Microbial pretreatment of lignocellulosic biomass for enhanced biomethanation and waste management

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Abstract

Biogas obtained from organic remains entails a developed technology and an appreciable methane yield, but its use may not be sustainable. The potential methane yield of various lignocellulose biomass and the operational conditions employed are inherently reviewed. Although of lower methane yields compared to conventional substrates, agricultural biomass is a cheap option. The major challenges encountered during its biogasification are its recalcitrance nature primarily due to the presence of crystalline cellulose and lignin. This necessitates an essential pretreatment step through physical, chemical or biological interventions for enhanced biomethanation potential. Various pretreatment—physical, chemical, and biological—strategies have been developed to overcome the inherent recalcitrance of lignocellulose to anaerobic degradation. Biological pretreatment approach, however, outcompete other pretreatments due to their application in milder conditions, little corrosiveness, and lower byproduct formation. Such pretreatment importantly aids in selectively reducing the lignin content and crystalline nature of the lignocellulosic biomass, which would evidently enhance the hydrolysis and production of monomers for their further anaerobic digestion (AD) for methanation. A variety of applied biological pretreatment strategies comprises microaerobic treatments, ensiling or composting, separation of digestion stages, and pretreatments using various lignocellulolytic fungi alongside. The net energy output through such approaches is substantially more and relatively inexpensive compared to other established chemical and mechanical approaches. The present review highlights the use of biological agents including bacterial, fungal and/or their enzymes which trigger biodegradation of wastes and utilization of lignocellulose for biofuel production. Additionally, the different physical, chemical, and biological pretreatment strategies for biogas yield enhancement are presented.

Keywords Anaerobic digestion · Biological pretreatment · Biomass · Biomethanation · Lignocellulose · Waste management

Introduction

To meet the rising global demands and most importantly as an alternative to fossil fuels several technologies for generating energy using lignocellulosic biomass have gained much attention. Extensive studies on the use and application of alternative energy resources are being emphasized to minimize the use of available conventional

energy reserves. According to studies, the steady rise in world population and energy demands are both contemporary (Kalyuzhnyi 2008). The energy requirements to some extent are fulfilled using renewable energy sources such as biogas and biofuels, actively produced from either agriculture waste, household refuses, municipal wastes, and or energy crops via anaerobic digestion technique (Gosselink 2002). Anaerobic digestion of organic refuse has gained improved consideration to produce energy and address environmental challenges including effective waste disposal (Ojha et al. 2015). Traditionally, production of biogas is mediated chemically or biologically through hydrolytic and anoxic methanogens, or until recently with possible combined biochemical and thermochemical hydrolytic processes in reduced time (Patinvoh et al. 2017). Biogas production has gained importance as it is considered to be an effective phenomenon for

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monitoring and mitigating global climate change. Importantly, biomethanation by anaerobic digestion is accredited with popularity worldwide as a foundation of renewable energy resource (Kalyuzhnyi 2008; Hartmann and Ahring 2006). Based on both economic and environmental benefits, biogas production from various biological wastes using anaerobic digestion is considered ideal. The major advantages of biogas in comparison to conventional fuels include (1) use of renewable resources, (2) little emission of greenhouse gases, (3) produced without requirement of either any foreign or available natural gases, (4) it reduces the level of pollution from organic waste and benefits waste biomass management (Malesu et al. 2016). According to Achinas et al. (2017), fulfilling enormous energy demands through anaerobic digestion technology under anoxic condition is considered an ideal approach to generate biofuels from diverse biowastes. Though biogas production from organic wastes such as kitchen refuse, animal and human excreta are reportedly being utilized for major activities such as cooking and lighting, yet the technology awaits popularization and universal acceptability (Malesu et al. 2016). Technically, the enhanced biomethanation of lignocellulose is affected by critical factors such as cellulose crystallinity, the inherent lignin content, pore size, available surface area for microbial interventions and available hemicellulose and cellulosic content in the biomass. According to Liew et al. (2011), hydrolysis activity of enzymes on lignocellulosic biomass is considered to be rate limiting in anaerobic digestion. Further, the process of biomethanation could be significantly enhanced by increasing biomass hydrolysis efficiency using enzymes through different pretreatments (Chandel and Singh 2011). On the basis of the total solid content of biomass, anaerobic digestion (AD) is majorly categorized into two types, solid-state AD (more than 15% total solid content) and liquid-state AD (total solid content less than 15%) (Zheng et al. 2014). Both types of AD are applicable to biogasification of different lignocellulose biomass (Li et al. 2010; Liew et al. 2011).

