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A comprehensive pre-treatment strategy evaluation of ligno-hemicellulosic biomass to enhance biogas potential in the anaerobic digestion process

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Effective pretreatment of ligno-hemicellulosic biomass has emerged as a pre-requisite for its efficient conversion into biogas through the anaerobic digestion (AD) process. Assessment of various pre-treatment methods shows microbial pretreatment to be the most promising, economically viable, and environment-friendly option. Microbial pretreatment offers the advantages of low energy consumption and minimal pollution generation, thus making it a promising avenue for enhancing biogas yields from biomass. Fungi and bacteria, along with their enzymes, play pivotal roles in this method. Fungal pretreatment, involving cellulose and lignin-degrading species like brown-rot and white-rot fungi, have shown improved biogas yield. Bacterial and enzymatic pretreatments offer quicker results, making them attractive options for shortening the reaction time. Microbial consortia have shown remarkable efficiency in biomass degradation and its anaerobic digestion under thermophilic conditions. Physical pretreatment methods, such as mechanical size reduction, have shown potential to increase biomass accessibility and enhance biogas production. However, due to its energy-intensive nature and for improving biogas yields, further research is needed to develop more cost-effective approaches. The combination of physical and biological pretreatment methods offers a promising approach to effectively pretreat ligno-hemicellulosic biomass for improved biogas production.

Sustainability spotlight

This review article focuses on the potential transition of lignocellulosic biomass into treasured sustainable & clean energy, *i.e.* biogas. First, it uncovers various relevant feedstock possibilities for the production of biogas. Secondly, it provides in-depth knowledge and recent developments on different pretreatment approaches for enhanced biogas yield. Furthermore, it delivers an insight into effective and sustainable pretreatment methods. The sustainability spotlight focuses on the UN Sustainable Development Goals (SDGs), including SDG 12: responsible consumption and production, SDG 9: industry, innovation, and infrastructure, and SDG 7: affordable and clean energy. It also highlights waste-to-energy initiatives and sustainable and clean energy ideologies. The advent of pretreatment strategies for the production of sustainable and clean energy is the emphasis herein.

1. Introduction

The renewable energy imperative and opportunities are growing as fossil fuels deplete and climate change accelerates. Biomass-based energy offers a crucial solution, utilizing organic wastes and residues to produce sustainable power, reduce greenhouse gas emissions, and mitigate our dependence on finite resources. According to the famous quote of Lao Tzu (the father of Taoism), "If you don't change direction, you will end up where you are heading," Where are we heading? Daily human activities and the advancement of industry have led to an astonishing amount of waste production. One striking illustration of this is the staggering quantity of municipal organic waste generated (∼2.2 billion tons by the year 2025) as forecasted by the World bank.[1] When raw materials and treatment technologies and ultimately production technologies are carefully chosen, biomass energy can be generated in a financially sustainable manner. Furthermore, by effectively managing carbon emissions and ensuring economic efficiency, biomass energy supply chains can be established as sustainable solutions. At present, one such technology that has already proven itself is anaerobic digestion (AD) technology for biomass utilization to produce biomethane, looking promising even for biohydrogen.[2,3] Anaerobic digestion is a widely adopted technology

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for conversion of organic waste into biogas, a renewable energy source comprising mainly methane and carbon dioxide. AD breaks down organic matter into biogas and a stabilized organic effluent through metabolic reactions involving a complex community of microorganisms (both facultative and strict anaerobes). These reactions occur under non-toxic conditions as highlighted by various researchers.[4–8] As a first step, complex organic substances that cannot be directly utilized by bacteria are broken down into soluble monomers through the action of extracellular hydrolytic enzymes produced by acidogens. This process, known as acidification or fermentation, serves as an intermediate step in substrate metabolism, acting as an electron acceptor. During this stage, acidogenic fermentation bacteria convert the soluble monomers into terminal products, such as volatile fatty acids (VFAs), along with the generation of cellular materials. In the third step, hydrogen-producing acetogens utilize the hydrolytic products to produce acetic acid while generating hydrogen and carbohydrates. A small population of homo-acetogenic bacteria also utilize CO_2/H_2 as substrates to produce acetic acid. Finally, strictly anaerobic methanogens transform the acidification products (such as acetic acid, formic acid, CO_2/H_2, and others) into methane through a complex process involving various bacterial species working synergistically.[2,6,9] Biomass, derived from plants, animals, and microorganisms, is a rational carbon-based feedstock that has gained prominence as a sustainable alternative to non-renewable energy sources. Its immense potential has led scientists, economists, and policymakers to envision a parallel economy known as a bio-based economy or circular bio-economy, highlighting its renewable nature.[3] Among the various biomass sources, lignocellulosic feedstocks (LCF) stand out with an annual production of 200 billion tons, offering abundant availability.[5,10] AD can be observed naturally in environments such as wetlands, swamps, and the digestive systems of various animals; its study has gained significant momentum due to the escalating energy crisis and mounting environmental concerns. Efforts to investigate this biochemical process have intensified, with a focus on utilizing effluents and residual substrates from diverse production chains. Notable examples include agriculture, livestock, agro-industries, municipal organic waste, and sewage sludge, as discussed in ref. 4. Apart from producing valuable high-energy biogas, AD also helps control the release of odours, reduces pathogens present in residual raw materials, and generates a stabilized liquid compound with beneficial properties suitable for use as a biofertilizer.[5,7]

There are three principal components in lignocellulosic biomass (LCB): cellulose, hemicellulose and lignin. These biopolymers are interlinked with each other in a hetero-matrix and at varying relative compositions depending on the type, species and even the origin of the LCB.[5,11,12] The relative abundance of cellulose, hemicellulose and lignin is a key factor in determining the optimum energy derivable from LCB. However, the efficient breakdown of complex organic compounds and the subsequent biogas production from lignocellulosic biomass can often be challenging due to its recalcitrant nature.[5,8,13]

The inherent characteristics of lignocellulosic biomass materials render them less suitable for anaerobic digestion as substrates. These biomass materials exhibit diverse shapes, sizes, moisture contents, and varying levels of lignin, cellulose, hemicellulose, and fixed solids.[14,15] Furthermore, due to the complex composition of lignocellulosic biomass, the enzymatic degradation of the biomass by hydrolytic microbes requires significant time due to its intricate properties.[15,16] In light of these challenges, it is necessary to pretreat lignocellulosic biomass before introducing it into an anaerobic digester. This pretreatment is essential to overcome operational difficulties that commonly arise, including clogging, the formation of floating layers, and the recalcitrance of solids to enzymatic breakdown.[16] Various pretreatment methods (physical, chemical, physiochemical, biological, enzymatic, *etc.*) have been explored to enhance the biodegradability and increase biogas yields. Among these, enzymatic pretreatment has gained considerable attention as a promising approach to overcome the limitations associated with biomass degradation.[1,3] Enzymatic pretreatment involves the application of specific enzymes to the biomass to break down complex carbohydrates, such as cellulose and hemicellulose, into simpler fermentable sugars.[3,5] This process aims to improve the accessibility of the substrate to microbial degradation during anaerobic digestion, thereby enhancing biogas production.

The microbial pretreatment approach is primarily centered around the utilization of either a single microbe or a consortium of microbes to treat the feedstock before it undergoes the AD process. This method offers several notable advantages, including minimal energy requirements and a low production of hazardous chemicals. However, a significant drawback of microbial pretreatment is the extended incubation time necessary to achieve substantial microbial growth, as well as the prolonged duration of the pretreatment process itself.[17,18] However, several studies have demonstrated the positive effects of microbial pretreatment from a variety of microorganisms on

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biomass degradation and biogas yields. Microbial pretreatment offers several advantages over other pretreatment methods. Firstly, it is a mild and environmentally friendly process, as it operates under mild conditions and does not require harsh chemicals. Moreover, microbial pretreatment can be tailored to target specific biomass types and compositions, allowing for customization based on the feedstock characteristics.[11,19,20] There has been extensive discussion on lignocellulosic biomass by several researchers in the past related to pre-treatment approaches and steps that can be followed for efficient bio-gas yields. However, this review is extensively focused on a hypothetic model of combined mechanical and biological treatment for practically applicable biomasses that should be followed for effective biogas/bio-CNG generation.

2. Lignocellulosic biomass: a potential source of biogas/bio-CNG

Lignocellulosic biomass encompasses different categories, including energy crops (such as perennial grasses), forest materials (like hard and softwood, sawdust, pruning, and bark residues), agricultural residues (including cereal straws, bagasse, and stovers), aquatic plants (like water hyacinth), and the organic portion of municipal solid waste.[5,14,21] The compositional contents of various LCB can vary in their quantity; however generally, the primary components of lignocellulosic biomass are cellulose (35–50%), hemicelluloses (20–35%), lignin (5–30%), and other extracted substances (1–10%).[21–23] The composition of lignocellulosic biomass varies depending on the substrate type and also within the same genera. Commonly utilized agricultural residues include waste materials like wheat straw, barley hull, barley straw, rice straw, oat straw, rice husks, sugarcane bagasse, corn cobs, corn stalks, and sorghum straw.

Cellulose fragments are composed of a linear chain of glucose units connected by 1,4-β-glycosidic bonds.[5,14,21,24] The cellulose molecules form a crystalline network through cross-linking of various hydroxyl groups within hydrogen bonds. Hemicellulose, on the other hand, is a heteropolymer consisting of different monomers, including hexoses (β-D-glucose, α-D-galactose, and β-D-mannose) and pentoses (β-D-xylose and α-L-arabinose).[24,25] It is attached to functional groups such as acetyl, methyl, glucuronic, galacturonic, and cinnamic acids. Lignin, which is insoluble in water, serves as a structural reinforcement and imparts resilience to plant tissues. The monomers of the lignin polymer consist of three phenolic compounds: coumaryl, coniferyl, and sinapyl alcohol.[16,21,26–28] These three prime components make the cell wall of any type of biomass very rigid in nature and subsequently hard to break.

