



Application of Microbes in Biogas Production

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Abstract

Uncontrolled production of organic waste due to rapid urbanization and growing population has become a global concern. Biogas is an economical, renewable, and eco-friendly source of energy produced by using various groups of microorganisms that work in a synchronized way. Virtually any type of solid organic wastes is transformable into biogas through anaerobic digestion (AD). This chapter discusses the importance of biogas and use of microbes for biogas production. The production processes and parameters influencing the yield are also discussed briefly. In addition, the challenges are faced by enhancement techniques and summarized.

Keywords

Biogas · Microbial community · Solid organic waste · Anaerobic digestion · Parameters · Bioaugmentation

Abbreviations

AD	Anaerobic digestion
C/N	Carbon nitrogen
GHGs	Greenhouse gasses

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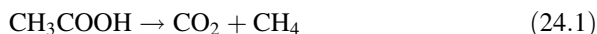
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HPH	Hydrodynamic pressure homogenization
HRT	Hydraulic retention time
MCFC	Molten carbonate fuel cell
MECs	Microbial electrolysis cells
MFCs	Microbial fuel cells
NREAP	National Renewable Energy Action Plan
OLR	Organic load rate
SOFC	Solid oxide fuel cell
SOWs	Solid organic wastes
VFAs	Volatile free fatty acids
WAS	Waste activated sludge

24.1 Introduction

Globally, the demands of energy have been growing gradually. For that reason, there is a need to enhance the growth of renewable and eco-friendly energy sources. The most important energy sources are fossil fuels that provide 80% of the total energy. Although the limited sources of fossil fuel also have some alarming impacts on the environment, it is necessary to reduce the use of fossil fuel because of global warming and other harmful pollutants. Around the world, the limited fossil fuel accessibility and the growing energy demands are the basic reasons that are compelling the governments to pursue the alternatives of renewable energy sources (Hijazi et al. 2016; Chuanchai 2018). Numerous methods including hydropower, solar heat, wind power, and anaerobic digestion (AD) can be used to produce renewable energy. However, bioenergy draws attention as renewable energy due to its viability and less production of CO₂. Generally, biogas consists of carbon dioxide (25–50%), methane (50–75%), water vapors, and some gases, i.e., N₂, H₂S, NH₃, and CO. The general equation of biogas production is as following: (Bo et al. 2014; Lee et al. 2017).



Conventionally biogas is produced through anaerobic digestion AD process by the microbial decomposition of organic matter. The organic matter including (crop residues, industrial wastes, municipal wastes, and animal manures) decomposed by microorganisms in anaerobic conditions. The AD process has been catalyzed by a wide variety of microbes. These microbes convert the macromolecules into smaller molecules. The first step of the AD process is hydrolysis; various microbial communities can be used for efficient hydrolysis process. Most of the species belong to the class of *Bacilli* and *Clostridia*. Clostridium species are common for degradation under anaerobic conditions. An extensive range of microorganisms such as *Thermomonospora*, *Actinomyces*, *Ralstonia*, *Shewanella*, *Methanobacterium*, and *Methanosarcina* contribute to the degradation and methane production. Recently

several species like *Clostridia*-36%, *Bacilli*-11%, both with the members of Mollicutes-3%, Bacteroidia-3%, Actinobacteria-3% and Gammaproteobacteria-3% are reported as the fermented bacteria in the digesters (Khalid et al. 2011; Wirth et al. 2012). Various Archaeal communities identified as methanogens, i.e., *Methanobacterium formicum*, *Methanosarcina frisia*, and *Methanosarcina barkeri*. Methanogens are uncultivable microorganisms that increase the production of methane (Goswami et al. 2016), whereas others are the member of thermophilic species (e.g., *Crenarchaea* and *Thermoplasma sp.*). Archaeal 16s rRNA gene clones associated with ArcI taxon have been recovered in large amount from a methanogenic digester to decompose sewage sludge. ArcI is reported as an acetate consumer that plays an important part in acetoclastic methanogenesis. About 16% of rRNA archaeal gene clones have been investigated in a mesophilic methanogenic digester that belongs to *Crenarchaeota* (the subphylum C₂). It also has been observed that by increasing the hydrogenotrophic species the production of methane increases (Chouari et al. 2005; Trzcinski and Stuckey 2010).

The process of AD involves four major steps, i.e., hydrolysis, acidogenesis, acetogenesis, and methanogenesis. The organic matter is converted into renewable bioenergy by the action of microbes in the presence of enzymes. A large variety of bacterial groups taking part in the AD processes, such as hydrolytic, fermenting, acid-oxidizing, and methanogenic archaea bacteria are used to degrade organic waste (Carballa et al. 2015; Tuesorn et al. 2013). This process is environmentally friendly, requires less energy, economically attractive, and produces high quality of biogas. On the other hand, it also has some limitations, such as low biogas production, destabilizing, and weak degradation of substrates. Various factors can affect the AD process like (temperature, pH, volatile fatty acids, C/N ratio, alkalinity, and substrate characteristics) (Cerrillo et al. 2016). To overcome these problems many physical and chemical methods have been established. Many techniques are used to increase hydrolysis efficiency that is a rate-determining step in the AD process. Currently, several new technologies, e.g., (MECs) and (MFCs) have been introduced to increase the efficiency of anaerobic digesters. These technologies use electric current from microorganisms to improve biogas production. The pretreatment of substrates along with micronutrients also improves gas yield. An improvement of discharge quality is also needed to avoid the adulteration of groundwater by nutrients and pathogens (Lee et al. 2017; Weiland 2010).

24.2 Historical Overview

Recently, one of the major environmental problems is the continuous production of organic material. This organic waste is managed and treated by AD which is a microbial anaerobic (absence of O₂) decomposition process to produce biogas in digesters (airproof reactor tanks). Biogas is a sustainable supply of renewable energy from organic waste. AD has attained global attention to lower the combustion of fossil fuel and to reduce the emission of greenhouse gases (Awe et al. 2017; Hosseini and Wahid 2014).