Although lignocellulose has a lower theoretical yield for biogas than its sugary/starchy counterparts, it is relatively free from the various problems associated with biofuel generation (Ahmad et al. 2017; Saini et al. 2015). To generate biofuels or bioenergy from biomass rich in lignocellulose, numerous studies have been conducted either to alter or modify the structural components of biomass using enzyme pretreatment method (Sindhu et al. 2015). Even though biomass pretreatment using mechanical means is successful in reducing the particle size and disruption of lignocellulose crystalline structure (Pommier et al. 2010), yet both thermal and chemical pretreatments are significantly important to enhance anaerobic digestion (Clarkson and Xiao 2000; Fox and Noike 2004; Fox et al. 2003; Teghammar et al. 2010;

Xiao and Clarkson 1997). As all such pretreatments require high energy inputs, they are not considered to be economically and environmentally beneficial technologies (Binod et al. 2010; Sun and Cheng 2002).

In contrast, biological pretreatment through the use of microbes is more environment friendly and has the advantage of utilizing very low chemical and energy source (Binod et al. 2010). Few biological pretreatments have demonstrably improved biogas production. Biological pretreatment is increasingly being advocated as an ecofriendly process that requires low energy input, low disposal costs, and milder operating conditions against chemical and physical pretreatment strategies that exhibit inherent drawbacks including the formation of inhibitory products (Wagner et al. 2018; Barik et al. 2000). No inhibitory molecule is produced during biological pretreatments at mild environmental conditions, but rather it includes benefits such as improved productivity of sugars, pH stabilization, enhanced rate of hydrolysis, adaptability and maximised substrate conversion rate (Kalyani et al. 2013). The metagenomics of related groups in methane cycle is also interesting (Meena et al. 2015). Bremond et al. (2018) has reported a table that indicates the research trends in biological pretreatment of anaerobically biodigested substrates including that of the lignocellulose biomass in the last 8 years till 2016 as published scientific articles. It tabulates that a higher (38%) percentage of biological pretreatment reports reduced over time to 16.5%. On the other hand, the articles on pretreatment strategies increased in number over the years during the said period, from 141 to 830. This fact reinforces the belief in the relevance of pretreatment in anaerobic digestion. The present work outlines the various individual and combined pretreatment strategies in lignocellulose biomethanation with special mention of biological pretreatment.

Structural components of lignocelluloses

The lignocellulosic components are more commonly found in naturally available substrates such as agricultural crop residues, kitchen wastes, and animal excreta. Lignocellulose refuse primarily contains 10–25% lignin, 20–35% hemicellulose and 35–50% cellulose (Sawatdeenarunat et al. 2015). A summary of composition of various lignocellulosic biomasses in terms of cellulose, hemicelluloses and lignin proportions is listed in Table 1. Cellulose consisting mainly of unbranched polysaccharide of about 10,000–15,000 β -1–4-linked glucose molecules is the most commonly available biopolymer for renewable energy generation. The β -units create a special physical structure that makes it different from starch and also digestible only by ruminant animals, where the bonds are cleaved by special enzymes, cellulases. Likewise, hemicellulose is represented

Table 1 Composition of lignocelluloses present in various biomass

S. no.	Biomass	Cellulose (%)	Hemicellulose (%)	Lignin (%)	References
1	Corn stover	37.5	22.4	17.6	Karthikeyan and Visvanathan (2012) and Li et al. (2014)
2	Wheat straw	38.2	21.2	23.4	Karthikeyan and Visvanathan (2012) and Brown et al. (2012)
3	Switch grass	31.0–45.0	20.0–31.0	12.0–18.0	Karthikeyan and Visvanathan (2012) and Brown et al. (2012)
4	Bagasse	38.2	27.1	20.2	Karthikeyan and Visvanathan (2012)
5	Sugarcane	25	17	12	Karthikeyan and Visvanathan (2012)
6	Rice straw	32	24	13	Ye et al. (2013)
7	Eucalyptus	38.0–45.0	12.0–13.0	25.0–37.0	Karthikeyan and Visvanathan (2012)
8	Giant reed stalk	33.1	18.5	24.5	Monlau et al. (2012)
9	Giant reed leaves	20.9	17.7	25.4	Monlau et al. (2012)
10	Sunflower stalk	31	15.6	29.2	Monlau et al. (2012)
11	Biomass sorghum	22.2	19.4	21.4	Monlau et al. (2012)
12	Barley straw	37.5	25.3	26.1	Monlau et al. (2013)
13	Rye straw	38	36.9	17.6	Monlau et al. (2013)
14	Napier grass	45.7	33.7	20.6	Reddy et al. (2012)