3. Applicable feedstock for bio-gas/bio-CNG production

Biogas and bio-CNG (compressed natural gas) are renewable energy sources that have gained significant attention due to their potential to reduce greenhouse gas emissions and provide a sustainable alternative to fossil fuels.[29] These biofuels are produced through the anaerobic digestion of organic materials, commonly known as feedstock. A study was carried out by researchers to evaluate the annual energy potential associated with biomass waste (like animal manure, crop residues, logging residues, and municipal waste).[30] The findings revealed that the technical bioenergy potential of these biomass resources amounts to 1.29×10^3 petajoules (PJ) in 2.31×10^4 cubic megameters (Mm^3) of biogas and 7.79×10^2 PJ in 3.49×10^4 million liters (ML) of cellulosic ethanol. The selection of suitable feedstock plays a crucial role in the efficiency, profitability, and environmental sustainability of biogas and bio-CNG production systems.[3,4] This section aims to explore various applicable feedstock options for the production of biogas and bio-CNG.

3.1 Agricultural residues

Agricultural wastes refer to waste materials generated from diverse agricultural activities. According to the UN, these wastes typically encompass manure and other byproducts from farms, poultry houses, and slaughterhouses, as well as waste from the harvesting process, fertilizer runoff from fields, and pesticides that find their way into water, air, or soils.[31,32] Additionally, according to the world energy council, spoiled food waste can also be considered a part of agricultural waste. Crop residue, commonly known as harvest waste, consists of both field residues, which are the remains left in the agricultural field or orchard after harvesting, and process residues, which are the leftovers from the processing of crops into usable resources. Examples of field residues include stalks, stubble, leaves, and seed pods, while process residues include materials like sugarcane bagasse and molasses.[32,33]

Agricultural residues are one of the primary feedstock sources for biogas and bio-CNG production. These residues include crop residues such as straw, husks, stalks, and cobs.[21,28] The residues from crops like wheat, rice, maize, and sugarcane are particularly suitable for anaerobic digestion.[22,28] These feedstock materials are abundantly available, making them cost-effective and environmentally friendly options for bio-gas/bio-CNG production. Additionally, their utilization helps to manage agricultural waste and reduces the need for traditional disposal methods such as open burning as commonly practiced across the world, especially in developing countries like India. In India, a diverse array of crops is cultivated, accompanied by the generation of substantial amounts of crop residues. Statistics indicate that India produces over 500 MT of residues each year from its primary crops. Notably, approximately 65% of these residues find purposeful applications such as animal feed, bio-manuring, soil mulching, temporary shelters, and fuel for both domestic and industrial consumption.[34] Additionally, each year, a staggering 140 billion tons of biomass are generated from diverse agricultural residues.[35] Insufficient implementation of sustainable management practices has resulted in the burning of a substantial amount of crop waste in India each year, estimated to be about 92 metric tons.[32] This practice leads to the

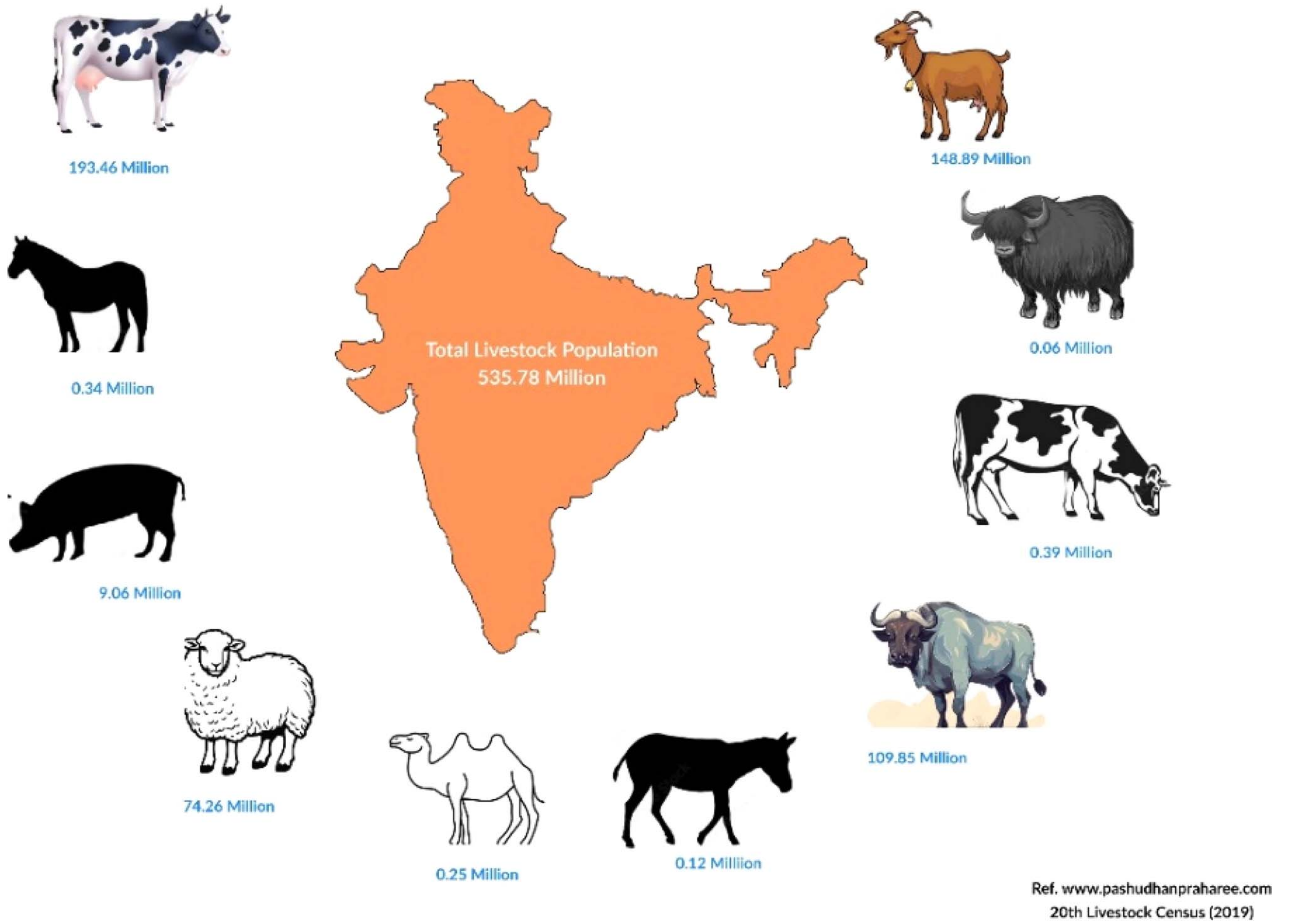

Fig. 1 Pictorial diagram representation of livestock availability of India.[38]

release of excessive particulate matter emissions and contributes significantly to air pollution.

3.2 Animal manure

Animal manure is another significant feedstock for biogas and bio-CNG production. Livestock operations generate substantial amounts of organic waste, which can be efficiently converted into biogas through anaerobic digestion. Manure from cattle, pigs, poultry, and other livestock contains high levels of organic matter, making it an excellent source of methane production. Biogas production from animal manure not only helps in waste management but also reduces odors and potential water pollution risks associated with manure storage.[36,37] According to a source from Pashudhan Praharee, India is home to approximately 535.78 million livestock, contributing to an annual production of around 3 million tonnes of livestock waste. This waste primarily consists of various materials such as dung, urine, placenta, bedding, feed wastage, and milk-house wastes, among others.[38] According to the 20th livestock census conducted in 2019, India boasts one of the largest livestock populations globally, with a total count of 536.76 million animals. This population includes 193.46 million cattle, 148.89 million goats, 109.85 million buffalo, 74.26 million sheep, 0.06 million yaks, 0.39 million mithun, 9.06 million pigs, 0.34 million horses and ponies, 0.08 million mules, 0.12 million donkeys, and 0.25 million camels,[39] as shown in Fig. 1. Thus, livestock manure plays an important role in economies, and its sensible and proper utilization through the adoption of contemporary processing technologies has the potential to greatly enhance earnings (especially in rural areas) by harnessing these enormous quantities of biomass for power generation and for organic recycling in general. India with its 60% agricultural sector has huge potential for biogas/bio-CNG generation from the abundant and wide range of feedstock materials. According to the Government of India (GoI), a total of 44 bio-CNG projects have been installed in the country during the last five years and the current year as of 30-11-2022 with a total capacity of 218 tonnes per day, for this renewable fuel[40] as shown in Fig. 2.

3.3 Energy crops

Energy crops specifically grown for biogas production are becoming increasingly popular as feedstock sources. Perennial grasses such as switchgrass, miscanthus, and reed canary grass are considered ideal energy crops due to their high biomass yield and ability to grow under diverse climatic conditions.[16,30,42] These crops require minimal fertilizers, pesticides, and water,

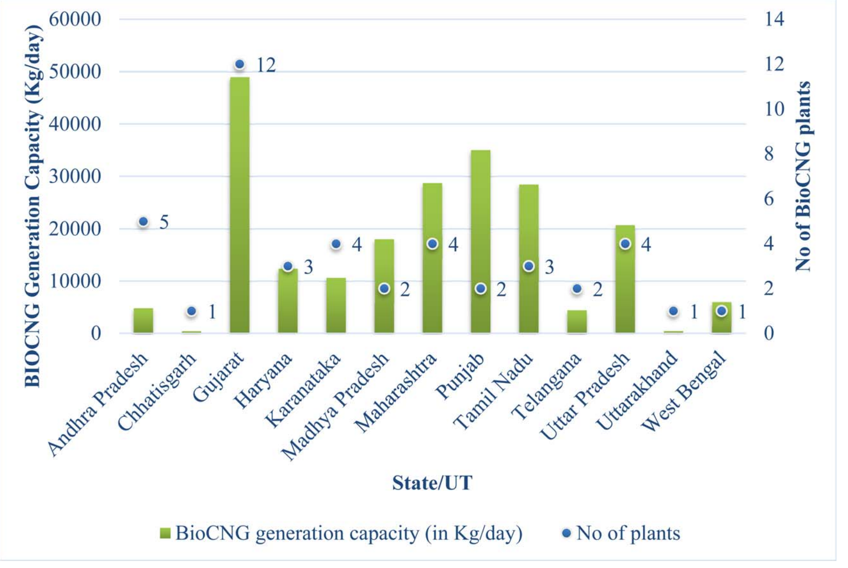

Fig. 2 Figure representing bio-CNG plants operational in various districts of India (updated data as on 6 January 2023).[41]

making them environmentally sustainable choices. Energy crops provide a consistent and reliable feedstock supply for biogas plants, ensuring a steady production of biogas or bio-CNG throughout the year.[11,43] In India, the production of crop residues from the 10 major Indian crops amounts to approximately 683 million tonnes annually, as reported in ref. 42 and 44, which also highlighted the use of *Opuntia ficus-indica* (L) Mill. and *Euphorbia tirucalli* L. as energy crops for anaerobic digestion. Over a period of four months, the plantations with the highest density, specifically *Euphorbia tirucalli* at 266 667 plants per hectare and *Opuntia ficus-indica* (L) Mill. at 20 000 plants per hectare, produced approximately 1791 m^3 of methane for *Euphorbia tirucalli* L. and 1860 m^3 for *Opuntia ficus-indica* (L) Mill. from one hectare of marginal land. However, according to ref. 41 and 45, typically, the methane yield obtained from anaerobic digestion of energy crops is lower compared to that from crop residues such as corn stover and wheat straw. Nevertheless, it is possible to improve the yield by pre-treating energy crops, which involves breaking down the cell-wall structure to facilitate cellulose hydrolysis.[28,45]