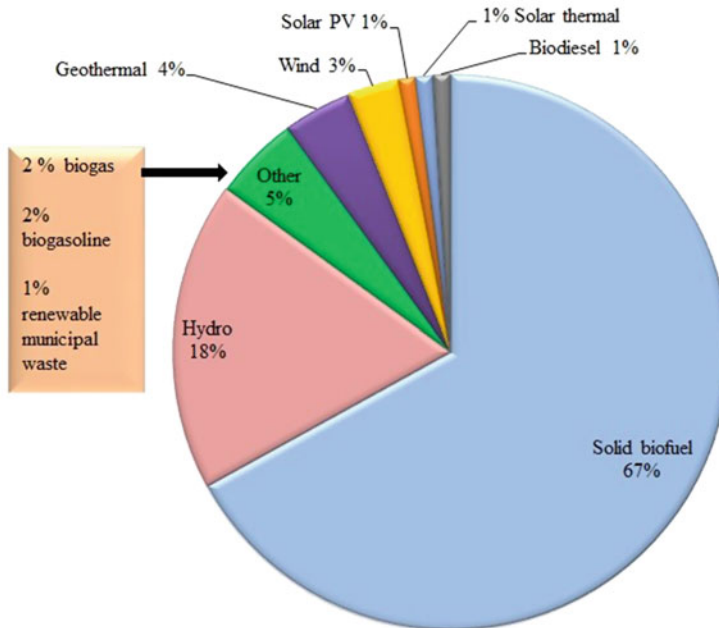


Fig. 24.1 Global energy source in 2013 (Atelge et al. 2018)

In France, Ad was first documented in 1891. In 1895, biogas was produced in the United Kingdom from municipal waste and it was used to harvest heat and light (Gashaw 2014). A comprehensive report in the USA about anaerobic digestion was published in 1936, by Hatfield and Buswell (Wett and Insam 2010). In the middle of the twentieth century, sustainable applications of biogas plants appeared. Currently, AD is a significant treatment of waste (industrial waste, aquatic biomass, sewage solid waste, and energy crops) and produces methane (García-González et al. 2019; Raucci et al. 2019). For years, the production of biogas has been applied in households and farms on a small scale. Since the 1930s, the production of biogas after viable stabilization requirements of sewage sludge became a standard process to treat sludge at large to medium scale treatment plans. In Europe particularly, over the last few years, biogas plant has developed an industrial scale largely by increasing the efficiency of biogas conversion. At the start of the twenty-first century, we came to know that biogas has the potential to eliminate many issues instantly. Taking methane in biogas can provide waste disposal management, reduction of GHGs emissions, and renewable energy production (Chiumenti et al. 2018; Hou and Hou 2019). Biogas is a common renewable energy source in developed countries. On the other hand in developing countries, this trend has not altered. Globally, the production of biogas was reported only 2% as displayed in Fig. 24.1, whereas in the EU, it was extended to 7% in 2013 as shown in Fig. 24.2 (Agency 2016).

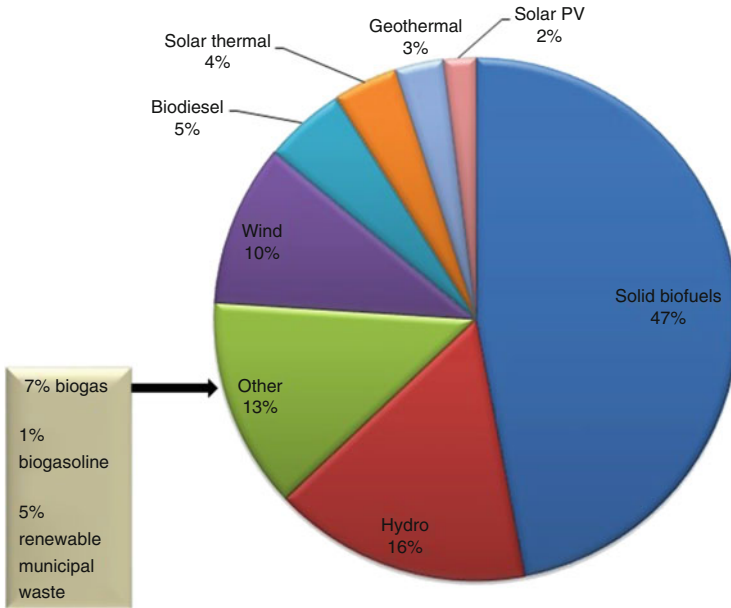


Fig. 24.2 EU-28 level energy source in 2013 (Atelge et al. 2018)

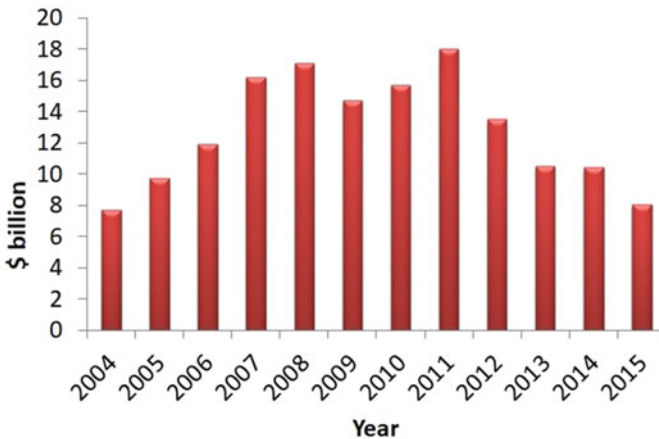


Fig. 24.3 Graphical representation of global investment in biogas production (Atelge et al. 2018)

The continuous increase in the growth of the biogas sector has been supported by the above facts since 1990. The sustainable energy investment trend during the era of 2004–2015 in the world is shown in the following Fig. 24.3.

Figure 24.3 illustrates that there is a continual increase during 2004–2008 where the trend remains relatively constant (Gonzalez-Salazar et al. 2016). The fewer investments made to be constant after 2011. While the rate of growth from

2004–2015 was 2%, the investment in waste and biomass to energy sector was 6 billion dollars in 2015. In developed countries like Denmark, Germany, and England, the energy sector has developed well, therefore investment in waste and biomass to energy lessened in the era of 2011 and 2015 (Solarte-Toro et al. 2018; Edenhofer et al. 2011). Conversely, in developing countries, the investment continues to increase progressively owing to their economic conditions (Offermann et al. 2011). In the EU, to meet the sustainable energy requirements of the National Renewable Energy Action Plan (NREAP), the sustainable energy sector has to develop 4% every year till 2020, to meet the anticipations. In Paris Agreement 2015, the target of the EU for 2050 was the reduction of greenhouse gasses emission to 85–90% from the volume produced in 1990 (Bausch et al. 2017). In the era of 2013–2020, electricity generation from biogas must be enhanced from 46.8 to 63.3 terawatt-hours in the EU to gain their NREAP target. Italy and Germany have achieved their goals because of their numbers of functional biogas plants, whereas other countries require economic investments and policies for the operation and development of more biogas plants (Repele et al. 2017).