as a branched heteropolysaccharide containing pentose (C5) and hexose (C6) sugars. The side groups of hemicellulose are attached to hydrogen bonds which prevents crystallization of hemicellulose unlike in cellulose. Both cellulose and hemicelluloses are composed of linear and branched heteropolymers of pentoses (viz., arabinose and xylose), hexoses (viz., glucose, galactose, mannose and rhamnose) and acids (viz., glucuronic, methyl glucuronic and galacturonic acids). The cellulose–hemicellulose–lignin matrix exhibits greater rigidity because of the network of short and branched chains of hemicelluloses with cellulose microfibrils and lignin. Importantly, the amorphous and branched hemicelluloses ensure the effectiveness to biological, thermal and chemical hydrolysis (Palonen et al. 2004; Grethlein 1985). In general, almost all biomass representing hemicellulose fractions upon hydrolysis produce a mixture of different sugars. The last and important composition of lignocellulose is lignin that comprises of phenylpropane polymers providing mechanical strength to the lignocellulosic machinery and also aiding in water impermeability of the secondary wall of plant cells. Studies suggest that lignin is the biggest barrier in renewable biofuel production from biomass (Sawatdeenarunat et al. 2015).

Biomethanation

The process of anaerobic digestion is one of the oldest and well-studied technologies of stabilizing biomass, leading to energy generation from organic wastes (Van Lier et al. 2001). According to reports of European Union Regulation EC1772/2002, biomass from different sources is subjected to sterilization or pasteurization prior to anaerobic digestion. Therefore, pretreatment methods could be

possibly applied for obtaining a higher energy recovery and eliminating the additional cost of pasteurization and/or sterilization (Hendriks and Zeeman 2009; Eggeman and Elander 2005). An elaborate summary of the previously used pretreatment methods is highlighted in Table 2.

The process of anaerobic digestion is quite complex and involves a group of fermentative microbes that decompose biodegradable substrates in the absence of oxygen. Several factors such as pH, temperature, C/N ratio of biomass, retention time, type of accelerants (biological pure culture and inorganic additives), green biomass (lignocellulose waste, municipal solid waste, food waste, livestock manure and waste activated sludge), and the specificity of anaerobic digestion processes significantly affect the efficiency of the biological pretreatment processes (Mao et al. 2015). All essential factors are critical to anaerobic digestion because they determine the metabolic state of the microbes and also influence the quality and quantity of biogas production. In the initial phase of digestion, acidogenic bacterial communities convert fermentable sugars and degradation products of proteins into CO₂, H₂, NH₃ and other organic acids, which thereafter are utilized by acetate-producing bacteria generating acetate. The methane fermenting microbes lastly transform acetate into CH₄ and CO₂ (Hartmann and Ahring 2006; Weiland 2010). Anaerobic digestion could treat numerous organic waste refuse such as wastewater (sewage), refuse from agricultural processes, food processing and the organic components of the municipal solid waste materials. The biological treatment for organic refuse (40% by mass) has been an established technology in various parts of Eurasia (Greben and Oelofse 2009). Many research studies and outcomes have determined the presence of multiple substrates in anaerobic digesters correlated to both

Table 2 A summary of conventional pretreatment processes involved in biogasification. Source: Patinvoh et al. (2017), Daroit et al. (2009) and Dererie et al. (2011)