3.4 Organic fraction of municipal solid waste (OF-MSW)

The effective management of Municipal Solid Waste (MSW) also referred to as the organic fraction of municipal solid waste (OF-MSW) remains a persistent challenge across all nations. It serves as a crucial component in the journey towards achieving a circular economy. According to ref. 46, the United States, China, and India rank as the top three contributors to municipal solid waste globally. Research indicates that developed countries generate approximately 107 kg of food waste per capita per year, while developing countries produce around 56 kg of food waste per capita per year. These figures clearly demonstrate that higher living standards are associated with increased waste generation.[47] Furthermore, the composition of solid waste exhibits variations based on income levels. Adhering to the principles of the circular economy, it becomes imperative to generate high-quality digestate suitable for agricultural applications. Achieving this outcome hinges upon the initial quality of the organic fraction of municipal solid waste (OF-MSW). Consequently, it becomes crucial to delve into the factors that influence the characteristics of the feedstock. In low-to-middle-income populations, the predominant type of waste generated is organic waste. Conversely, high-income populations tend to produce larger amounts of waste paper, metals, and glass. According to ref. 48 and 49, MSW is composed of various types of waste. These include organic waste, which consists of food scraps, yard leaves, grass, brush, wood, process residue paper, and similar materials. Paper waste, on the other hand, encompasses paper scraps, cardboard, newspapers, magazines, bags, boxes, wrapping paper, telephone books, shredded paper, and paper beverage cups. Although paper is technically organic, it is not classified as organic unless it is contaminated by food residue. Another category is plastic waste (PW), which includes bottles, packaging, containers, bags, lids, and cups. Glass waste consists of bottles, broken glassware, light bulbs, and colored glass. Metal waste encompasses cans, foil, tins, non-hazardous aerosol cans, railings, bicycles, and similar items. Lastly, there is the category of other waste, which includes textiles, leather, rubber, multi-laminates, e-waste, appliances, ash, and other inert materials. MSW can also be further divided into different streams. Recyclables encompass paper, glass, plastic, metals, and other materials that can be recycled. Compostable organic matter consists of food waste, fruit and vegetable waste, and similar organic materials suitable for composting. Toxic substances include paints, pesticides, medicines, used batteries, and other hazardous materials. Lastly, hazardous solid waste includes items like blood-stained cotton, disposable syringes, sanitary napkins, and other potentially harmful waste materials.

According to ref. 50, the generation of municipal solid waste is projected to increase to 3.4 billion tonnes by 2050.

Additionally, there is a concerning prediction that the number of individuals lacking adequate access to essential waste management services may rise to 5.6 billion by the same year. This alarming situation needs to be managed effectively. Organic waste from municipal sources, including food waste and green waste, can be effectively utilized as feedstock for biogas and bio-CNG production.[51,52] The organic fraction of municipal solid waste contains significant amounts of organic matter that can be anaerobically digested to produce biogas.[45,53] Overall, methane emissions from municipal solid waste (MSW) landfills are recognized as a significant contributor. Currently, unsegregated MSW is indiscriminately dumped in these landfills, posing grave environmental and groundwater risks.[52] By diverting organic waste from landfills, the production of biogas from municipal waste helps in reducing methane emissions, minimizing landfill space requirements, and promoting a circular economy approach. With the projected increase in population and economic growth, conversion of bio-waste can offer multiple co-benefits, in addition to mitigating greenhouse gas emissions. These benefits include (i) utilizing biogas as a substitute for fossil energy, thereby reducing direct methane emissions from landfills, (ii) freeing up valuable land occupied by landfills for other productive activities, (iii) reducing health hazards associated with landfills, and (iv) recovering organic fertilizer through improved waste and slurry management.[49,53,54] Accurately estimating future waste generation in urban areas and determining the potential energy that can be derived from these waste resources through anaerobic digestion are crucial steps towards building sustainable cities in developing countries like India, which are undergoing significant rural to urban transitions.

3.5 Industrial waste

Certain industrial wastes can also serve as feedstock for biogas and bio-CNG production. For example, wastewater from food processing facilities, breweries, and distilleries often contains high organic content, which can be anaerobically digested to generate biogas.[47,55] This offers a dual benefit of waste treatment and renewable energy generation, contributing to the sustainability goals of industries. For example, the largest proportion of waste generated in the brewing industry is spent grain, also known as exhausted malt. It accounts for approximately 85% of the total waste produced during the brewing process. Spent grain has a composition that is rich in carbohydrates, cellulose (15–25%), hemicellulose (28–35%), lignin (28%), proteins (15 to 26.2%), free amino acids, lipids (10%), phenolic compounds, vitamins, and minerals such as calcium, selenium, phosphorus, and magnesium.[47,56] It also contains a high moisture content ranging from 75% to 80%. The specific composition of spent grain can vary depending on the type of barley used and the conditions during the technological process. Ref. 57 and 58 highlight that anaerobic digestion (AD) is commonly employed for the treatment of both liquid and solid waste streams. These include industrial wastewater with a significant organic content, the organic fraction of municipal solid waste (OFMSW), and sewage sludge.

The selection of suitable feedstock is vital for the successful implementation of biogas and bio-CNG production systems. Agricultural residues, animal manure, energy crops, organic municipal waste, and industrial wastes offer diverse and abundant feedstock options. Utilizing these organic materials through anaerobic digestion helps in waste management, reduces greenhouse gas emissions, and provides a renewable energy source. Furthermore, the production of biogas and bio-CNG from these feedstock sources promotes a circular economy by transforming organic waste into valuable energy resources. Continued research and technological advancements in feedstock treatment will lead to enhancement of bioenergy generation.

4. Microbial approach towards a high BMP yield

The breakdown and conversion of biomass into biogas/bio-CNG can be enhanced through various processes, including physical, physio-chemical, and biological methods. Each approach has its own set of advantages and disadvantages. However, among these methods, the biological process has emerged as the most economically viable and feasible option.[5,59,60] Biological pretreatment offers unique advantages, such as low energy consumption and a high level of environmental friendliness. Studies have shown that environmentally friendly pretreatment methods do not generate significant pollution, particularly in terms of water, air, and soil, following the pretreatment process.[59] Cellulose, a major component of biomass, is relatively resistant to microbial degradation compared to hemicelluloses, which are more easily broken down. Lignin, another component, is highly resistant and requires specialized fungi for its degradation. Different types of fungi, such as brown rot, white rot, and soft rot fungi, are commonly utilized to break down these biomass components, each employing unique mechanisms to do so.[61] As a result of the lower energy consumption, biological processes are always under research and areas of focus. Processing of the feedstock requires a high level of residence time, and the activity of the reaction also decreases with increasing temperature, since microorganisms are unable to withstand high temperatures.[62] The utilization of fungal and bacterial strains or their enzymes is a prominent approach in this method. It is gaining increasing attention owing to its remarkable ability to operate within a relatively short reaction time while requiring minimal nutritional resources for enzymatic reactions.[43,63] It is to be noted that fungal pretreatment necessitates an extended incubation period, ranging from weeks to months, whereas bacterial and enzymatic pretreatments can be accomplished within a few hours.[64] In the biological route, anaerobic digestion and fermentation are the two main categories. In anaerobic digestion, microorganisms break down biomass and lignocellulosic materials in an oxygen-free environment, resulting in the production of biogas, also known as biomethanation. Anaerobic digestion consists of four steps mainly (i) hydrolysis, where the initial breaking of polysaccharide molecules takes place, (ii) acidogenesis, where the products of hydrolysis are converted into volatile fatty acids, alcohols, hydrogen, and carbon dioxide, (iii) acetogenesis, where the volatile fatty acids and alcohols are further broken down into acetic acid, hydrogen, and carbon dioxide, and (iv) methanogenesis, where the acetic acid, hydrogen, and carbon dioxide are converted into methane and water by methanogenic archaea.[60,64] This process mimics the natural decomposition of organic matter, leading to the creation of a valuable fossil fuel alternative. By leveraging the potential of biological pretreatment methods and utilizing anaerobic digestion or fermentation, the biomass–bioCNG sector can effectively convert biomass resources into biogas, contributing to a sustainable and eco-friendly energy solution.

Furthermore, fungal pretreatment is extensively used as a pretreatment approach for lignocellulosic biomass.[61,65] The fungi may be categorized as cellulose or lignin degrading species, where cellulolytic fungi are mainly brown-rot-fungi[61] and for delignification, white rot fungi[65] are used during the pre-treatment process. For better CH_4 yield, several researchers have used fungi during their pre-treatment process. In a study conducted on corn silage, pretreatment with *Trametes versicolor* showed an improved production of biogas. Total solids (TS) of corn silage estimated were 35.75%. Corn silage was co-digested with cow manure along with corn grits under mesophilic conditions, whereas the methane production rate improved up to 0.236 m^3 CH_4 per kg VS from 0.167 m^3 CH_4 per kg VS of untreated corn silage.[66] Ref. 67 showed the impact of a combination of metals ($MnSO_4$; $CuSO_4$ and $FeSO_4$) along with the fungus *Polyporus brumalis* BRFM985 on wheat straw. They found an improved biogas production of 52%. V. Wyman *et al.*[68] reported and screened different fungi (*Pleurotus eryngii*, *Pleurotus ostreatus*, and *Trametes versicolor*) for their pretreatment productivity towards ligno-hemicellulosic biomass. According to the study the production of ligninolytic enzymes is high in *Pleurotus eryngii* resulting in a 19% increase in biogas yield. Several studies were reported on the disintegration of lignin and hemicellulose by different fungal species. The effects of moisture content on the pre conditioning of *Agropyron elongatum* with *Flammulina velutipes* were assessed, and at 65% optimum moisture content, a remarkably 120% higher biogas production was observed.[69] After 48 days of pretreatment of Albizia chips with *Ceriporiopsis subvermispora*, lignin removal from biomass reached a maximum of 24% and methane production increased by 3.7 times to approximately 123.9 L per kg VS.[70] The fungal pretreatment on rice straw for biogas production was explored using two species namely *Pleurotus ostreatus* and *Trichoderma reesei* by solid state anaerobic digestion. After the pre-treatment process, a methane yield of 263 L per kg VS achieved which was 120% higher than that of the untreated rice straw. Whereas, in another study over rice straw, pretreatment with *Trichoderma reesei* resulted in a methane yield of 214 L per kg VS.[71] Hence, fungal treatment can be an effective choice for disintegration of biomass and a subsequently high biogas yield.