Animal waste has been alleviated by AD unless the middle of the 1970s in North America (Abbasi et al. 2012). The biogas plant number with well-developed AD has increased in the USA. Recently, the number of AD plants in operation are around 2100, it is still lesser compare to their model potential (Wang et al. 2019). Japan is also using this technique to manage and treat its waste. At this time, thermophilic AD is used only in Japan in the world. 200 mL biogas was formed in 2006. Many cities in Japan like Kobe, Nagaoka, and Kanazawa are producing biogas from sewage sludge with various capacities, e.g., 800,000, 600,000, and 280,000 m³/year, respectively (Yolin 2015; Gubaidullina and Kargina 2015). In developing countries, AD has been becoming more suitable and standard technique due to high energy costs compared to developed nations. At present, India and China have a large number of operated biogas plants with 4.7 million and 42.6 million correspondingly as shown in Fig. 24.4 (Tongia and Gross 2018).

Other countries in Asia like Bangladesh, Kenya, Nepal, Cambodia, and Vietnam have installed progressively more domestic biogas plants (Geng et al. 2016). In 2016, the numbers of small scale biogas plants installed in these nations are in the range of 360–15,000. In this year Asia has invested more for AD technologies compared to any other region. African Biogas Partnership Program operated almost 68,000 biogas plants in 2016, in Africa. In developing countries, more than 700,000 plants have been installed in 2015 (Appavou et al. 2017).

24.3 Importance of Biogas

Fossil fuels are the renewable source of energy but their formation process is very slow and current consumption is rapidly draining the reserves. Biogas is formed during the process of anaerobic digestion and is a reliable and flammable gas with short formation time (Hosseini and Wahid 2014). Biogas has versatile applications

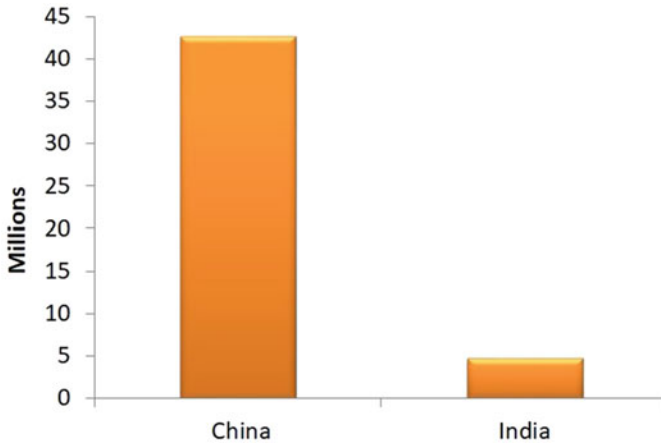


Fig. 24.4 Domestic number of biogas plants established in 2016 by India and China (Atelge et al. 2018)

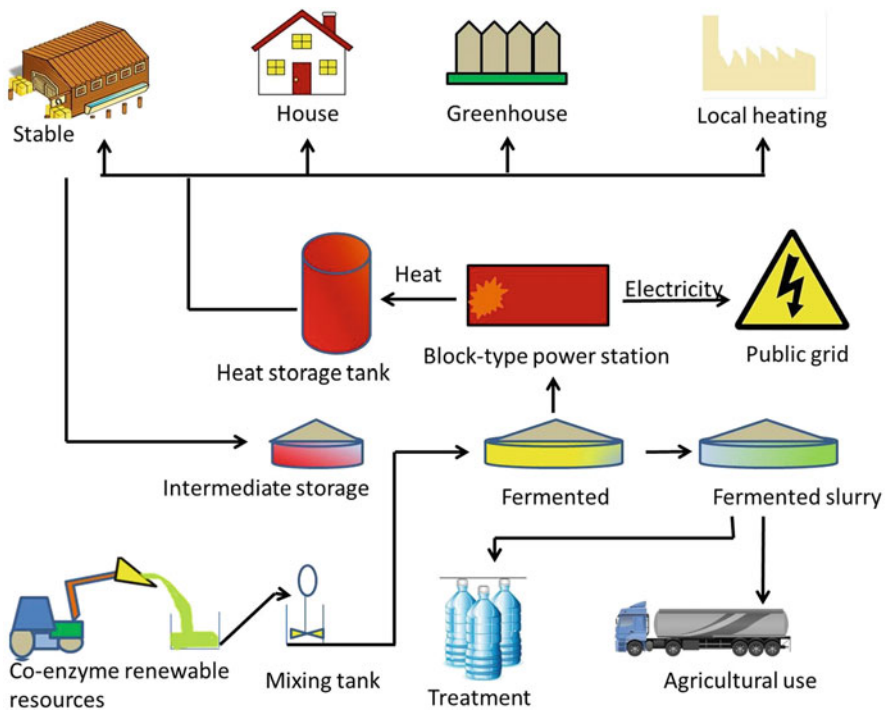


Fig. 24.5 Utilizations of biogas (Ferreira et al. 2019)

as shown in Fig. 24.5, e.g., due to controlled combustion its chemical energy can be transformed into mechanical energy.

Thermal energy can also be generated from biogas when it burns to yield heat energy in boilers. In stationary engines as well as in automotive it is used as fuel. It is a promising source of H_2 that is loaded into fuel cells (Alves et al. 2013).

Use of Biogas for Sludge Treatment The sludge sanitization process can be performed with the help of a boiler worked on biogas and a heated concrete tank. Once a day, the tank might be aided to avoid the necessity of a large gas holder. A heat exchanger is fitted in the tank to heat the sludge for 30 min at 70 °C. Therefore, excess thermal heat (up to 70%) can be used for cooking and water heating (Passos et al. 2020).

Use of Biogas in Fuel Cells The techniques to change H_2 into electrical energy and desirable power levels are near to commercialization. In fuel cells, the direct biogas use is termed as internal reforming (Ohkubo et al. 2010; Membrez and Bucheli 2004). (SOFC) and (MCFC) are high-temperature fuel cells. They have a greater ability of internal reforming (use of biogas directly) due to better capacity of thermal integration and great tolerance of H_2 contaminants. In the literature, various studies indicated that in fuel cells, biogas reforming is used frequently. However, some studies revealed that biogas can be converted into electricity without a humidifier, ancillary fuel, external reformer, and metal catalyst (Shiratori et al. 2008). During internal reforming, CO is also produced which is a poison for fuel cells (Xuan et al. 2009).

Use of Biogas as Biofuels Biogas is a high octane fuel. The components of biogas can be categorized in the following ways:

- Combustible.
- Non-combustible.