Pretreatment	Processes	Function	Economic challenges
Physical	Milling, pyrolysis, ultrasonic and irradiation	Increases pore size and surface area, disruption of biomass along with crystallinity and reduction in polymerization	High electricity consumption and equipment maintenance can be highly expensive
Rapid decompression	Steam explosion, ammonia fibre explosion, CO ₂ and SO ₂ explosion	Partial hemicellulose hydrolysis, breakdown of biomass, increased hydrolysis rate, partial lignin solubilization and partial decrystallization	Ammonia recovery can be expensive
Acid	Hydrochloric, sulphuric, phosphoric carbonic and nitric acids	Increment in hydrolysis of hemicellulose	Corrosion repair of instruments can be expensive along with the recovery of acids
Autohydrolysis	Liquid hot water	Cellulose hydrolysis at high and low temperature, high removal of lignin and hemicellulose	Cost of energy consumption is moderate
Alkali	Potassium, sodium, calcium hydroxide and ammonia	Reduction in degree of polymerization, decrystallization, increased internal surface area, lignin structure disruption and breakdown of linkages between carbohydrates and lignin	High cost of bases
Leaching	Organic solvents (diethyl ether, hexane, ethyl acetate and dichloromethane)	Removal of inhibitors in citrus fruit waste	Solvent recovery is costly
Solvent	Organic solvents (ethylene glycol, methanol, ethanol etc.) Cellulose dissolving solvents, e.g. concentrated mineral acids, dimethyl sulfoxide and <i>N</i> -methylmorpholine- <i>N</i> -oxide	Solubilization of lignin	Solvent recovery is costly and high cost of solvent
Oxidative	Oxidative compounds (hydrogen peroxide and peracetic acid)	Increment in cellulose accessibility, removal of lignin and hemicellulose	High chemical cost
Supercritical fluid	Carbon dioxide, sulphur dioxide and water utilization	Increase in biomass digestibility and lignin removal	Highly expensive

considerable advances in biogasification processes and nutrient loss (Park et al. 2011).

Stages of biomethanation

Anaerobic digestion represents a complex biological phenomenon of biomass degradation and conversion, categorized into four distinctly important phases (Zheng et al. 2014; Ojha et al. 2012) as indicated in Fig. 1.

Hydrolysis phase

The process of anaerobic digestion (AD) begins with hydrolysis controlled by the microbially secreted extracellular enzymes. Hydrolysis results in the degradation of (1) complex organic matter to simpler monomer, (2) long-chain fatty acids to short-chain fatty acids, and (3) proteins to amino acids and (4) carbohydrates to simple sugars. Facultative and

obligatory anaerobic bacteria secrete exoenzymes that split covalent bonds resulting in the formation of water-soluble monomers, during which the degradation of lignocellulose and lignin are relatively slow and incomplete (Gerardi 2003). Most importantly, the available dissolved oxygen (DO) utilized by facultative anaerobic microbes leads to the formation of low redox potential, thereby triggering the activity of obligate anaerobic microbes.

Acidogenic phase

In the second stage of AD, the acidogens convert the smaller simple molecules to volatile fatty acids (VFAs). These VFAs are further utilized by acetogenic group of microbes to produce acetate, CO₂ and H₂. The imbalance between any stages of this complex process may result in the formation of inhibitors, possibly leading to slower biogasification. During the biodegradation process, the types of products generated primarily depend on the H⁺ ion concentration. It is observed

Fig. 1 A flowchart depicting the biological process chain in (anaerobic) biogasification with possible biological pretreatment intervention point

that higher the H_2 partial pressure, the slower is the formation of the reduced compounds like acetate. Overall, in this important phase, the organic acids and alcohols are produced from a mixture of simple sugars, fatty acids and amino acids (Gerardi 2003).

Acetogenic phase

The products generated from the earlier metabolic reactions act as important substrates for subsequent microbial communities. In this phase, the homoacetogenic microbial population reduces exergonic H_2 and CO_2 to form acetic acid. Both acetogenic microbial group and methane-generating microbes grow symbiotically. Importantly, the organic acids and alcohols are converted to acetate in this phase which subsequently serve as the substrate for methane-producing microbial communities.

Methanogenic phase

The methanogenesis and hydrolysis are assumed as rate-limiting steps at this stage (Zheng et al. 2014). The methanogenic group of bacteria metabolically performs exergonic reactions to generate methane using carbon dioxide as the terminal electron acceptor (Cheremisinoff 1997). The production of methane via anaerobic digestion is achieved by two different ways: acetoclastic methanogenesis that converts acetic acid to methane, and hydrogenic methanogenesis which converts carbon dioxide to methane in association with hydrogen (Fig. 1). Importantly, the slow microbial growth of methanogenic microbes and complexity of

lignocellulosic biomass directly influence methane production (Chen et al. 2008; Cirne et al. 2007).