Pretreatment of ligno-hemicellulosic biomass with microbial consortia is an efficient approach to improve biomass degradation. A consortium is a group of two or more diversified microorganisms living symbiotically. *Cellulomonas* and *Cytophaga* groups of bacteria and various fungi such as *Humicola*, *Penicillium*, *Aspergillus* and *Trichoderma* are studied mostly for their ability to secrete extracellular proteins.[72] In a study conducted in ref. 73, microbial consortia WSD-5 was used for pretreatment of Napier grass which improved the methane yield by 49% during the AD process. Consortia WSD-5 is a combination of fungal and bacterial communities, the main fungus is *Coprinus cinereus* and bacterium is a Gram negative, *Ochrobactrum* sp. Along with these, two more microbial consortia were studied for the pretreatment of Napier grass, XDC-2 and MC1. XDC-2 was primarily composed of genera *Clostridium*, *Bacteroides*, *Alcaligenes* and *Pseudomonas* and MC1 was composed of thermophilic bacteria, majorly cellulose degrading bacteria such as as *Clostridium straminisolvens*. The pretreatment of rice straw was performed with rumen fluid, which showed the improved biogas production with an 82.6% higher methane yield.[74] Also, C. Zhong *et al.*[75] studied pretreatment of wheat straw with a microbial consortium during the AD process and obtained an 80.34% higher methane yield. A degradation study was reported on catalpa saw dust (cellulose 45.89%, hemicellulose 18.11%, and lignin 21.75%) with the help of two consortia such as an aerobic consortium (CS-5) and an anaerobic consortium (BC-4) to enhance biogas production.[76] The CS-5 aerobic consortium consists of individual species such as *Micrococcus luteus* SR-1, *Citrobacter freundii* SR-3, *Exiguobacterium acetylicum* SR-5, *Acidisoma tundrae* strain SR-14 and *Dyella* sp. Strain SR-16 whereas BC-4 has *Thermoanaerobacterium aciditolerans* strain SR-4, *Ruminococcus flavefaciens* strain SR-7, *Caproiciproducens galcititolivorans* strain SR-8 and *Methanobrevibacter thaueri* stain SR-13. The synergistic effect of both the consortia CS-5 and BC-4 resulted in a 113.7% higher methane yield. Under thermophilic conditions due to high lignocellulolytic enzyme activities, anaerobic digestion of ligno-hemicellulosic biomass was advantageous for its degradation and subsequent high yield of CH_4 production.[77]

The microbial pretreatment approach has been of utmost priority among the scientific community, and is basically centered around the use of either a single microbe or a consortium of microbes to treat the feedstock before it undergoes the AD process. For instance, S. O. Thong *et al.*[78] investigated the enzymatic hydrolysis of cassava starch waste water using a thermophilic mixed culture (natural microbial consortia of samples of a hot spring located in Southern Thailand) and observed a significant increase in biohydrogen production. Similarly, fermentation of cassava wastewater by using *Clostridium acetobutylicum* resulted in up to 42% COD (chemical oxygen demand) removal and COD concentrations of 10.7, 7.5, and 5 g L^{-1} provided 1.34, 1.2 and 2.41 mol H_2 per mol of glucose.[79] Again, according to ref. 80 rumen bacteria isolated from a barn for the treatment of cow manure feedstock resulted in a CH_4 enhancement of 103.3% (CH_4 yield: 138 mL per g VS). The effectiveness of the xylanase enzyme isolated from *Aspergillus niger* LC1 in saccharifying various feedstocks was investigated. Feedstocks including barley husk, groundnut shell, pearl millet husk, rice husk, rice straw, sugarcane bagasse, wheat bran, and wheat straw were studied and, the highest saccharification yield of up to 34.5% was achieved with rice straw.[6] K. Sophanodorn *et al.*[81] used 2% (2% v/v) of cellulase enzyme with 2398 units per g, β-glucosidase 577 units per g, and pH 4 (Union Science Company, Chiang Mai Thailand) over hydrothermally pretreated dried tobacco stalks and on tobacco stalk residue, which resulted in total and reducing sugar concentrations of dried tobacco stalks of 27.97 g L^{-1} and 5.43 g L^{-1}, respectively. Also, it was observed that when hydrothermal pretreatment was applied, the total sugar (complex sugar) concentration increased. Meanwhile, the reducing sugar (simple sugars) was observed to be lower compared to that in the untreated biomass with values ranging from 4.07 to 4.55 g L^{-1} according to the study.

The microbial community residing in the rumen, comprising bacteria such as *Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, *Roseovarius albus*, and others, possesses the ability to attach to lignocellulosic materials. These microorganisms produce enzymes that effectively remove the waxy layer and lignin, facilitating the hydrolysis of approximately 65% of cellulose within a span of 48 hours.[11,82] Also, the pretreatment of unsterilized yard trimming by *C. subvermispora* followed by anaerobic solid-state digestion yielded 44 L kg^{-1} of methane. T. Rangseesuriyachai *et al.*[43] studied anaerobic co-digestion of elephant dung (ED) and Napier grass (NG) with and without the biological pretreatment approach at various mixing ratios. The biological pretreatment of Napier grass was performed using the microbial activator super LDD1 (mixed culture microbial sludge) enzyme before further biogas production. The results confirmed that LDD1 enzymatic microbes increased methane production capacity by 1.95 times compared to untreated napier grass. Furthermore, the findings from the data analysis demonstrated that employing a NG/ED ratio of 1 : 1 for a 14-day pretreatment period resulted in the highest cumulative methane production, reaching 234.8 ± 5.9 mL CH_4 per g VS. This represents a substantial increase of 99.2% compared to the baseline. In a comprehensive investigation, L. A. Fdez-Guelfo *et al.*[83] explored the composting pretreatment of the organic fraction of municipal solid waste (OF-MSW) within a thermophilic dry batch anaerobic digestion (AD) system. Their findings indicated a substantial enhancement in the specific microbial growth rate, ranging from 160% to 205%. This suggests that the composting pretreatment can significantly promote the microbial activity in the subsequent AD process. In contrast, previous studies, such as that conducted in ref. 84, reported a reduction in volatile solids by 19.5% during composting, alongside a 40% loss of methane, emphasizing the complexity and variability of these processes. Y. Ueno *et al.*[85] furthered the investigation by examining synthetic OF-MSW subjected to pre-hydrolysis pretreatments under both mesophilic and thermophilic conditions. Their results revealed that mesophilic pre-hydrolysis was more efficient in hydrogen production, whereas thermophilic pre-hydrolysis led to better solubilization. Additionally, a continuous two-stage AD system achieved the highest methane production, reaching 341 mL CH_4 per g VS. Furthermore, during thermophilic pre-hydrolysis of OF-MSW in a continuous two-stage AD system, C. Escamilla-Alvarado *et al.*[86] reported an impressive 81.5% COD removal with 95.7% utilization of volatile suspended solids (VSS). This process resulted in twice the biogas production compared to the untreated sample, as demonstrated in ref. 85.

Numerous aerophilic bacterial species have been studied and stated to have high degrading potential towards ligno-hemicellulosic biomass with the foremost advantage of a faster growth rate than fungi.[87] V. B. Barua *et al.*[88] reported a bacterial strain isolated from the gut of silverfish such as *Citrobacter werkmanii* VKVVG4 which enhanced the solubilization of water hyacinth during anaerobic digestion and also showed a cumulative biogas production of 3737 ± 21 mL for pretreated water hyacinth on the 50th day. The co-culture of *Bacillus* sp. with rice straw showed a substantial reduction in lignin content and 76% rise in methane production.[89] Along with aerophilic bacterial treatment, microaerobic pretreatment also exists. Commonly microaerobic refers to giving little oxygen to the digestion system to boost the rate of hydrolysis and acidification.[90] It is being recommended to execute the microaerobic treatment at the pretreatment stage to avoid the risk of mixing of oxygen with biogas during the AD process. Some of the biogas ventures have implemented microaerobic technology to achieve the best methane yields.[91] During these large-scale biogas projects, livestock manure and biogas slurry can be chosen as a microaerobic inoculum.[92] Studies showed that on utilization of cow manure as a microbial additive during microaerobic pretreatment, the rate of hydrolysis of ligno-hemicellulosic biomass increased by two times and estimated VFAs were 6–7 g L^{-1} resulting in 419 mL per g VS at 5%.[93] The utilization of microaerobic technology at the pretreatment stage is a simple and user-friendly approach. Table 1 represents all types of biological pretreatments mainly used for disintegration of biomasses used and their CH_4 yield capacity.