The combustible components include CO_2 , H_2 , and CH_4 while CO and N_2 are non-combustible components. Various factors such as the source of substrates and preparation techniques may change the composition of the biogas. Biofuel is a biomass-based fuel. It has various advantages compared to fossil fuel. Primarily, biofuel is readily available from biomass. Furthermore, biofuel circulates the carbon between the fuel and air, as a result, many problems, i.e., energy scarcity and greenhouse gas emission can be resolved. Thirdly, various kinds of biofuel like ethanol and biodiesel have physicochemical characteristics for combustion in the internal combustion engine (Raheem et al. 2015; Brown and Brown 2013). Similarly, bioethanol (a renewable substitute) has been used for gasoline in the system isolated engine. As compared to natural gas and LPG, biogas has a lower heating value and lower flame speed. Secondly, the autoignition temperature is also greater than that of natural gas and LPG. Their chemical and physical properties have a greater effect on the use of biogas in the spark-ignition engine (Qian et al. 2017) (Table 24.1).

Table 24.1 Shows the contents of organic matter and their theoretical yield (Braun 2007)

Substrates	Biogas(Nm ³ /tTS)	CO ₂ (%)	CH ₄ (%)
Carbohydrates	790–800	50	50
Raw fat	1200–1250	32–33	67–68
Raw protein	700	29–30	70–71
Lignin	0	0	0

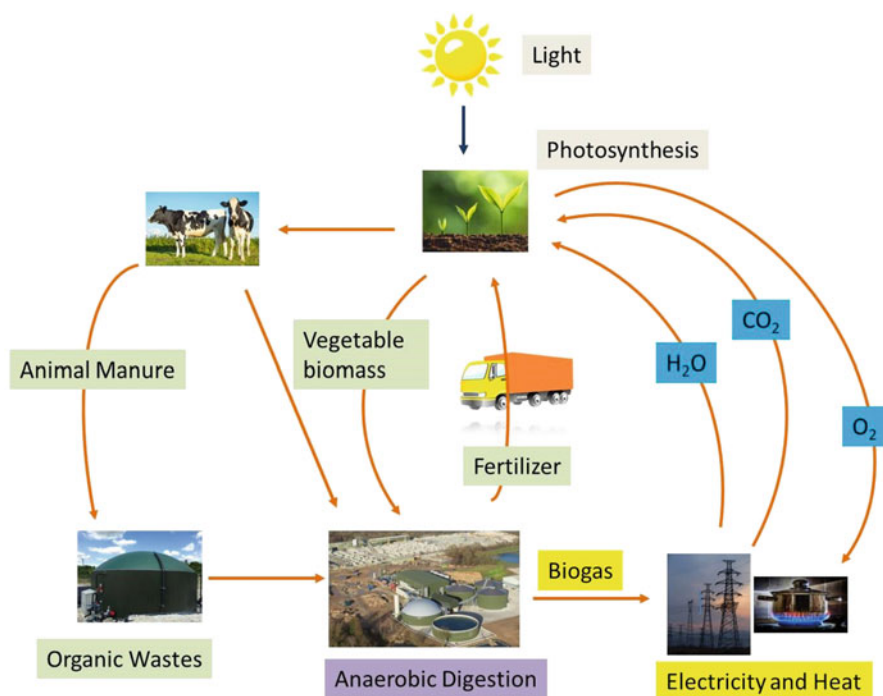


Fig. 24.6 A conventional biogas production cycle (Al Seadi 2001)

24.4 Commonly Used Substrates for Biogas Production

For renewable energy (biogas) biomass is the most commonly used substrate in AD. Some substrates are shown in Fig. 24.6. The biomass consists of proteins, carbohydrates, hemicelluloses, celluloses, and fats. However, some co-substrates are also used to obtain the highest gas yield. These co-substrates are agricultural wastes, food wastes, harvesting residue, i.e., leaves, and top of sugar beet and household municipal wastes. The composition and total yield of biogas depend on the type of feedstock and substrate used in the anaerobic plant to determine the composition and yield of biogas (Braun 2007; Achinas et al. 2017). Contents of organic matter and their theoretical yield are listed in the table.

Methane production from different feedstocks is very difficult to compare. Experimental conditions, i.e., temperature, volatile solids, total solids are analyzed for the maximum performance of particular raw material. Thus it is useful to relate different feedstocks by their methane yield (B_{20}) (Owen et al. 1979).

Manures are used as the substrate in AD which is an abundant source of organic matter. The use of manures as feedstock also reduces the emission of greenhouse gases. Some biochemical methane potential assays showed that the potential yield of methane differs among livestock types. Various factors take part in the potential of methane, e.g., animal growth stage, type of bedding, species, breed, feed, amount, and any decomposition process (Møller et al. 2004). Farm manures have a high concentration of NH_3 that may be an inhibitory factor in the AD process. The feedstocks having low nitrogen concentration involve high ammonia concentration for effective degradation. Beyond this manures usually consist of recalcitrant fiber that is hard to degrade. The pretreatment of manures gives up to 20% increase in methane production by reducing the particle size (Sung and Liu 2003; Angelidaki and Ahring 2000).

Biomass contains straws from rice, wheat, sorghum, and other waste products of food. It is the most favorable feedstock for the AD process. Their methane yield (B_{20}) is high. However, the high amount of recalcitrant material usually needs pretreatment to completely comprehend the potential yield. The biogas yield is also affected by the harvesting time (Pettersson et al. 2007).

It is the most capricious feedstock because the production of methane is influenced by the location (source of material), sorting method, and time of collection. Cultural values, beliefs, the lifestyle of communities impact their recycling practices and waste disposal approaches (Cho et al. 1995). When the municipal solid waste is not differentiated by source then the process of pretreatment is mandatory to remove metals, glass, plastics. The pretreatment process can be done manually or mechanically, i.e., pressing, screening, and pulping. Sewage sludge is another form of industrial or municipal waste. It has a high methane yield due to the presence of high organic matter for AD (Ward et al. 2008).

Food waste has a high content of volatile solids, low total solids, and its degradation is easy in an anaerobic digester. These substrates during hydrolysis may accumulate acid in the digester and inhibit methanogenesis consequently. In the early 1980s, it was revealed that the various carbohydrate comprising wastes required alkali buffer as well as co-digestion for stable performance (Hills and Roberts 1982; Knol et al. 1978) (Table 24.2).

