic loading rate of 0.5 kg/m³ d. Zeynali et al. [42] studied the efficiency of ultrasonic pretreatment in improving biogas production from fruits and vegetable waste. They adopted three sonication times of 9, 18, 27 min operating at 20 kHz and amplitude of 80 μm on the substrate. The highest methane yield they obtained was at 18 min sonication with specific energy 2380 kJ/kg TS (Total solids) for a 12 d batch period, while longer exposure to sonication led to lower methane yield. The energy content of the biogas obtained by them was twice that of input energy for sonication. Alzate et al. [65] reported that, the sonication applied to macro algae at a specific energy input of 75 MJ/kg TS produced just 20% of the methane production. Upon increasing the specific energy to about 100–200 MJ/kg TS, they reported an increase in the methane production rate between 80 and 90%.

In hydrodynamic systems, cavitation is generated by forcing fluid flow through cavitating devices, where pressure substantially drops. Many microbubbles formed as a consequence of this pressure drop subsequently collapse. The collapse of the cavitation, results in release of large magnitudes of energy which helps in dissolution of biomass and makes it more suitable for subsequent bacterial decomposition, improving biogas yield during the AD process [66]. They investigated the application of hydrodynamic cavitation (HC) for the pretreatment of wheat straw with an objective of enhancing the biogas production. They observed the methane yields of 31.8 ml with untreated wheat straw, 77.9 ml with HC pre-treated wheat straw and a maximum yield of 172.3 ml with the combined pre-treatment using KOH and HC.

4.1.3 Microwave irradiation

During microwave irradiation the destruction of the microbial cells is caused by the disruption of the chemical (hydrogen) bonds in the cell walls and membranes, by polarized parts of macromolecules aligning with the poles of the electromagnetic field, which results in denaturation. Microwaves can induce an athermal effect in addition to their thermal effect due to dipole orientation, which results in possible breakage of hydrogen bonds and subsequently leads to the disintegration of the floc matrix [17]. They observed that, microbial cells exposed to MW showed greater damage at similar applied temperatures compared to conventional heating. Rincón et al. [67] studied the effect of a MW pre-treatment on olive mill solid residue to enhance its anaerobic digestibility. They carried out the experiment at a power of 800 W and temperature 50°C and observed a maximum methane yield of 395 ± 1 ml CH_4/g VS for an applied specific energy 7660 kJ/kg TS. Beszédes et al. [16] focused on the effects of MW irradiation at different power levels on biodegradation and subsequent AD of sludge from the dairy and meat industry. Compared to their results obtained from conventional heat treatment of the same sludge, the MW treatment proved to increase the methane yield.

4.1.4 Extrusion

In extrusion pretreatment, the biomass is allowed to experience heat, compression and shear force, which creates physical damage and chemical alterations of biomass cells while passing through the *extruder*. The extruder arrangement consists of single or twin screws that spin into a tight barrel, which is equipped with temperature control. When a biomass material passes through the barrel, it is exposed to friction and vigorous shearing causing an increase in temperature and pressure. When it exits the finishing end, the biomass material experiences a pressure release, which causes structural changes in the processed biomass enabling easy digestion in the subsequent step [61].

Maroušek [68] evaluated extrusion parameters of pelleted hay for maximal cumulative biogas production, and reported that, at optimal conditions of pressure 1.3 MPa, reaction time 7 min, and 8% dry matter, the maximal biogas production was 405 m^3/ton TS (with 52.3% methane), which was about a 33% increase over the biogas yield of control. Novarino and Zanetti [69] employed extrusion pretreatment to improve biogas production from the organic fraction of municipal solid waste, resulting in a biogas yield of 800 L/kg VS containing about 60% methane content.

4.2 Thermal

Thermal pretreatment improves hydrolysis, with increased methane yield during subsequent anaerobic digestion. A wide range of temperatures has been studied, ranging from 60 to 270°C, but temperatures above 200°C have been found responsible for the production of recalcitrant soluble organics or toxic/inhibitory intermediates during the pretreatment process [70]. Many studies employed at an optimum thermal range of 160–180°C for hydrolysis of wastewater sludge have proved an increase in methane yield during AD. Higher temperatures lead to a sharp reduction in biodegradability of sludge hydrolysate, due to production of recalcitrant soluble organics or toxic/inhibitory intermediates during the process [71]. The effect of thermal treatment of anaerobic sludge on the disintegration of the remaining organic fraction was evaluated by Borges and Chernicharo [18]. At 75°C, they observed an increase of 30–35 times increase in the concentrations of protein,

carbohydrate, lipid and COD and an increase of 50% in the biogas production, thus characterizing a higher biodegradability of the remaining organic fraction.