It is well known that enzyme pretreatment has a quick rate of reaction and very slight loss of sugars during digestion due to which it becomes more and more appealing. Before anaerobic digestion, exogenous application of enzymes of the hydrolytic or oxidative classes can hasten the degradation of ligno-hemicellulosic biomass under anaerobic conditions.[114] The biological breakdown of cellulose takes place in the presence of enzymes with exoglucanase, endoglucanase and β-glucosidase activities whereas hemicellulose breakdown involves a lot of enzymes such as *endo*-xylanase, *endo*-mannanase and α-glucuronidase.[5,115] There are various factors on which efficiency of enzyme pretreatment is based on such as the activity of the enzyme, enzyme specificity towards the substrate, tolerance of the enzyme to various inhibitors, amount of enzyme used for the treatment, incubation time, the anaerobic digestion system, enzymatic stability at several temperatures and pH.[116,117] The enzyme pretreatment approach can enhance biogas production from recalcitrant ligno-hemicellulosic biomass.[114] Y. Lin *et al.*[110] studied pulp & paper sludge, and observed the effect of endoglucanase and laccase enzymes isolated from *Pleurotus ostreatus*, and obtained 34% higher methane yield. In another pretreatment study reported on corn stover, cellulase improved the rate of substrate breakdown and better biogas was produced by up to 36.9% after 24 h of incubation time.[105] K. Ziemiński *et al.*[106] investigated the utilization of spent hops pretreated with a combination of enzymes endoglucanase, xylanase and pectinase

Table 1 Biological pre-treatment effects on biogas/methane yield from available recent data in the literature

Pre-treatment methods	Microorganism used/inoculum	Biomass	AD conditions	Effect on methane or biogas production	References
Fungal pre-treatment	*Polyporus brumalis* and *Trametes versicolor*	Wheat straw	Batch, 36 °C, 57 days	52% higher methane yield	66 and 67
		Corn silage	Semi-continuous, 37 °C, 21 days	Methane generation rate 0.236 m^3 CH_4 per kg VS (control 0.167 m^3 CH_4 per kg VS)	
	Pleurotus eryngii	Corn stover	Batch, mesophilic, 40 days	19% higher biogas production	68
	Flammulina velutipes	*Agropyron elongatum* (tall wheat grass)	Batch, 37 °C, 24 days	120% higher biogas production	69
	Pleurotus ostreatus	Rice straw	Batch (SS), 37 °C, 45 days	120% higher methane yield	71
	Trichoderma reesei	Rice straw	Batch (SS), 37 °C, 45 days	78.3% higher methane yield	71
	Ceriporiopsis subvermispora	Albizia chips	Batch (SS), 37 °C, 58 days	3.7-fold higher methane yield	70
	P. flavido-alb	Grass of verge		No effect	94
	Ceriporiopsis subvermispora	Yard trimmings	Batch (SS), 37 °C, 45 days	106% higher methane yield	95
Bacterial pre-treatment	Composting	OF-MSW	Mesophilic dry batch	19.55 VS loss, 40% loss of methane	84
	Composting	OF-MSW	Thermophilic dry batch	160–205% higher specific microbial growth rate	83
	Mesophilic and thermophilic pre-hydrolysis	OF-MSW (synthetic)	Mesophilic and thermophilic (continuous 2-stage)	Mesophilic resulted in better hydrogen production, while thermophilic resulted in better solubilization	86
	Thermophilic pre-hydrolysis	OF-MSW (synthetic)	Thermophilic (continuous 2-stage)	81.5% COD removal with 95.7% VSS destruction and 2 times higher biogas production	85
	Thermophilic pre-hydrolysis	Food waste	Mesophilic AD	61.3% VS destruction, 280 mL per g VS methane yield	96
	Mesophilic culture pre-hydrolysis	Food waste	Mesophilic continuous (2 stage system)	9% and 13% higher biogas production than mesophilic and thermophilic AD	97
	Bacillus sp.	Rice straw	Batch (SS), 37 °C, 50 days	76% higher biogas production	89
	Bacillus subtilis	Corn straw	Batch (SS), 37 °C, 50 days	17.35% higher methane yield	87
	Citrobacter werkmanii VKVVG4	Water hyacinth	Batch (SS), mesophilic, 80 days	3.07 times higher biogas production	88
	Anaerobic inocula (manure & crops)	Grass silage	55 °C, 63 days	405 $mLCH_4$ per g VS	98
	Anaerobic sludge	*Typha latifolia*	Batch assay, 37 °C, 60 days	151 mL CH_4 per g VS	99
	Cattle dung	Duckweed (aquatic plant) : cattle dung in a 1 : 1 ratio	Batch assay, 38 °C, pH 7.2	580 mL per days	100
	Biogas slurry	Rice straw	35 ± 1 °C	233.3 mL methane per g VS	101
	Anaerobic sludge	*Potamogeton maackianus*	Semi continuous operation	255.9 mL CH_4 per g VS	102
	Bioaugmentation with *Piromyces rhizinflata* YM 600	Corn silage and cattail	60 days	No effect on the improvement of methane production, improved the VFA degradation rate	99

Table 1 (*Contd.*)

Pre-treatment methods	Microorganism used/inoculum	Biomass	AD conditions	Effect on methane or biogas production	References
Microbial consortium	Microbial consortium TC-5	Wheat straw	Batch, 45 °C, 35 days	36.6% higher methane yield	77
	Microbial consortium	Saw dust	Batch, mesophilic, 28 days	25.6% higher biogas production	103
	Rumen fluid	Rice straw	Batch, 35 °C, 30 days	82.6% higher methane yield	74
	Microbial consortium MC1	Napier grass	NA	39% higher methane yield	104
	Microbial consortium WSD-5	Napier grass	NA	49% higher methane yield	
	Microbial consortium XDC-2	Napier grass	NA	32% higher methane yield	
	Microbial consortium CS-5 & BC-4	Catalpa sawdust	50 °C	113.7% improved methane	103
Enzyme pre-treatment	Microbial consortium	Wheat straw	Batch, 37 °C, 20 days	80.34% high methane yield	75
	Cellulase	Corn stover	Batch, 37 °C, 18 days	36.9% higher biogas production	105
	Endoglucanase + xylanase + pectinase	Spent hops	Semi-batch, 37 °C	13% higher biogas production	106
	Cellulase + cellobiase	Switch grass	Batch, 50 °C, 30 days	Methane yields 274.28 mL per g (VS), (control 197.39 mL per g (VS))	107
	Endoglucanase + xylanase + pectinase	Sugar beet pulp silage	Batch, 37 °C, 30 days	27.9% higher biogas production	108
	Laccase (*Trametes versicolor*)	Willow	NA	33% higher methane yield	109
	Endoglucanase/laccase (*Pleurotus ostreatus*)	Pulp & paper sludge	37 °C, 50 days	34% higher methane yield	110
	Endoglucanase + exoglucanase + xylanase	Sorghum forage	Batch, 35 °C, 30 days	15% higher methane yield	111
	Lipase (*Candida rugosa*)	Butter	NA	84% methane yield improved	112
	Laccase	Corn stover	Batch, 37 °C, 30 days	25% higher methane yield	113
	Cellulolytic enzymatic cocktail (novozymes)	Corn cob	Mesophilic	14% higher methane yield	104
	Mn peroxidase + versetile peroxidase	Corn stover	Batch, 37 °C, 30 days	17% higher methane yield	113

(volume ratio of 3 : 1 respectively) which aids in an improved biogas production of 13%. Lignin was a hindrance to improve pretreatment efficacy when ligno-hemicellulosic biomass was pretreated by laccases and versatile peroxidases. Willow was pretreated with laccase isolated from *Trametes versicolor* which showed a 33% higher methane yield.[109] The ideal incubation conditions for these enzymes are 37–50 °C and 2–72 h. Limited use and high cost of commercial enzymes is the major reason for inadequate applications during digestion of ligno-hemicellulosic biomass. Lately, the solid-state digestion method, which relied on agricultural and industrial waste as the primary medium components to produce affordable enzymes was proposed.[68]

5. Physical pretreatment towards effective digestion

The physical pretreatments are those methods that do not use external compounds like chemicals or microbes during the process. This approach can increase the surface area of ligno-hemicellulosic biomass by reducing the particle size.[6] Size reduction can encourage the digestion of biomass during the AD process by making it more accessible to microbial and enzymatic exposure. Prominently, physical pretreatments do not produce any noxious compounds, which constrain the AD process.[118,119] Physical pretreatment includes mechanical, irradiation and microwave-based methods.[6,120] Mechanical pretreatment methods have also gained attention in OF-MSW processing. Researchers have employed techniques such as rotary drums, disc screens, screw presses, and shredders with magnetic separation. B. Zhu *et al.*[121] reported methane yields ranging from 457 to 557 mL CH_4 per g VS and methane content between 57.3% and 60.6% through rotary drum pretreatment, sometimes combined with semi-composting. Similarly, T. Subramani *et al.*[122] observed 18–36% higher biogas production with the same approach. T. L. Hansen *et al.*[123] explored the use of screw presses, disc screens, and shredders with magnetic separation for mechanical pretreatment of sorted OF-MSW

Table 2 Physical pre-treatment effects on biogas/methane yield from available recent data in the literature

Substrates	Particle size	Methane yield (untreated)	Methane yield (after treatment)	Reaction system	References
Barley straw	5 mm	240 mL per g VS	370 mL per g VS	Glass reactor 2 L	130
	20 mm	240 mL per g VS	339 mL per g VS		
	50 mm	240 mL per g VS	286 mL per g VS		
Crop feedstocks (winter rye, sorghum, forage rye, maize, and triticale)	6–33 mm	278 mL per g ODM	403 mL per g ODM	Stirred tank reactor 3 L	128
Maize stalks	2 mm	246 mL per g VS	272 mL per g VS	Glass reactor 2 L	130
	20 mm	246 mL per g VS	254 mL per g VS	Glass reactor 2 L	
	2 mm	297 mL per g VS	376 mL per g VS	Bottle 0.5 L	133
OF-MSW	16 mm (shredder with magnetic separation)	487 mL CH_4 per g VS	NA	Thermophilic batch	123
	16 mm (use of disc screen)	428 mL CH_4 per g VS	NA	Thermophilic batch	
	16 mm (use of screw press)	461 mL CH_4 per g VS	NA	Thermophilic batch	
Meadow grass	<200 mm	—	347 mL per g VS (increase 20%)	Bottle 0.5 L	11
	0–200 mm	303 mL per g VS	372 mL per g VS	Bottle 0.5 L	134
	0.3 mm	58.1 mL per g VS	62.7 mL per g VS	—	131
Rice straw	0.75 mm	58.1 mL per g VS	65.7 mL per g VS	—	
	50 mm	197 mL per g VS	203 mL per g VS	Glass reactor 2 L	130
Switch grass	2–10 mm	127.4 mL per g VS	170.7 mL per g VS	Bottle 0.5 L	135
	0.001 mm	—	Increase 20% (from 50 to 70%)	Digester 0.45 L	136
Water hyacinth	0.05 mm	—	Increase 16% (from 50 to 66%)	Digester 0.45 L	136
	1.0 mm	—	Increase 10% (from 50 to 60%)	Digester 0.45 L	
	2.5 mm	—	Increase 5% (from 50 to 55%)	Digester 0.45 L	
	0.3 mm	167.8 mL per g VS	245.6 mL per g VS	Reactor 2 L	132
	1.2 mm	167.8 mL per g VS	264.7 mL per g VS	Reactor 2 L	
	0.3 mm	67.1 mL per g VS	70.3 mL per g VS	—	131
Wheat straw	0.75 mm	67.1 mL per g VS	93.1 mL per g VS	—	
	0.088–0.759 mm	183.4 mL per g VS	252.8 mL per g VS	Lab flask	137
	2 mm	182 mL per g VS	334 mL per g VS	Glass reactor 2 L	130
	50 mm	182 mL per g VS	285 mL per g VS	Glass reactor 2 L	138
Wastepaper	60 min of beating time	132 mL per g VS	215 mL per g VS	Flask 0.5 L	104