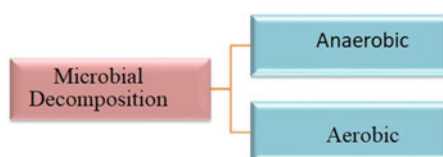
24.5 Application of Microbes in Biogas Production

24.5.1 Decomposition

It is an incessant and intricate microbial decomposition of complex organic biomass into its mineral forms. Decomposition is categorized by various physical and biological processes like biological fragmentation, respiration, and leaching

Table 24.2 Biogas production feedstocks (Krishania et al. 2012)

Feedstocks	Total waste (Kg/day/head)	Gas yield(m ³ /kg)	Requirement of pretreatment
Poultry	0.75	0.46	No
Pig	1.3	0.39	Yes
Sheep	0.75	0.37	No
Cattle	10–15	0.34	No
Kitchen	0.25	0.30	Yes
Night soil	0.75	0.38	No
Wheat straw	3.5	0.41	Yes
Rice straw	1.2	0.61	Yes
Marine algae	3.3	0.40	No
Water hyacinth	5	0.40	Yes

Fig. 24.7 Types of microbial decomposition

(Hahn-Hägerdal et al. 2007; Busing et al. 2008). These processes work synergistically as they are very closely related to each other. Many factors affect decomposition processes such as the concentration of O₂/CO₂, temperature, humidity quality of substrate containing components, species, position, and size. Generally, decomposition has two types: abiotic and biotic. Biotic decomposition is the microbial (fungi, bacteria, and protozoa) disintegration of the complex substrate into simpler units. On the other hand, abiotic decomposition uses physical and chemical methods to breakdown complex organic substrate (Rahman et al. 2013). The microbial decomposition occurs in either anaerobic or aerobic environment as shown in Fig. 24.7.

Anaerobic Decomposition

It is an anaerobic symbiotic microbial conversion of organic waste to biogas, salts, nutrients, refractory organic matter and additional cell matter, etc. It is an environmentally friendly technique.

The main components of the raw biogas are 60% CH₄, 40%CO₂ trace amount of H₂S and water vapors. It is a colorless and odorless gas. When it burns a blue color flame is made which is similar to the flame of LPG gas. Archaea and bacteria are two basic kinds of microbes used for the conversion of biogas strictly in an anaerobic environment (Adekunle and Okolie 2015; Kusch et al. 2012). AD reduces pathogens, organic wastewater solids, and the odor by producing biogas from fractions of volatile solids. The product of this process has not only stabilized solids but also has some nutrients like ammonia-nitrogen. AD is applied in waste management including industrial wastewater, agriculture waste, sludge digester, municipal

wastewater, septic tank, and waste treatment (Zhou and Wen 2019). It is used in both domestic and industrial fermentation to produce food and drink products. Different factors influencing biogas conversion may include the nature of substrate, volatile free fatty acids, carbon-nitrogen ratio, temperature, hydraulic retention time, digester design, pH and loading rate (Kusch 2008). It can be either a batch process or a continuous process. The organic waste is added continuously in continuous AD to the reactor. On the other hand, organic biomass is added in the batch process at the start of the process to the reactor.

Aerobic Decomposition

It is a decomposition of organic biomass in the presence of oxygen by microorganisms into SO_4^- , CO_2 , NO_3^- , H_2O , etc. It is the most common process that occurred in the forest to produce stable organic compounds from dropping animals and trees. It can also take place in bins, piles, pits, stacks, etc. and insufficient O_2 environment. Some compounds cannot decompose well in an aerobic environment that is the major disadvantage of this process. These unreactive compounds contain insoluble materials which require chemical oxygen demand up to 70%. To overcome these problems versatile AD technique is used to treat organic waste.

24.5.2 The Biochemical Process of Biogas Production

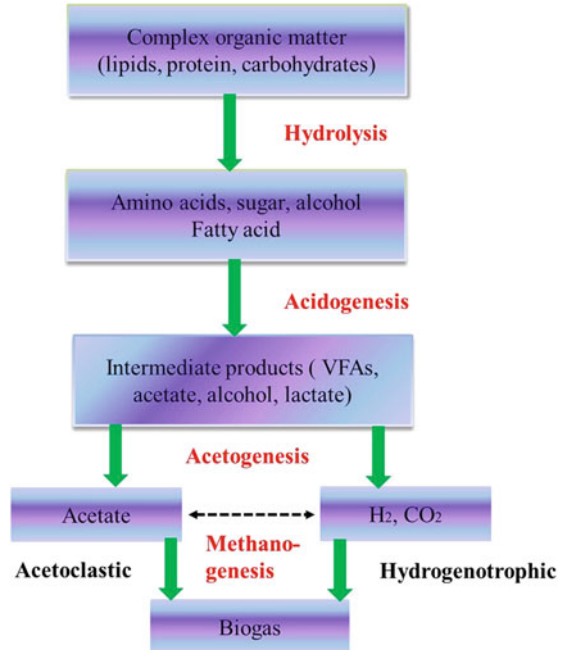
Biogas production through the AD process has significant advantages. It is a versatile source of energy that reduces the emission of greenhouse gases. Various types of organic substrates (agricultural remains, animal wastes, municipal solid wastes, and market wastes) are converted into biogas and digestate (Hijazi et al. 2016; Weiland 2010). The process of anaerobic digestion has been carried out by various independent progressive and biological reactions in anaerobic conditions (Parawira 2012). It is an enzyme-driven process during which organic matter is converted into CH_4 and CO_2 . AD process consists of four main steps which are as follows and described in Fig. 24.8: (Weiland 2010).

- Hydrolysis.
- Acidogenesis.
- Acetogenesis/Dehydrogenation.
- Methanogenesis.

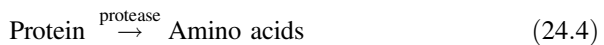
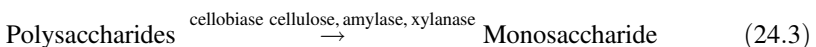
Hydrolysis

Hydrolysis is a process that transforms the complex organic macromolecules (lipids, polysaccharides, proteins) into smaller ones with the help of microbes secreted from different enzymes (Cirne et al. 2007). The different degradation steps involve diverse groups of microscopic organisms, which work in a closely related way. Hydrolyzing microorganisms are initially attacking polymers and converting them into long-chain fatty acids, monosaccharides, and amino acids. However, many