Operational factors for biological pretreatment of lignocellulosic biomass

Biological pretreatment activity includes application of both anaerobic and aerobic methods, and as well as use of specific enzymes such as peptidase, carbohydrase and lipase for digestion and biodegradation. As biological pretreatment of substrates do not produce any inhibitors during the overall process, the pretreatment is considered ecofriendly though it is a moderately time-consuming process compared to other pretreatments. Interestingly, certain studies (Ge et al. 2010, 2011) consider the hydrolytic–acidogenic step in biomethanation as biological pretreatment method, whereas others refer to it as process configuration of anaerobic digestion, not a pretreatment method (Carlsson et al. 2012; Parawira et al. 2005) but rather a process optimization for enzyme activity to induce hydrolysis.

It is observed that pretreatment processes in the presence of oxygen, such as composting or microaeration prior to AD, are effective to obtain extensively hydrolysed complex substrates via hydrolytic enzymes, yet production of certain intermediates may lower net generation of energy. Contrarily, a two-stage AD consists of a hydrolytic–acidogenic followed by methanogenic stage with a specific advantage of increased activity of methanogens resulting in higher methane yield. According to Verrier et al. (1987), a dual-stage reactor converts almost 90% of the waste biomass to biogas using microbial groups such as mesophilic and thermophilic

decomposers outperforming the single pretreatments. However, during the process, the loss of sugars and time efficiency could be improved by selecting appropriate microbial culture and operating parameters. A number of factors influencing the pretreatment processes during biogas generation are pH, temperature, microbial strains used, the nature of biomass, aeration, particle size of biomass and the duration of incubation.

pH

The pH is considered as an important parameter during biological pretreatment of biomass. Biomethanation process is most effective within a pH range 6.5–8.5 (Weiland 2003). The pH is relatively an essential factor as it influences the growth of the microbes and their metabolic reactions. The pH optima for methanogenesis is reportedly 7.0, whereas the hydrolysis and acidogenesis steps are most effective between pH 5.5 and 6.5 (Heo et al. 2003). Smaller variation in pH ensures that the biomethanation phenomenon works effectively generating maximum energy output (Weiland 2003). Studies outline that besides the bacterial and archaeal communities, many white-rot fungi exhibit ligninolytic activity at pH between 4.0 and 5.0, and help in the reduction of the medium acidity during digestion. The change in pH beyond a desired range affects the structure and production of laccase which eventually leads to the reduction of the enzymatic activity (Patel et al. 2009; Sindhu et al. 2015).

Temperature

To facilitate an effective biological pretreatment process, the temperature optimal range for mesophilic, thermophilic and psychrophilic groups of microorganisms is essential (Chae et al. 2008). Any minimal change in digester temperature affects the microbial activity and may result in an altered gas production (Deublein and Steinhauser 2008). Maintenance of incubation temperature is also vital during the pretreatment and it may change according to the microbes involved. Most Ascomycetes grow at 39 °C whereas Basidiomycetes are active between 25 and 30 °C. Isroi et al. (2011) described that fungal physiology helps in heat generation that increases the temperature of the media in addition to regulating the optimal temperature of biomass pretreatment.

Fatty acids

The presence of different volatile fatty acids such as acetic, propionic or butyric acids is considered as important intermediates in the process of biomethanation. Ideally, at higher concentrations of volatile fatty acids, the methanogenesis processes are inhibited (Weiland 2010). A sudden rise or accumulation of volatile fatty acids (VFA) retards the

growth of the acid-producing bacteria resulting in decreased acidogenesis and methane formation. According to reports, the fermentation of different sugars is strictly inhibited when the total VFA concentration in the biomass reaches 4 g/L (Angelidaki et al. 2003; Špalková et al. 2009; Sirohi et al. 2009), and in turn affects biogas formation.

Type of microbial strains

The selection of microbial strains for pretreatment depends solely on their microbial activity. According to the research conducted by Gao et al. (2012) and Sindhu et al. (2015), fungal strains such as *Gloeophyllum trabeum* release some enzymes when used for pretreatment process that help in the depolymerisation of hemicellulose and cellulose along with the modified form of lignin as brown residues. Also, it has been observed that the enhanced enzymatic hydrolysis with maximum lignin degradation is observed when pretreated with white-rot fungi. Interestingly, the use of a microbial consortia significantly helps in better delignification, improved xylan degradation and increased accessibility of cellulosic surface in biomass as compared to a single microorganism when used during pretreatment (Sindhu et al. 2015). Several groups of facultative anaerobes such as *Enterobacter* sp. during pretreatment produce a mixture of acids, alcohols, CO₂ and H₂ from carbohydrates, lipids and proteins (Gerardi 2003). Obligate anaerobes such as methanogens, particularly *Methanococcus* and *Methanobacterium*, participate in the conversion of acetate and associated intermediates to methane. According to Klocke et al. (2007), an estimate of around 90% archaeal and 60% bacterial species can be effectively used in anaerobic digestion.