4.3 Chemical

4.3.1 Acid

Acid pretreatment causes sludge disintegration and cell lysis which releases the intracellular organics, which become more bioavailable and thus increases the rate and efficiency of the digestion process [17]. In lignocellulosic biomass, the pretreatment results in the disruption of the Van der Waals forces, hydrogen bonds and covalent bonds that hold together the biomass components, which consequently causes the breaking of hemicellulose and the reduction of cellulose [72]. Devlin et al. [73] showed the improved effects of HCl pretreatment at pH 2 on subsequent digestion of WAS. In semi-continuous digestion experiments conducted for 12 day hydraulic retention time at 35°C, they found a 14.3% increase in methane yield compared to untreated WAS. Taherdanak et al. [74] used dilute sulfuric acid pretreatment, to improve the biomethane production from wheat plant under mesophilic anaerobic digestion. At 121°C, they obtained a maximum methane yield of 15.5% higher than that of the untreated wheat plant after pretreatment for 120 min.

4.3.2 Alkali

The mechanism of alkaline pretreatment mainly induces swelling of particulate organics at elevated pH, enabling the biomass cellular substances more susceptible to enzymatic action [24]. The complex cell gets damaged by the hydroxyl anions available in the alkali. In macroalgae, it enhances hydrolysis of RNA, organic liquefaction of proteins and saponification [28]. In lignocellulosic biomass, it causes swelling, delignification and de-esterification of intermolecular ester bonds. With the disintegration of the bonds the porosity and internal surface area of the biomass increases, the degree of polymerization and crystallinity decreases. This makes it more accessible for enzymes and bacteria [6]. Regarding WAS, at higher pH, the microbial cell walls are broken and intracellular material is released into the liquid phase.

Studies were explored by Banu et al. [13] to evaluate the advantage of sodium hydroxide (NaOH) for its higher sludge solubilization potential and lime. They conducted experiments at a fixed alkali strength (35 meq/l) and varying concentration of NaOH and lime to demonstrate the role of alkalis in solubilizing sludge. The highest solubilization they achieved, was at an optimum dosage of NaOH and lime 1.6 and 0.7 g/l respectively at time 3 h. Sambusiti et al. [75] investigated the effect of alkaline (NaOH) pretreatment on ensiled sorghum forage in semi continuous digesters. They observed that pretreatment with 10 g NaOH/100 g TS increased the methane yield by 25% compared to untreated sorghum without experiencing any inhibition of the process.

4.3.3 Oxidative

Wet air oxidation is a pretreatment option that enhances contact between molecular oxygen and organic matter for the complete degradation of organic compounds into carbon dioxide and water. In order to achieve this, high temperature (and subsequently high pressure) conditions are required [22]. The correspondingly high pressure required is to maintain the high temperature conditions,

as well as to help increase the concentration of dissolved oxygen, and thus oxidation rate. Chandra et al. [76] employed wet air oxidation to enhance the biodegradability of the complex biomethanated distillery effluent. They reported an enhanced biogas yield of pretreated effluent up to 2.8 times higher than the untreated effluent with methane content up to 64.14%.

4.3.4 Ozonation

Ozone is a strong oxidant and hence powerful in oxidizing substrates. It has potential to degrade lignin in diverse feedstocks. It reacts with the polysaccharides, proteins, lipids and other recalcitrant compounds and transform them into biodegradable molecules. The ozonation process can result in efficient cell wall rupture and release of more soluble and easily biodegradable organics, which can be easily accessed and assimilated by anaerobic microorganisms. Thus it leads to improvement in the AD process [15].

AD of ozone pretreated excess sludge was studied by Goel et al. [14] through long-term operation of laboratory-scale reactors. They found that ozone pretreatment was effective in partially solubilizing the sludge solids and leading to subsequent improvement in anaerobic degradability. The extent of solubilization and digestion efficiency depended on the applied ozone doses. At 0.05 g O_3/g TS, the AD efficiencies improved to about 59% as compared to 31% for the control run. Different process indicators like specific methane production and ammonia concentration in the reactor, also specify the higher observed solid degradation rates for ozonated sludge.