Fig. 24.8 Schematic diagram of the biochemical process of biogas production



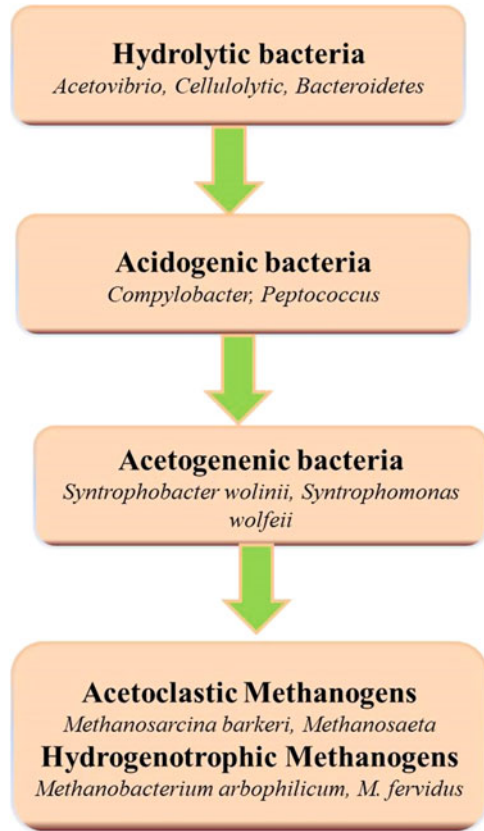
hydrolytic enzymes that are secreted by microorganisms, e.g., cellobiose, cellulose, amylase, protease, xylanase, and lipase are taking part in hydrolysis (Weiland 2010; Bagi et al. 2007). Various bacterial groups are also included in the hydrolysis of polysaccharides, most of them are strictly anaerobic, i.e., *Clostridium*, *Bacteroides*, and *Acetivibrio*. The resulted products of hydrolysis are further decomposed by other microorganisms (Heeg et al. 2014).



Acidogenesis

In acidogenesis, the final products of hydrolysis, i.e., fatty acids, sugars, and amino acids are further decomposed by fermenting organisms. Some facultative and various hydrolyzing microorganisms (i.e., *Paenibacillus*, *Ruminococcus*, *Streptococci*) are taking part in fermentation (Ziganshin et al. 2013; Zheng et al. 2014). However, microorganisms, e.g., *Acetobacterium*, *Enterobacterium*, and *Eubacterium* along with the hydrolyzing microbes are also included to carry out the fermentation.

Fig. 24.9 Shows an overall view of bacteria involved in the AD (Goswami et al. 2016)



These fermented bacteria (acidogens) convert the hydrolyzing products into numerous organic acids (i.e., butyric acid, acetic acid, propionic acid, succinic acid, lactic acids), alcohols, NH_3 , CO_2 , and H_2 . The resulting compound depends on the type of microorganism's present, the kind of substrate used, and on environmental conditions (Schnurer and Jarvis 2010).

Acetogenesis

In acetogenesis, the fermented products are further oxidized into methanogenic substrates. The obligate acetogenic hydrogen-producing bacteria convert the high VFAs, amino acids, and alcohol into acetate and hydrogen. *Syntrophus*, *Clostridium*, *Syntrophomonas*, and *Syntrobacter* are the microorganisms that carried out acetogenesis as shown in Fig. 24.9 (Bagi et al. 2007; McInerney et al. 2008).

Methanogenesis

In the biochemical process, the very last step of AD is methanogenesis in which fermentation of various organic compounds synthesized methane gas. The process of

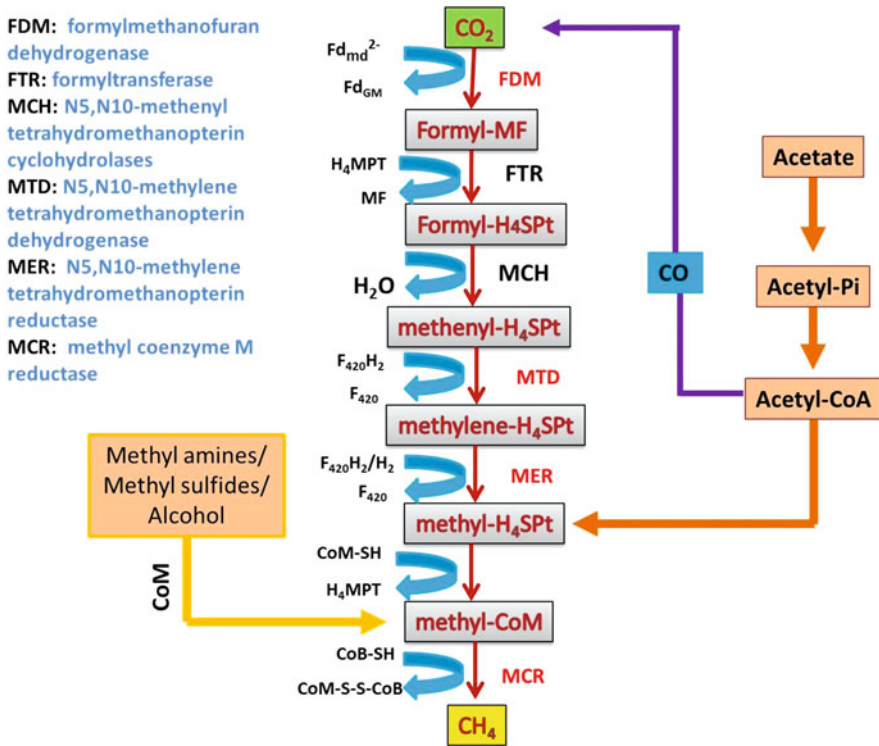


Fig. 24.10 shows the complex process of methanogenesis including three pathways (Goswami et al. 2016)

methanogenesis has been compelled by six different pathways, in which three are the major pathways, i.e., methylotrophic pathway, hydrogenotrophic pathway, and acetoclastic pathway. Every pathway is differentiated by other pathways by the source of energy and nature of the substrate used for methane. These substrates are formic acid, carbon dioxide, methylamine, dimethyl sulfate, and methanol. The common pathway of methanogenesis is the reduction of CO₂ into CH₄. However, according to methanogenic cofactors other five pathways may be assembled into two (Slonczewski and Foster 2013; Garcia et al. 2000). The three basic pathways are described in Fig. 24.10.

Methylotrophic pathway: In this pathway, methane is produced by the decarboxylation of methylamine/methyl sulfides/alcohols.

Hydrogenotrophic pathway: In this pathway, methane is produced by the reduction of CO₂.