Table 3 Chemical pre-treatment effects on biogas/methane yield from available recent data in the literature

Substrate	Pre-treatment conditions	Methane yield (untreated)	Methane yield (after pre-treatment)	Reaction system	References
Corn straw	Sulfuric acid 2% v/v	100.6 mL per g VS	175.6 mL per g VS	Flask 1 L	152
	Hydrochloric acid 2% v/v	100.6 mL per g VS	163.4 mL per g VS		
	Acetic acid 4% v/v	100.6 mL per g VS	145.1 mL per g VS		
	Hydrogen peroxide 3% v/v	100.6 mL per g VS	216.7 mL per g VS		
	Sodium hydroxide 8% v/v	100.6 mL per g VS	163.5 mL per g VS		
	Calcium hydroxide 8% v/v	100.6 mL per g VS	206.6 mL per g VS		
	Ammonia 10% v/v	100.6 mL per g VS	168.3 mL per g VS		
Pennisetum hybrid	4% NaOH, 37 ± 0.5 °C	249.3 mL per g VS	281.4 mL per g VS	NA	153
Food waste	Addition of HCl until pH = 2 at room temperature	13 ± 7% higher COD solubilization and 48% higher biogas production	NA	Thermophilic batch	154
Household waste	KOH until pH = 10, 70 °C for 60 min	Methane yield of 500 mL CH_4 per g VS, no enhancement due to pretreatment	NA	Thermophilic batch	155
OF-MSW	Alkaline	11.5% higher COD solubilization, methane	Methane yield of 0.15 m^3 CH_4 per kg VS (172% higher than untreated)	1 L batch reactor	148
Corn stalk	2% NaOH, 35 ± 1 °C	187 mL per g VS	196 mL per g VS	NA	156
Cotton gin waste	Citric acid 0.5 mmol per g VS substrate	95.4 mL per g VS	147.1 mL per g VS	Flask 0.25 L	157
	Hydrogen peroxide 0.5 mmol per g VS substrate	178.0 mL per g VS	247.5 mL per g VS	Flask 0.25 L	
	Ethanol 0.5 mmol per g VS substrate	172.5 mL per g VS	241.5 mL per g VS	Flask 0.25 L	
	Citric acid 0.5 mmol per g VS substrate	180.4 mL per g VS	353.5 mL per g VS	Flask 0.25 L	
	Hydrogen peroxide 0.5 mmol per g VS substrate	275.0 mL per g VS	385.6 mL per g VS	Flask 0.25 L	
Olive pomace	Ethanol 0.5 mmol per g VS substrate	214.3 mL per g VS	332.8 mL per g VS	Flask 0.25 L	157
	Citric acid 0.5 mmol per g VS substrate	200.1 mL per g VS	183.2 mL per g VS		
	Hydrogen peroxide 0.5 mmol per g VS substrate	178.7 mL per g VS	172.3 mL per g VS		
	Ethanol 0.5 mmol per g VS substrate	193.0 mL per g VS	157.5 mL per g VS		
	Sulfuric acid 2% v/v	11.2 mL per g VS	16.6 mL per g VS	Bottle 0.6 L	143
Salvinia molesta	Sulfuric acid 4% v/v	11.2 mL per g VS	17.4 mL per g VS	Bottle 0.6 L	
	Sulfuric acid 6% v/v	11.2 mL per g VS	17.8 mL per g VS	Bottle 0.6 L	
Switch grass	Sodium hydroxide 7 g L^{-1}	112.4 mL per g VS	132.5 mL per g VS	Bottle 0.5 L	135
Water hyacinth	Sulfuric acid 5% v/v; 60 min residence time	58 mL per g VS	64 mL per g VS	Flask 1 L	158
	NMMO 85% 120 °C; 3 h	274 mL per g VS	304 mL per g VS	Serum bottles 0.125 L	159
Wheat straw	Ethanol 50% 180 °C; 1 h	274 mL per g VS	316 mL per g VS	Serum bottles 0.125 L	159
	Sodium hydroxide 1.6% w/w 30 °C; 24 h	274 mL per g VS	315 mL per g VS	Serum bottles 0.125 L	
	Citric acid 0.5 mmol per g VS substrate	159.1 mL per g VS	163.7 mL per g VS	Flask 0.25 L	157
Winery waste	Hydrogen peroxide 0.5 mmol per g VS substrate	171.3 mL per g VS	190.2 mL per g VS	Flask 0.25 L	157
	Ethanol 0.5 mmol per g VS substrate	190.7 mL per g VS	239.5 mL per g VS	Flask 0.25 L	

under thermophilic batch conditions, achieving methane yields of 461 mL CH_4 per g VS, 428 mL CH_4 per g VS, and 487 mL CH_4 per g VS, respectively. Mechanical pretreatment is a familiar method to improve biogas production, though it is still considered to be an expensive method, due to its high energy requirements.[124] There are widely used mechanical techniques for pretreatment which are chipping, grinding, milling, knife milling, hammer mill, extrusion, *etc.*

Several studies were reported on the effect of particle size of different feedstocks. S. K. Sharma *et al.*[125] reported and studied the effect of different particle sizes 0.088, 0.40, 1.0 and 6.0 mm of numerous agricultural residues like wheat straw, rice straw, cauliflower leaves, *Ipomoea fistulosa* leaves, banana peel, *etc.* and obtained the highest biogas yield with a particle size of 0.88 mm with *Ipomoea fistulosa* leaves followed by cauliflower leaves. M. A. De la Rubia *et al.*[126] carried out a study on sunflower oil cake feedstock, and reported the maximum methane yield of 17% at a particle size of 1.4–2.0 mm. Some of the reports are on the different mechanical pretreatments which tested the particle size in the range of 0 to >20 cm.[127] The study indicates no significant increase in methane yield against the untreated biomass. Correspondingly, C. Herrmann *et al.*[128] compared various particle lengths of the same feedstock and discovered a strong link between particle length and methane yield. N. Pérez-Rodríguez *et al.*[104] compared 30 and 60 min duration of pretreatment, and found a 21% rise in methane yield for 60 min whereas no effect was recorded for 30 min. S. Sumardiono *et al.*[129] studied the effect of bagasse size reduction up to 0.85 mm (22 mesh) with the use of cow dung as an inoculum. The study resulted in the highest biogas yield of 51.04 L kg^{-1} substrate from a combination bagasse treated with 2% NaOH solution for 24 hours and as much as 20% cow's rumen. The difference between experimental and control groups can be summarized as, the experimental groups involving longer pretreatment durations and specific size reduction and treatment methods showed significant improvements in methane and biogas yields, whereas the control groups showed little to no improvement. According to ref. 127, importantly, the equipment's speed must be taken into account in order to reduce the cost of energy used during physical pretreatments. S. Menardo *et al.*[130] carried out a study on barley straw and reported an increase of 54% CH_4 yield at 5 mm particle size and 41% methane yield at 20 mm particle size separately. Additionally, it was found that methane yield decreased with particle sizes greater and smaller than the range of 2–5 mm.[130–132] The methane yield with several feedstocks pretreated mechanically is presented in Table 2.

6. Chemical approach as a pre-treatment approach

The most preferred approach is chemical pretreatment of ligno-hemicellulosic biomass with chemicals like acids, alkalis and solvents because they can be very effective at degrading complex substrates.[139,140] Acids and alkalis are commonly used to solubilize the hemicellulose and lignin present in biomass. Several studies demonstrated that the most promising method for treating ligno-hemi-cellulose is the dilute acid treatment.[45,141] M. Germec *et al.*[140] discovered that 1.58% v/v of dilute sulphuric acid (DSA) was the ideal acid concentration for the pretreatment of spent tea leaves. Furthermore, in the study, it was also observed that 131 °C for 20 min is more effective than 121 °C for 1 h in terms of temperature and reaction time respectively which was also suggested in ref. 142. I. Syaichurrozi *et al.*[143] suggested dilute acid pretreatment under room temperature conditions for a duration of 2 days. Besides acid treatment, alkaline pre-treatment is also commonly used to disrupt the biomass surface layer. During delignification of biomass, alkaline pretreatment causes the cell wall to swell, increasing the internal surface area while simultaneously reducing the degree of polymerization and cellulose crystallinity.[10,22] To boost the methane yield, researchers in ref. 144 and 145 studied and optimized the NaOH loading rate. During the pretreatment of giant reed with varied NaOH concentrations between 0.5% and 2% w/v an increase in methane yield at 2% NaOH from 217 mL per g VS to 355 mL per g VS was observed. M. S. Romero-Güiza *et al.*[146] reported that 0.7% w/w KOH pretreated wheat straw produced 128% more methane than untreated wheat straw. Other studies were investigated with different concentrations of $Ca(OH)_2$ at 6%, 8%, 10%, 12% and 15% out of which the maximum biogas production was found for 8% and 10% pretreated rice straw which also recorded the biogas production value of 34.3% and 36.7% higher than that of the control.[147] Numerous studies have also explored the effects of chemical pretreatment on OF-MSW. M. Lopez Torres *et al.*[148] investigated alkaline $[Ca(OH)_2]$ pretreatment and observed an 11.5% higher COD solubilization. They achieved a methane yield of 0.15 m^3 CH_4 per kg VS, which was 172% higher than that of the untreated sample. Conversely, another study used HCl addition at pH 2 under room temperature conditions and achieved a 13 ± 7% higher COD solubilization and a 48% increase in biogas production. Water hyacinth was tested in ref. 149 and reported to have a 97.6% higher methane yield when pretreated with 1-*N*-butyl-3-methyimidazolium chloride/dimethyl sulfoxide (DMSO) at 120 °C for 120 min. N. R. Katukuri *et al.*[150] considered the pretreatment of *Miscanthus floridulus* by using 0.8% of H_2O_2 and recorded 49% increment in methane yield over the untreated substrate. Similarly, B. R. A. Alencar *et al.*[151] explored a method to recover H_2O_2 which was used during the pretreatment, and also tested the efficacy of recovered H_2O_2 for reusing it. Methane yields from different residues after chemical pretreatment are presented in Table 3 and Fig. 3.