4.4 Biological

The biological mediated pretreatment process is based on the function of multiple form of heterotrophic microbes. Complex biopolymers such as protein and carbohydrate can be transformed into simpler end products due to the action of various enzymes produced by the bacteria. The significance of biological pretreatment lies in the fact that is solubilizes the organic compounds present in the biomass with minimum energy, with no severe changes in substrate environment. Biological pretreatment is done with or without enzyme addition some of which can be produced endogenously by microorganisms present in the sludge. Some of the enzymes like protease, lipase, cellulase, alpha-amylase and dextranase [11] can effectively improve the hydrolysis rate and release of biopolymers to a large extent. Contrarily, these enzymes are more costly and difficult to preserve. Bonilla et al. [77] evaluated the potential for enzymatic pretreatment of pulp mill biosludge with protease from *B. licheniformis* for biodegradability. Carrying out BMP test, they arrived at a maximum improvement of 26% in biogas yield.

Saranya et al. [10] studied the impacts of phase separated disintegration pretreatment using calcium chloride ($CaCl_2$) and bacteria. For their study a pH of 6.5, temperature of 40°C and treatment period of 42 h were the optimum conditions for pretreatment. In the initial phase, they achieved the floc disruption (deflocculation) with 0.06 g/g SS of $CaCl_2$ and in the latter phase, cell disintegration through potent biosurfactant producing bacteria, *Planococcus jake 01*. They were able to achieve 17.14% SS reduction and 14.14% COD solubilization for deflocculated and bacterially pretreated sludge, which were comparatively higher than for sludge treated with bacteria alone. They observed a biogas yield potential for pretreated sludge of 0.322 L/g VS as against 0.145 L/g VS for control. Kavitha et al. [43] investigated the bacterial-based biological pretreatment on liquefaction of microalga *Chlorella vulgaris* with cellulase-secreting bacteria prior to anaerobic

biodegradation. The biomethanation studies implied that bacterial pretreatment increased the bioavailability of biomass and hence methane generation. They arrived at a methane yield of nearly twice that of control.

Fungal pretreatment improves degradation of lignin and hemicellulose and hence result in increased digestibility of cellulose, which is preferably essential for AD process. Several fungal classes, including brown-, white- and soft-rot fungi, have been used for pretreatment of lignocellulosic biomass for biogas production, with white-rot fungi being the most effective. Amirta et al. [78] employed four fungal species to pretreat Japanese cedar wood chips in the presence of wheat bran which supplements nutrition for fungal growth. They revealed that wood chips pretreated by *Ceriporiopsis subvermispora ATCC 90467* produced the highest methane yield, which was 4 times higher than that of the control biomass at the end of 8 weeks.

4.5 Combined treatment

4.5.1 Steam explosion

Steam explosion pretreatment is an effort to expose the biomass to high temperature and pressure for short period of time and then reducing the pressure rapidly. This stops the reactions, causing the biomass to decompose explosively. This pretreatment condition may involve temperatures as high as 260°C and pressure up to 4.5 MPa. A study was investigated by Nges et al. [79] to improve the anaerobic biodegradability of *Miscanthus lutarioriparius* for biogas production. Employing steam explosion pretreatment with 0.3 M NaOH with particle size reduced to 0.5, they achieved a methane yield of 57% higher than that for the untreated samples. Their result was estimated to be 71% of theoretical methane yield of the biomass. Wang et al. [80] achieved a 24% higher methane yield than untreated bulrush at 1.72 MPa steam pressure, 8.14 min residence time, and 11% moisture content employing steam-explosion treatment of bulrush. During the pretreatment they observed the breakage, disruption, and redistribution of the rigid lignin structure which was proved by thermos gravimetric analysis. Srisang and Chavalparit [81] optimized a pre-treatment condition of 1.0% acetic acid, 17.45 min reaction time of sugarcane bagasse using steam explosion at 180° C. They achieved a maximum biogas production (434.47 L/kg VS) which was 91.88% higher than that of control (226.42 L/kg VS).