Inoculum concentration

The concentration of the inoculum plays a key role in microbial pretreatment process. For effective colonization of the substrates, the time factor is the key as it is dependent on the type and amount of inoculum (Kuijk et al. 2015).

C/N ratio of the biomass

Renewable energy from lignocellulosic biomass primarily depends on the energy crops, forest residues, household and agricultural wastes. The C/N ratio of a substrate in general and lignocellulose in particular is a direct indicator of its ease of biodegradability. A summary of the C/N ratios of various lignocellulosic biomass is provided in Table 3. The proximate of lignocelluloses vary among biomass varieties, species, growth conditions and feedstock maturation, and these are critical while selecting either individual microbial species or a consortium for biological pretreatment (Sindhu et al. 2015). Mital (1996) opined that the optimum C/N ratio

Table 3 C/N ratio in different lignocellulosic biomass as common biomethanation candidates. Sources: Chandra et al. (2012), Krishania et al. (2013) and Kader et al. (2015)

Biomass	Dry matter (%)	C/N ratio
Saw mill waste	20–80	511
Household waste	40–60	18
Straw	70	90
Paper	85–95	173
Sewage sludge	0.5–5.0	6
Green grass	24	37
Swine excreta	18	18
Human excreta	20	8
Poultry waste	47	10
Cattle manure	17	24
Water hyacinth	18	25

that is effective for biomethanation ranged between 20 and 30, and Gerardi (2003) suggested a value of at least 25 for biogas production.

Aeration

Aeration is considered as essential in biological pretreatment of biomass using viable cells, particularly for the production and activity of ligninolytic enzymes. Reports about the oxidative processes involving microbes suggest that O₂ is pretty essential in lignin degradation. Aeration has a host of functions including gaseous exchange, removal of CO₂, regulation of heat and uniform dispersion of volatile matters that are produced through microbial metabolic activities, and thereby helps in generating biogas (Isroi et al. 2011).

Particle size of biomass

The particle size of biomass is an important factor in biological pretreatment. Larger the particle size, more it prevents water diffusion, microbial surface colonization and metabolite movement to inside the substrate. Contrarily, too small a particle is detrimental to the system as it risks heat and mass transfer, and clogging, thereby incapacitating the biomethanation process. Since small particle size adversely affects the gaseous exchange, appropriate particle size is required for enhanced biomass hydrolysis (Kuijk et al. 2015).

Pretreatment duration

The microbial strains and the biomass composition determine the incubation duration for pretreatment. Large-scale biological pretreatment of lignocellulose is affected by low delignification rate as it consumes more time for the process to complete. Reports suggest that corn stalk pretreated with

the wood-decaying Basidiomycete *Irpex lacteus* resulted in 37.6% lignin removal in 42 days while there was significant glucan (37.5%) and xylan (59%) loss as well (Du et al. 2011; Sindhu et al. 2015). Thus, it was observed that *Irpex lacteus* pretreatment helped in reducing the recalcitrant nature of corn stalk while negatively impacting the sugar production. Therefore, the type of strain and the incubation time must be optimized prior to pretreatment for maximum sugar recovery and microbial activities (Du et al. 2011; Sindhu et al. 2015) for effective biomethanation.