Acetoclastic pathway: In this pathway, methane is produced by the decarboxylation of acetate. This pathway has been reported as the major pathway to produce methane in anaerobic conditions. It has been stated that during the AD process of domestic sewage about 70% of the total CH₄ is produced through this process. The

Parameters

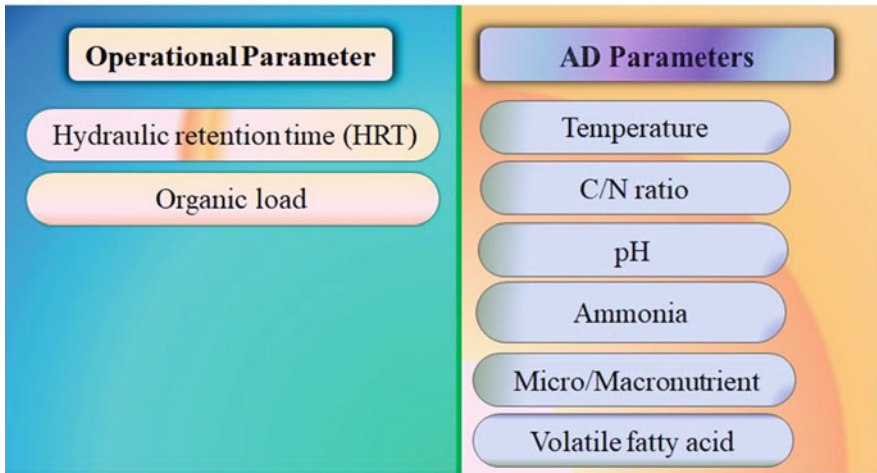


Fig. 24.11 Important parameters in AD

process of methanogenesis is very complex, it requires various substrates and cofactors to take place (Goswami et al. 2016; Lettinga 1995).

24.5.3 Parameters Influencing Microbial Growth and Biogas Yield

There are two main parameters such as operational and AD parameters to enhance biogas yield. These parameters are described in Fig. 24.11.

Anaerobic Digestion Parameters

1. Temperature.

Temperature is a fundamental factor significantly influencing different functions such as hydrolysis rate, biogas conversion, sludge quality, enzyme and its related coenzyme activities in the AD process. In that process, various anaerobic microorganisms work well at different temperature ranges (Yan et al. 2015). Three thermal stages are described in Fig. 24.12.

Enzymes may not show their optimal catalytic activity at very low temperatures, whereas sensitive enzymes may become denature at very high temperatures, as a result, lead to process failure. From literature, we come to know that ammonia accumulation, endergonic metabolic reactions, and biogas yield accelerated at the thermophilic thermal range compared to the mesophilic thermal range (Sikora et al. 2019; Keating 2015). It was also noted that thermophilic thermal conditions could not be promising for exergonic metabolic reactions and specific substrates like co-digestion of sugar beet pulp with sewage waste (Montañés et al. 2015). The

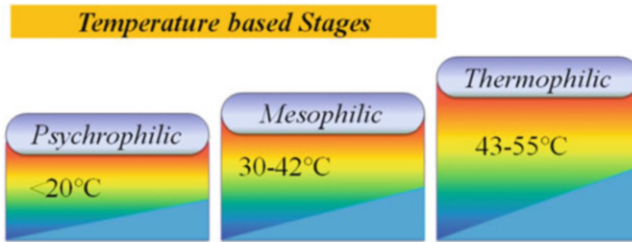


Fig. 24.12 Important thermal stages in AD

thermophilic condition also causes adverse environmental effects. During the digestion, process temperature must be kept constant because temperature fluctuations affect biogas yield negatively. Thermophilic bacteria are more sensitive than mesophilic bacteria.

2. C/N ratio.

This ratio plays a significant role in anaerobic digestion (Mathew et al. 2015). For the growth of anaerobic microorganism in a stable environment, optimum ratio of C/N is required. Commonly, a range of 20–30 C/N ratio is suitable for the AD process (Meegoda et al. 2018). Wang et al. executed anaerobic co-digestion of three substrates (wheat straw, dairy and chicken manure) at a low concentration of free ammonia and ammonium ion and stable pH; as a result, he found that the maximum yield of methane produces at 27.2 C/N ratios (Wang et al. 2012). Zeshan et al. were also found that digestion accomplished well at a C/N ratio of 27 than 32 (Karthikeyan and Visvanathan 2012). Whereas according to modern study, AD performed well at 15–20 C/N ratios. For co-digestion, Zhong et al. found that the most favorable C/N ratio was 20 (Zhong et al. 2013). Anaerobic co-digestion for cattle manure and food waste was done by Zhang et al. at C/N ratios 15.8 (Zhang et al. 2013a). The optimum C/N ratios depend on inoculum and feedstock for the anaerobic digestion process. For a long-term AD operation, suitable C/N ratios are enforced.

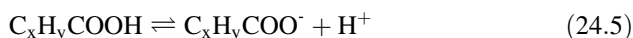
3. pH

In the AD process, pH is an indispensable parameter to regulate and stabilize the activities of methanogenic and acidogenic bacteria because their activities are greatly affected by pH changes. Usually, an optimum pH between 6 and 8 is reported for higher biogas yield (Deepanraj et al. 2014; Zhao et al. 2008). Acidogenesis and hydrolysis take place at pH 6.5 and 5.5, respectively. The amount of volatile fatty acids (VFAs) and CO_2 formed during the digestion process affects significantly the pH of matter present in a digester. Typically to ensure fermentation in AD, then the concentration of CH_3COOH and volatile free fatty acid should be <2000 mg/l. In 1998, Mattiasson and Jain reported that the efficiency of methane yield was

enhanced >75% at above pH 5. In the co-digestion of two substrates such as dairy manure and cheese whey, when pH was uncontrolled, a two-phase anaerobic digester worked as a single-phase reactor, whereas in the methanogenic phase, when whey pH was controlled, then the digester functioned as a two-phase two-stage reactor (Bertin et al. 2013; Venetsaneas et al. 2009). From previous reports, we come to know that the pH of anaerobic reactor affects VFAs in a great manner, at low pH butyric acid and acetic acids are dominant VFAs however at pH 8 main VFAs are propionic acid and acetic acid (Horiuchi et al. 1999). Similarly, with the help of optimum pH, we should control acidogenic bacteria and their number (Horiuchi et al. 2002).