4.5.2 Physico chemical

The combination of thermal and chemical pre-treatments have been investigated in a number of studies in which the enhancement of the anaerobic digestibility of sludge was reported. Yi et al. [19] has used combined alkaline and low-temperature thermal pretreatment to enhance the subsequent AD of WAS. Different combinations of these two methods were investigated and biochemical methane potential (BMP) test was used to assess the anaerobic digestibility of pretreated WAS. With the combined treatment of adding 0.05 g NaOH/g TS and temperature maintained at 70°C for 9 h, they achieved a ratio of 72.8% soluble carbohydrate/total carbohydrate. Biogas production achieved through their BMP experiment was six times higher than the control and the average value of methane content of the produced biogas was 64%. In another study, Kavitha et al. [56], employed microwave irradiation to disintegrate the dairy WAS biomass after deagglomerating the sludge using a mechanical device, ultrasonicator. The outcomes of their study revealed that a higher biomass lysis efficiency of about 33.2% was possible through

ultrasonic assisted microwave disintegration (UMWD) when compared to microwave disintegration MWD (20.9%). Their results of BMP test showed that UMWD has better amenability towards AD with 50% higher methane production representing enhanced liquefaction potential of disaggregated sludge biomass.

Jang and Ahn [5] determined the effect of MW irradiation with NaOH pretreatment on AD of thickened municipal WAS in semi-continuous mesophilic digesters at HRT of 15, 10, 7, and 5 days. They combined MW pretreatment at temperature of 135°C with the input power of 1000 W with 60 ml of alkaline (20 meq NaOH/l) pretreated sludge. The degree of substrate solubilization arrived was 18 times higher in pretreated sludge (53.2%) than in raw sludge (3.0%). With HRT reduced to 5 days, they observed an improvement in biogas production (205% higher) for pretreated sludge compared with the control. The results show that MW irradiation combined with alkali pretreatment is effective in increasing mesophilic anaerobic biodegradability of sewage sludge. Ebenezer et al. [58] reported an increased COD and biopolymers release of WAS treated with Sodium citrate, a cationic binding agent, followed by microwaves pretreatment. They also concluded that the above pretreatment made the biomass more amenable for batch AD and hence higher biogas production with a methane content of 60–70% of biogas volume. Tamilarasan et al. [28] has made an attempt, by coupling a mechanical disperser with a chemical Sodium tripolyphosphate (STPP) for pretreatment of macroalgal biomass. They arrived at a 15% liquefaction and more than 5 times higher methane production compared to control at an optimal disperser-specific energy input of about 3312.67 kJ/kg TCOD (total COD) and an STPP dosage of about 0.04 g/g COD. Thus the combined pretreatment showed a greater biodegradability and biomethanation properties.

4.5.3 Ammonia fiber expansion

Ammonia fiber expansion is a promising method especially to pretreat agricultural materials for bioenergy production. Ammonia can be easily recovered and presents a high selectivity towards the lignin reactions, while preserving the carbohydrates. Ammonia can also penetrate the crystalline structure of cellulose and causes swelling [30]. The method involves treating the lignocellulosic biomass with liquid ammonia under mild temperature (70–200°C) and pressure (100–400 psi) for a specific time. This explosion results in several physical and chemical alterations in the structure of biomass. Jurado et al. [32] studied the effect of aqueous ammonia soaking (AAS) as a method to disrupt the lignocellulosic structure and increase the methane yield of wheat straw, miscanthus and willow. In all three cases, with AAS they observed an increase in methane yield from 37 to 41%, 25 to 27% and 94 to 162% for wheat straw, miscanthus and willow, respectively. Antonopoulou et al. [30] employed AAS as a pretreatment method, for the AD of three lignocellulosic biomass—poplar sawdust, sunflower straw and grass. In their study, they arrived at an increase in the ultimate methane yield being 148.7, 37.7 and 26.2% of poplar, sunflower straw and grass, respectively. They did not observe any toxic compounds such as furaldehydes, during AAS pretreatment.