Biological pretreatment of lignocellulose

To achieve enhanced biogas production, several pretreatment processes have been recommended. Although there are numerous approaches for generating increased energy from lignocellulose biomass, pretreatment involving the use of microbes and their enzymatic constituents could be demonstrably effective. Such pretreatments result in the change of lignocellulosic structure either through partial or complete removal of lignin. According to Zheng et al. (2014), biological pretreatments for increased biomethanation during anaerobic digestion are mainly characterized by fungal, enzymatic and microbial pretreatment methods, as outlined in Fig. 2. This pretreatment requires less energy compared to the physical and chemical approaches and also results in the generation of less harmful chemicals (Wagner et al. 2018), with certain moderation in the anaerobic digestion process (Taherzadeh and Karimi 2008). Biological pretreatments cause less carbohydrate loss and more microbial accessibility for cellulosic fractions of feedstock for improved hydrolysis. Pretreatment by biological measures are mainly performed as solid-state fermentation process (Isroi et al. 2011). It has been observed that ligninolytic activities of fungi are more in solid-state fermentation in comparison to submerged fermentation (Barik et al. 2000). Moreover, there are several factors which influence the activity of enzyme and lignin degradation such as nutritional availability, fungal strains, aeration, pH, temperature and moisture content (Isroi et al. 2011; Šnajdr and Baldrian 2007; Patel et al. 2009).

Microbial pretreatment

Microbial pretreatment involves the process of screening and use of microorganisms isolated from natural environment that helps particularly in the hydrolysis (the first stage of methanogenesis) of biomass during anaerobic digestion. Microbial consortia being engaged in pretreatment should evidently have potential cellulose- and hemicellulose-degrading activities. A summary of the microbial pretreatment methods is outlined in Table 4. A microbial consortium isolated from thermophilic straw-decomposing landfill

Fig. 2 Schematic representation of (fungal and bacterial) biological pretreatment of lignocelluloses for enhanced biomethanation

showed 96% more methanation at 55 °C in anaerobic digestion of distillery wastewater-fortified cassava refuse compared to its untreated counterpart (Ziemiński et al. 2012). In addition to the conventional strategy of using screened microbes isolated from natural environments, Zheng et al. (2014) opined that a mixture of lyophilized yeast and cellulolytic microbial strains could be effective in biological pretreatment. They reported 36% less retention time and 75% additional methane production compared to the unamended biomass when the autoclaved corn straw was treated with freeze-dried microbial consortia.

Fungal pretreatment

The process of fungal pretreatment for biomethanation on lignocellulose substrates emphasizes on their evaluation of lignin and hemicellulose degradation, as cellulose is recalcitrant to fungal attack. Hemicellulose and lignin degradation enhances the cellulose digestibility by microorganisms (Zheng et al. 2014). Fungal species such as white-rot fungi are accredited as the most effective amongst all fungal types employed in generating biogas from lignocellulosic biomass (Sun and Cheng 2002) although effective fungal pretreatment requires sterilization of the lignocellulosic residue. Chestnut leaves and hay at 1:2 ratio pretreated for 2–3 weeks at 37 °C using wood-decomposing fungus *Auricularia auricula-judae* increased biogasification by about 15% as compared to the untreated biomass (Mackulak et al. 2012). When treated with white-rot fungus *Ceriporiopsis subvermispora* at 60% moisture content for 30 days, backyard refuse significantly enhanced the methanation by 154% (Zhao 2013). The key for enhanced biomethanation by white-rot fungi is that other than the essential lignin-degrading enzymes they also produce hemicellulases, cellulases and xylanases. Although

nearly all fungi secrete laccase and manganese peroxidase, few of them produce lignin peroxidase during the pretreatment of lignocellulosic feedstock (Isroi et al. 2011; Barik et al. 2000).

Enzyme pretreatment

The hydrolysis of hemicellulose and cellulose is considered as a rate-limiting step during anaerobic digestion (Romano et al. 2009). To achieve enhanced biogas production from lignocellulosic biomass, key enzymes with hydrolytic activity such as hemicellulases and cellulases can be applied prior to or during the process of biomethanation. Enzymic pretreatment of biomass for biomethanation is limited and not very popular because of the substrate selectivity/specificity and the cost factor (Zheng et al. 2014). An earlier study by Yunqin et al. (2010) reported that paper sludge and paper pulp treated with mushroom compost extract that was rich in laccase and carboxymethylcellulase (CMCase) activity enhanced methane production by 34.2% compared to the untreated one. Enzymatic pretreatment partially hydrolysed the spent hops and sugar beet pulp enhancing their biomethanation potentials (Ziemiński et al. 2012). The pretreatment aided in removal of organic matter content by 7% and 23% in pretreated hydrolysates of spent hops and sugar beet pulp, respectively with about 13–19% enhanced methanation.