7. Hypothetical combined physical and biological strategy for efficient biogas yield

Biogas production from biomass is a sustainable and renewable energy solution that holds immense potential to address global energy and environmental challenges. Combining physical and biological methods in the biogas production process can enhance efficiency, optimize yields, and ensure a more

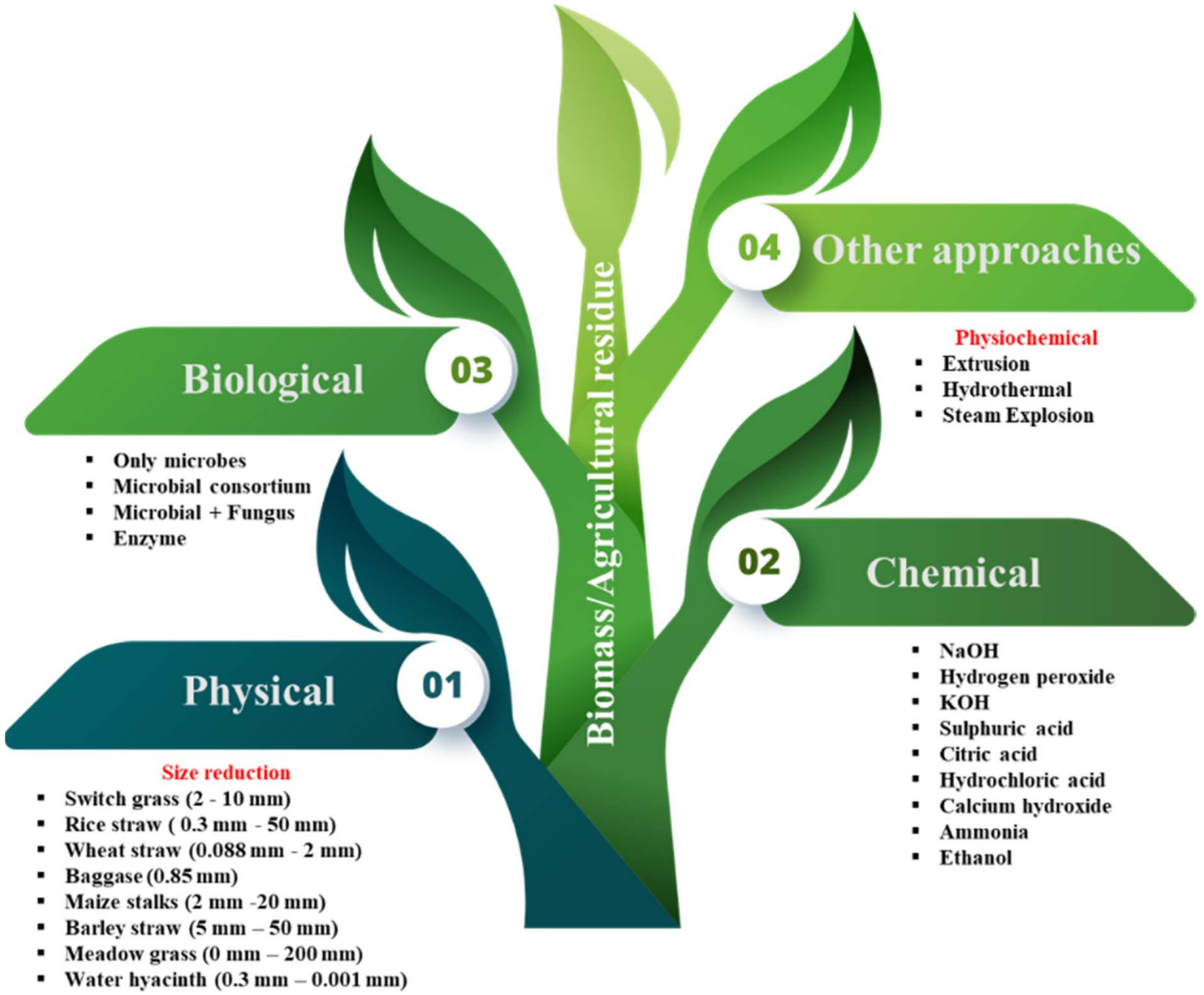

Fig. 3 Preferred pre-treatment process for efficient biogas yield as per literature studies cited in this article.

sustainable and economically viable approach for harnessing this valuable energy source. In this hypothetical scenario, a cutting-edge biogas production system is being considered that integrates physical and biological techniques to maximize the decomposition of organic matter and subsequent biogas yield. This innovative approach seeks to overcome the limitations of conventional anaerobic digestion systems and significantly improve the conversion of biomass into biogas.

The physical aspect of the proposed system focuses on pre-processing biomass before its introduction into the anaerobic digestion process. According to several studies mentioned, advanced mechanical techniques, such as hydrothermal pretreatment after mechanical milling through use of a shredder (one way or through use of two shredders, primary and secondary) that can convert the biomass up to average 2–5 mm size, can effectively disrupt the complex lignocellulosic structures of biomass. Furthermore, using hydrothermal treatment at temperatures up to 90 °C for several hours to a day, followed by cooling the pulpy slurry to the mesophilic range in a mixing tank, increases the surface area and creates a pulpy biomass. This process enhances the accessibility of substrates for microbial degradation within the mixing tank. Optimizing the pulpy material after hydrothermal treatment will also help to maintain the temperature inside the reactor, which subsequently maintains the growth of the population for anaerobic microorganisms (especially methanogens). Additional pre-treatment such as the use of an optimized microbial consortium may be applied inside the mixing tank that will enhance the biogas yield. This approach may be more suitable for complex lignocellulosic materials such as rice straw, sorghum grass, *etc.* However, from an economical point of view large plants with this approach still need to be addressed properly. But, in the successful longer run of the plants, this technology seems more suitable as compared to existing processes.

Consequently, the breakdown of complex organic compounds becomes more efficient, accelerating the overall biogas production process. To complement the physical approach, the hypothetical system incorporates a tailored consortium of specialized microorganisms (*Bacillus*, *Pseudomonas*, and *Citrobacter* are the most commonly preferred) during the biological (hydrolytic) phase. The microbial community is carefully selected and optimized (with the same feedstock on which the methanogens are going to act) to efficiently decompose different types of biomasses, ensuring a diverse and robust population. Genetic engineering and synthetic biology techniques could also be employed to enhance the metabolic capabilities of the microorganisms, making them more efficient in converting specific organic compounds into biogas. Additionally, a carefully balanced nutrient (macro, micro and optimized vitamin solution) supply and pH control system would be implemented to maintain favorable conditions for the microbial community throughout the anaerobic digestion process. By closely monitoring and adjusting these parameters, the hypothetical system would create an environment conducive to increased biogas production while minimizing the risk of process inhibition. Moreover, the integration of sensors and real-time monitoring systems would enable precise control and optimization of the process. These sensors would provide valuable data on key parameters like temperature, pH, and biogas composition. Artificial intelligence algorithms could be employed to analyze this data and fine-tune the operation of the system, leading to continuous improvements in biogas yield and system efficiency. Furthermore, the hypothetical approach emphasizes the use of a diverse range of biomass feedstocks (like herbs, shrubs, *etc.*). This ensures a constant supply of organic material to the system and reduces dependence on specific feedstock sources. By incorporating agricultural residues, food waste, and organic by-products from various industries, the system enhances the circular economy and contributes to waste reduction.

Biological additives play a vital role in scrubbing hydrogen sulfide (H_2S) and carbon dioxide (CO_2) during anaerobic digestion processes. One such example is the use of sulfur-oxidizing bacteria, like *Thiobacillus*, which convert H_2S into elemental sulfur or sulfate, reducing the odorous and corrosive effects of H_2S.[160,161] Additionally, methanotrophic bacteria, such as *Methylococcus capsulatus*, consume methane and CO_2, preventing methane loss and mitigating greenhouse gas emissions.[162,163] These biological additives enhance the overall efficiency of anaerobic digestion by reducing environmental hazards, ensuring stable biogas production, and promoting a sustainable waste-to-energy conversion process.

Another approach can be pretreatment of chopped biomass with boiling water followed by the addition of a hydrolytic culture and incubation, which has been expected to be a method to enhance biogas production in anaerobic digestion. The proposed pretreatment approach is relatively simple and can be implemented using conventional equipment. Boiling water is readily available and easy to use, making it feasible for small-scale biogas production units (not sure about big plants). Additionally, the optimized hydrolytic culture developed in the laboratory, provides flexibility in its application. This approach can be insightful in a number of ways such as, boiling water pretreatment can break down the lignocellulosic structure of the biomass, increasing its surface area and making it more accessible to hydrolytic enzymes. This results in higher