4.6 Electrical

In this pretreatment, a very short burst (~100 μs) of rapidly pulsed (several kHz), high voltage (about 20 kV) electric field is utilized to disrupt and break up the cell membrane of microorganism. This focused pulse (FP) induces a critical electrical potential across the cell membrane, causing cell lysis by direct attack on phospholipids and the peptidoglycan, respectively. Once the cell membranes get

damaged, the intracellular organic material are released, making complex organic macromolecules more biodegradable [82]. They evaluated the effects of FP treatment and SRT on WAS in laboratory-scale digesters operated at SRTs of 2–20 days. They achieved an increased methane production rate and TCOD removal efficiency of about 33% and 18%, respectively, at a SRT of 20 days. They also concluded that, an increase in the hydrolysis rate was caused by FP-treatment of WAS, particularly at lower SRTs. Salerno et al. [83] applied FP to WAS and pig manure for increasing the production of methane during AD. In their work, methane production increased 200% for sludge and 80% for pig manure as compared to untreated sludge and manure. Thus PEF technology is advantageous due to low energy requirement for very short pulse time.

5. Future challenges and conclusion

The global energy supply is highly relying on fossil sources (crude oil, coal, natural gas) till now. According to the current energy policies and management, world market energy consumption is forecast to increase by 44% from 2006 to 2030 [84]. At the same time, concentrations of greenhouse gases in the atmosphere are rising rapidly, with fossil fuel-derived CO_2 emissions being the most important contributor. Nowadays, increasing attention has been gained on various strategies for the bioconversion of biomass into methane-rich biogas, due to increased global warming, the need for sustainable waste management and high energy costs [41]. The production of biogas through AD offers significant advantages over other forms of bioenergy production. Unlike fossil fuels, biogas from AD is permanently renewable, as it is produced from biomass, which is a living form of storage of solar energy through photosynthesis [85]. It has been evaluated as one of the most energy-efficient and environmentally beneficial technology for bioenergy production [86]. It can drastically reduce GHG emissions compared to fossil fuels by utilization of locally available resources.

Many sources, such as crops, grasses, leaves, manure, fruit, and vegetable wastes or algae can be used, and the process can be applied in small and large scales in many parts of the world. Energy crops digestion requires prolonged HRT of several weeks to month to achieve complete fermentation with high gas yields and minimized residual gas potential of the digestate [4]. For an increased dissemination of biogas plants, further improvements of the process efficiency, and the development of new technologies for mixing, process monitoring, and process control are necessary. Pretreatment of substrates and the addition of micronutrients offers a major potential for increasing the biogas yield. With the increasing number of biogas plants, also an improvement of the effluent quality is necessary, in order to avoid a contamination of ground water with pathogens and nutrients [3]. The choice of a pretreatment should be made not only based on energy balance and economy, but also various environmental factors such as pathogen removal, use of chemicals, and the possibility for a sustainable use of the residues, impacts on human health and the environment [8]. Carballa et al. [87] evaluated the environmental aspects of different pretreatment methods in terms of abiotic resources depletion potential, eutrophication potential, global warming potential, human and terrestrial toxicity potential through a life cycle assessment.

The profitable operation of a biogas plant relies on low capital and operational expenditures [28]. The frequent approaches including physical, thermal and chemical processes have been commercially implemented nowadays with a number of patented technologies. But research on biological techniques is still undergoing investigations from bench scale to full scale applications. Many pretreatment

methods are expensive or have a high energy demand. The performance of any pretreatment method is quantified based on the economic feasibility of the method in terms of the cost of pretreatment versus the value of added methane yield. The effect of the pretreatment is however mostly dependent on the biomass composition and operating conditions. The investment costs for pretreatment of recalcitrant substrates are high at the moment due to high expenditure in process engineering. Biological disintegration is devoid of chemical contamination and energy inputs and the use of an enzyme secreting bacterial consortium for biomass is beneficial, as commercial enzymes are expensive [55]. But the need for long reaction times renders biological pretreatment unsuitable for large scale plants where land space is expensive or restricted.

Most studies reviewed assessed the impact of pretreatment processes on the biogas yield on a laboratory scale with a few determining the net energy gain/loss obtained after pretreatment [11, 28, 58]. 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. Hence, there is a continuous need for newer and cleaner methods of biomass processing with less energy demand and lower waste generation.

This chapter concludes the effect of various biomass pretreatment for enhancement of biogas production and the future challenges for an energy efficient and eco-friendly manner. Therefore, optimizing the pretreatment conditions in order to lower production costs, improving the process performance and production of fewer residues is needed. A pretreatment method optimized based on the above situations may enhance the performance of individual pretreatments and achieve technical, environmental and financial feasibility. However, a further research on combined pretreatments is necessary in the future to get useful information that may lead to the necessary improvements in the AD industry.

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