Socioeconomics

Among the various bioenergy forms, biogasification anaerobic digestion is recognized the most cost efficient, less skill set requiring and chemically and environmentally friendly (Chandra et al. 2012). As biogas production promises to

Table 4 Biological pretreatment interventions for enhanced biomethanation

Biological pretreatment	Microorganism/enzyme	Biomass	Pretreatment conditions	Results	References
Fungal	Fungi (white, brown and soft rot) and Basidiomycetes	Agricultural residue; chestnut leaves and sisal leaves	28–37 °C for 12 days to 8 weeks Autoclave/no autoclave Aerobic	15% to fivefold increased methanation	Take et al. (2006), Amirta et al. (2006) and Mackulak et al. (2012)
Microbial	Complex mixture containing yeast and cellulolytic bacteria, heat treated sludge, <i>Clostridium thermocellum</i> , mixture of fungi and compost forming microorganisms	Agricultural residue; corn straw, corn stalk, cassava residue and cotton stalk	20–55 °C for 12 h to 20 days Autoclave/no autoclave Aerobic	25–96.63% enhanced methane production	Bruni et al. (2010), Zhang et al. (2011) and Zhong et al. (2011)
Enzymatic	Laccase, mixture of cellulases, hemicellulases and β -glucosidase, pectinase, <i>Trichoderma</i> enzyme complex	Agricultural residue; sugar beet pulp, spent hops, manure, biofiber, MSW, paper pulp, wheat grass	37 °C for 4–24 h Autoclave Aerobic/anaerobic	0–34% improved methane production	Bruni et al. (2010), Yunqin et al. (2010) and Ziemiński et al. (2012)

fulfil more than 30–40% of the fuel demands, a significantly large number of studies worldwide using different technologies are reported. Since the use of traditional fuels produces smoke and particulates leading to air pollution and consequently several health issues, biomethanation, therefore, significantly helps in reducing air pollution and respiratory diseases. Likewise, most chemical, physical and physico-chemical pretreatments require high energy inputs, which results in the production of various harmful byproducts that adversely affect methane production and also the environment (Paudel et al. 2017). To address such issues, various industrial, lignin-degrading and cellulose-hydrolysing enzymes can be applied to degrade lignocellulosic biomass for biomethanation (Lopez et al. 2007). Though application of industrial enzymes in biological pretreatments are not desirable due to their high unit price, the use of raw lignocellulose-degrading enzymes indirectly through the biological routes using microbial consortia can be potential candidates for biomass pretreatment and biogas production.

Conclusion

The growing concerns about the ever-growing generated wastes, rise in energy demands and elevated instances of global warming effects have necessitated accelerated and enhanced anaerobic digestion process. Various physical, chemical and biological pretreatment strategies have been standardized in recent times to make lignocellulosic biomass more amenable to biomethanation by overcoming its inherent resistance to anaerobic degradation. The eco-friendly biological pretreatment strategies outcompete their counterparts due to their requirement of milder operational conditions, lesser byproduct formation and less corrosiveness. The array of applied biological techniques include, not essentially restricted to, microaerobic treatments, ensilaging or composting, the separation of digestion stages, and additional pretreatments using various fungi. Fungal pretreatments allowing the use of recalcitrant, second-generation substrates for biogas production have achieved particular success. Simple and sustainable biological pretreatment of lignocellulosic biomass through the use of microbes or their metabolites facilitate higher net energy yield. Microbial pretreatment ensures enhanced biomethanation while lowering the retention time by converting complex structure of the biomass into simpler monomers. Several critical parameters include the biomass complexity, pH, temperature, microbial strains used, and the C/N ratio of the biomass optimization for better conversion rate. Fungal pretreatment selectively enhances the cellulose accessibility and removal of lignin. Such applications assist in less sugar loss during the process as compared to other established physical and chemical pretreatment strategies. As biological pretreatment consumes

less energy than other methods without generating any inhibitory molecules, it can be considered as an ecofriendly approach and an efficient waste management strategy. Thus, biological pretreatment strategies offer great potential to improve the digestibility of different biogasifiable substrates. Despite all the benefits and advantages of using biological pretreatment for biogas formation, further adequate research is required to make the process duration efficient by reducing the effective time required for the process and selecting/engineering effective microbial strains for better and efficient biogas production. Further detailed investigations of the mode of action, the application of different substrates, field demonstration, full-scale implementation, and possible byproduct formation are called for.

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Compliance with ethical standards

Conflict of interest No potential is reported by the authors.

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