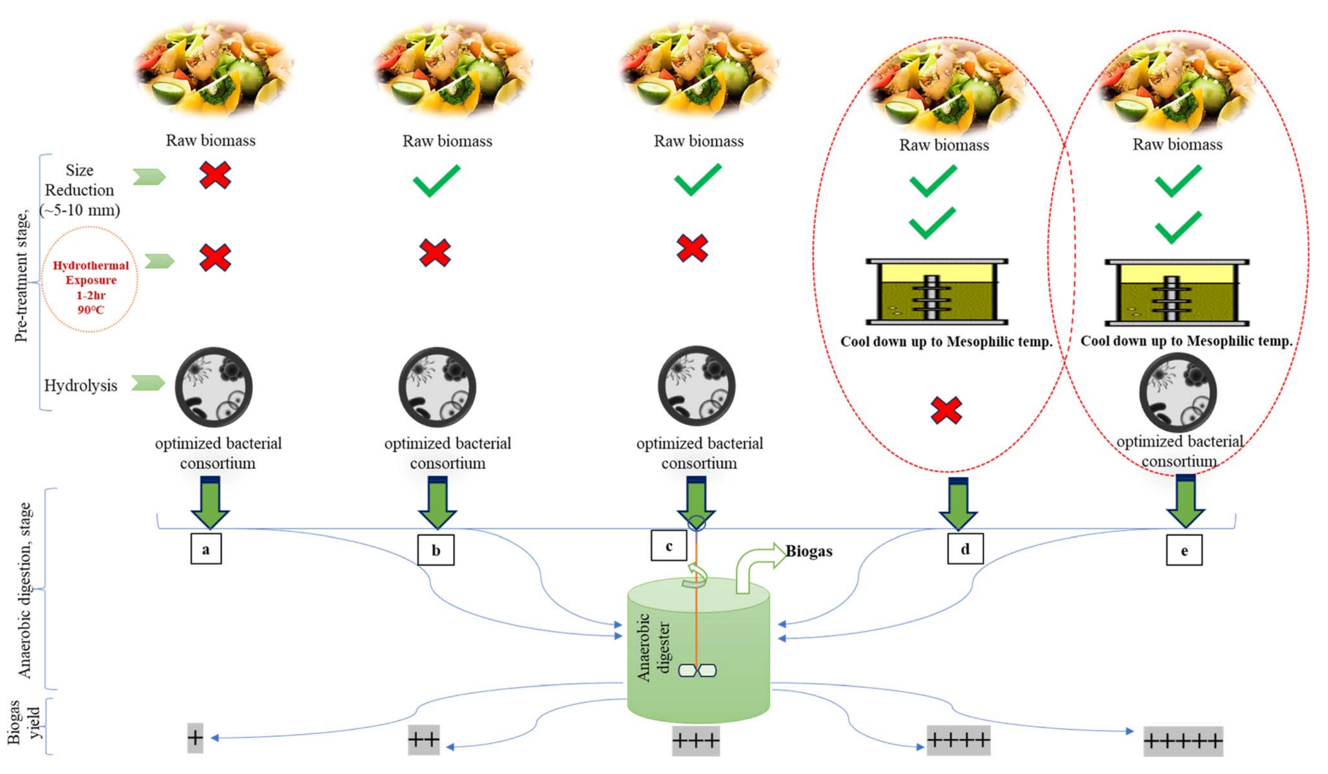

Fig. 4 Hypothetical pre-treatment process for efficient biogas yield, where steam exposure can be an effective method (+ sign denotes increasing efficiency). (a) No use of mechanical and hydrothermal pre-treatment technology; (b) use of mechanical pretreatment and microbial pretreatment technology but not hydrothermal technology; (c) no use of mechanical pretreatment but use of hydrothermal and microbial treatment approach; (d) use of mechanical, hydrothermal but not microbial pre-treatement; (e) use of mechanical, hydrothermal and microbial pretreatment technology.

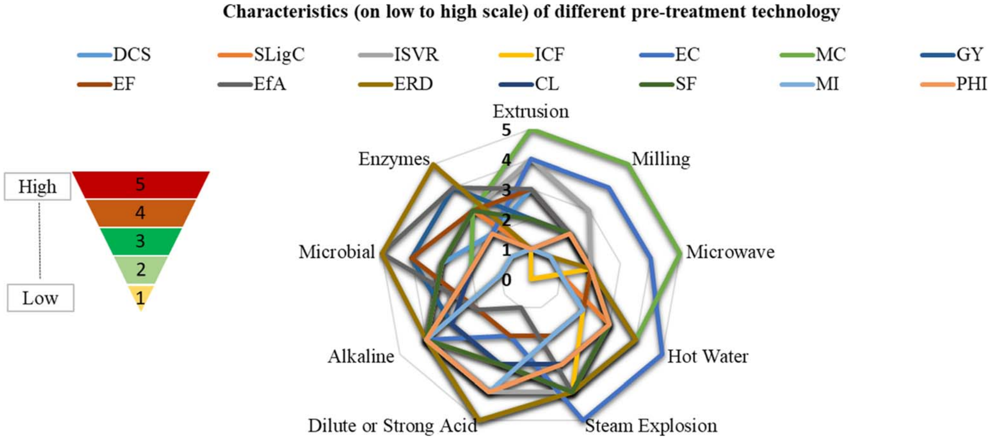

Fig. 5 Conceptualization based on literature studies of different characteristics (low to high scale) in pre-treatment technologies, where DCS = destruction of cellulose; SLigC = dolubilization of ligno-hemicellulosic components; ISVR = increase in the surface to volume ratio; ICF = inhibitor compound formation; EC = energy consumption; MC = maintenance cost; GY = gas yield; EF = economical feasibility; EfA = eco-friendly approach; ERD = establishment of R&D facilities; CL = carbon loss; SF = slow/fast process; MI = methanogen inhibition; PHI = pH imbalance.

efficiency during the subsequent hydrolysis step. By introducing an optimized hydrolytic culture, the breakdown of complex organic compounds into simpler sugars is accelerated. This improves the availability of substrates for anaerobic microorganisms, leading to increased biogas production.

Boiling water is a low-cost pretreatment method, and the hydrolytic culture can be obtained at a relatively low cost or even cultured in-house, making it economically viable. The use of an optimized hydrolytic culture and boiling water pretreatment can help in the efficient digestion of a wider range of biomass feedstocks, including agricultural residues and organic waste, thereby contributing to waste reduction and management (Fig. 4). However, there are some cons as well with this approach, such as, boiling water pretreatment requires a significant amount of energy, especially when dealing with large quantities of biomass. This could increase the overall operational cost and environmental impact of the process. The addition of a hydrolytic culture may introduce the need for additional nutrients to support microbial growth and activity. Ensuring a proper balance of nutrients is essential for optimal performance. A very important factor is that the pretreatment process and subsequent hydrolysis phase might take several days to complete. This extended time frame could affect the overall biogas production rate and may not be suitable for applications with a demand for rapid energy generation. Furthermore, managing the process requires careful control of temperature, pH, and nutrient levels to maintain favorable conditions for the hydrolytic culture. Deviations from optimal conditions could lead to reduced performance. Also, the introduction of a hydrolytic culture might alter the microbial community structure within the anaerobic digester. If not carefully managed, this could lead to potential competition among different microbial groups, impacting overall process stability. Overall, the pretreatment of chopped biomass with boiling water followed by the addition of a hydrolytic culture has both advantages and disadvantages. While it can improve biogas production efficiency and expand feedstock options, the approach's energy intensity, time consumption, and potential changes in microbial dynamics need to be carefully considered during the design and operation of the anaerobic digestion system. Based on the literature cited and analyzing the positive and negative sides of biomass pre-treatment technologies, a concept of low to high factor of characters is presented in Fig. 5.[138,164,165] Comparatively, the most economical and easier method among the given options for biomass conversion depends on the specific criteria. For economic feasibility (EF) and energy consumption (EC), alkaline treatment scores high, offering efficient lignocellulosic breakdown with lower costs. However, for gas yield (GY) and an eco-friendly approach (EfA), steam explosion may be preferred, delivering a good balance between economic feasibility and environmental sustainability. This also minimizes carbon loss (CL) and has moderate maintenance costs (MC). Dilute or strong acid treatment may be ideal in a surface to volume ratio increase (ISVR) and establishment of R&D facilities (ERD), making it a viable option for specific research-focused applications. This concept of characteristic scaling may help in the selection of pre-treatment technologies. Additionally, it's crucial to conduct thorough research and optimization to assess the feasibility and benefits of this method for specific applications.

The benefits of this combined physical and biological approach are manifold. Firstly, it would lead to a substantial increase in biogas production efficiency compared to conventional anaerobic digestion systems. This would, in turn, reduce the land and resource requirements for biogas plants, making them more economically viable and scalable. Secondly, the approach would contribute to waste management by utilizing various organic residues that would otherwise end up in landfills, mitigating their environmental impact and reducing greenhouse gas emissions. The hypothetical combined physical and biological approach for efficient biogas yield from biomass presents a promising avenue for sustainable energy production. By integrating advanced physical preprocessing techniques with tailored microbial communities and advanced monitoring systems, this approach holds the potential to revolutionize biogas production and contribute significantly to a cleaner and more sustainable future. However, it is essential to recognize that such an approach is theoretical and would require extensive research, development, and testing before it could be practically implemented on a large scale.

8. Conclusion

The successful conversion of lignocellulosic biomass into valuable biogas and bio-CNG through anaerobic digestion (AD) requires efficient pretreatment methods. Among the various approaches, the biological pretreatment method stands out as the most economically viable and environmentally friendly option. Microbial pretreatment offers advantages such as low energy consumption and minimal pollution generation, making it a promising avenue for enhancing biomass yield suitable for the AD industry. Fungi and bacteria, along with their enzymes, play pivotal roles in this method. Fungal pretreatment, involving cellulose and lignin-degrading species like brown-rot and white-rot fungi, has shown great promise in improving biogas yield. Studies have demonstrated significant increases in methane production after fungal pretreatment of various biomass sources, such as corn silage, wheat straw, and rice straw. While fungal pretreatment may require an extended incubation period, bacterial and enzymatic pretreatments offer quicker results, making them attractive options for shortening the reaction time. Moreover, microbial consortia, consisting of diverse microorganisms living symbiotically, have shown remarkable efficiency in biomass degradation. Co-culturing different bacteria and fungi has led to higher methane yields, enabling effective ligno-hemicellulosic biomass disintegration and AD under thermophilic conditions. Chemical pretreatment using acids, alkalis, and solvents has also proven effective in disrupting the biomass structure, particularly with dilute acid treatment and alkaline pretreatment. Optimizing the concentration and duration of chemical pretreatment has led to significant increases in methane production from various feedstocks like spent tea leaves, giant reed, wheat straw, and rice straw. Physical pretreatment methods, such as mechanical size reduction, have shown potential in increasing biomass accessibility to microorganisms and enzymes, enhancing biogas production. However, due to its energy-intensive nature, further research is needed to develop more cost-effective approaches.

In conclusion, the combination of physical and biological pretreatment methods offers a promising approach to effectively pretreat ligno-hemicellulosic biomass for improved biogas production. By leveraging the potential of these pretreatment methods and utilizing anaerobic digestion or fermentation, the AD industry can contribute to a sustainable and eco-friendly energy solution while effectively converting biomass resources into valuable biogas. Continued research and development in this field will drive the advancement of pretreatment technologies and enhance the feasibility of biogas production from various ligno-hemicellulosic biomass sources.

Data availability

No primary research results, software or code have been included and no new data were generated or analysed as part of this review.

Author contributions

Rajesh Kumar Prasad: conceptualization, methodology, writing – original draft preparation, visualization. Anjali Sharma: assisting in draft preparation, and sorting tables and figures. PB Mazumder: reviewing. Anil Dhussa: supervision, reviewing and editing.

Conflicts of interest

No conflict of interest exists.

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