4. Ammonia.

Ammonia and ammonium ions are obtained by degrading nitrogen-rich organic waste and protein (Yenigün and Demirel 2013; Whelan et al. 2010). Ammonia is a crucial nutrient for bacterial growth but in higher concentrations, it can be very toxic to bacteria (Walker et al. 2011). A recent study revealed that during anaerobic digestion ammonia could increase buffer capacity by neutralizing VFAs (Scherer et al. 2009). Zhang et al. have reported reaction equations between VFAs and ammonia as follows:



In the above equations, C_xH_yCOOH symbolizes VFAs. With the increase of organic load rate (OLR), the amount of VFAs increases which inhibits the AD process therefore to avoid this inhibition NH_3 could react with VFAs and allow enough fatty acids for biogas production. Ammonia is directly proportional to both pH and temperature. It means that free ammonia concentration rises with increasing temperature and pH values such as at 35 °C and pH 7 the amount of free ammonia formed is <1%. Conversely, free ammonia at pH 8 and the same temperature increase to 10% (Fernandes et al. 2012). Bacteria grow at low ammonia concentration, whereas its higher concentration can inhibit bacterial growth. To regulate AD functions various techniques are used to remove excess ammonia such as microwave (Lin et al. 2009a, b), ion exchange (Wirthensohn et al. 2009), electrochemical conversion (Lei and Maekawa 2007), ammonia stripping (Böhm et al. 2011), membrane contractor (Lauterböck et al. 2012) and biological nitrogen elimination processes (Hsia et al. 2008), etc. We can calculate concentration free ammonia from the following formula.

$$[NH_3] = \frac{[T - NH_3]}{\left(1 + \frac{H^+}{K_a}\right)} \quad (24.8)$$

Here, K_a is the dissociation parameter, while $[NH_3]$ and $[T-NH_3]$ represent free ammonia and total ammonia, respectively.

5. Volatile Fatty Acid.

Valeric acid, acetic acid, butyric acid, and propionic acid are the basic VFAs intermediates that identify the stability of the AD process (Buyukkamaci and Filibeli 2004; Pham et al. 2012). Among these acids, propionic acid and acetic acids are essential for biogas production (Zhang et al. 2013b). During acidogenesis these intermediates are formed with a chain of carbon up to 6 atoms. Mainly, methanogens and acetogenic bacteria converted VFAs finally into CO_2 and CH_4 . However, volatile fatty acids are directly proportional to organic load. High organic loading can increase VFAs concentration inside the reactor as a result of pH value drops which inhibit the AD process (Zhang et al. 2013a; Palacio-Barco et al. 2010).

6. Macro- and Micronutrients.

Trace elements such as nickel (Ni), cobalt (Co), molybdenum (Mo), iron (Fe), tungsten (W), selenium (Se) and macronutrient carbon (C), phosphorus (P), sulfur (S), and nitrogen (N) are important equally for the survival and growth of microorganism in anaerobic digestion (Aglar et al. 2008). These nutrients not only maintain the activities of enzymes but also help in their synthesis (Moestedt et al. 2013; Facchin et al. 2013). The optimum ratio of microelements S: P: N: C for AD is 1: 5: 15: 600.

Operational Parameters

1. Organic Load Rate.

It is defined as the amount of organic waste fed continuously to anaerobic reactor per day per unit working volume as shown in the equation below:

$$B_R = m \times \frac{c}{V_R} \quad (24.9)$$

where B_R , V_R , c , and m are the organic load ($Kg/d \cdot m^3$), digester volume (m^3), organic matter concentration (%), and mass of substrate fed per time unit (Kg/d), respectively.

In diverse AD operations, the OLR differs because of variances in feedstock properties, operating temperature, and hydraulic retention time (Divya et al. 2015a). An optimal amount of OLR is required because too high organic load could accumulate VFAs in AD reactors that inhibit bacterial growth resulting in process failure; on the other hand, too low organic load could lead to the malnutrition of

fermenting microbes consequently reducing the efficiency of the AD process. Generally, to some extent, OLR is directly proportional to the biogas yield. Various factors influence significantly OLR like operational cost and conditions as well as the type of SOWs fed (Meegoda et al. 2018).

2. Hydraulic Retention Time.

Hydraulic retention time (HRT) is defined as the time (days/hours) required for the complete degradation of SOWs. It is expressed in the following equation:

$$HRT = \frac{V_R}{V} \quad (24.10)$$

In this equation, V and V_R are substrate volume fed per unit time (m^3/day) and digester volume (m^3) correspondingly. It is inversely proportional to the organic load as shown in the above equation. It is a very important parameter influencing microbial growth in anaerobic reactor; therefore, it should be optimized (Mao et al. 2017). In the presence of very low HTR, volatile fatty acids could accumulate that lead to process failure by inhibiting bacteria while a very high HRT could result in insufficient feedstock usage. It depends upon the specific fed feedstock in the digester (Dareioti and Kornaros 2015). For SOWs treatment a 15-30 days HRT is required for AD operation (Mao et al. 2017).

24.6 Current Trends in Biogas Production

The general process of anaerobic digestion to produce biogas still requires intensive research. However, the information about the process has been increasing throughout recent years. The recently achieved methodological and technological advancements in that facet area, i.e., biogas upgrading (Angelidaki et al. 2018), use of new substrates (Vergara-Fernández et al. 2008), ammonia toxicity (Westerholm et al. 2009), process monitoring tools, i.e., VFAs sensors (Boe et al. 2007) and membrane reactors (Vyrides and Stuckey 2009). Reduction in the cost and time required for sequencing techniques played an important role in comprehending the complex microbial AD process. Nowadays various omics tools are used to decode the anaerobic digestion black box (Kougias and Angelidaki 2018).

Use of Pretreatment Techniques To make the AD system economically viable national systems have been supported to use an array of various substrates. However, several studies examined that biogas synthesis is directly affected by various interacted waste streams. So the researchers try to improve the arrangement of different waste streams for the optimal production of biogas also called co-digestion. Advance studies illustrated that the co-digestion of crops, lignocellulosic and sewage sludge wastes give the better quality as well as quantity of biogas. Despite these, the different pretreatment technologies help to improve the biogas

yield, speed of the AD process and also provide a wide variety of substrates (Mahanty et al. 2014; Igoni et al. 2008).

Modifications in Biogas Digesters Biogas digesters are the air-tight bioreactors that are used to produce the biogas by the AD process. In the past, the basic model of digesters faced many problems and failures including high cost and unsteady gas pressure. Recently a new digester named puxin digester has been developed by China to contain all the qualities to improve biogas production. By the changing trends, the small household digesters holding the 5000 m³ capacity have been designed to produce biogas for vehicular fuel (Bharathiraja et al. 2018; Rajendran et al. 2012). On large scales to preclude system failures the biogas plants have been modified to work in a programmed manner. These modifications (i.e., heating accouterments, mechanical agitators, performance monitoring systems, and temperature regulators) in response help to lessen the system failures (Ward et al. 2008).

Biogas Upgradation The conventionally used upgradation methods are pressure swing adsorption, pressure water scrubbing, amine adsorption, and biological methods. Even though, the latest cryogenic upgradation technology is becoming popular day by day. It is designed for the purification and bottling of biogas. In this technology boiling or sublimation points of different gases are used at very low temperature and high pressure. It is a very demanding technology because it yields 99% methane (Petersson and Wellinger 2009; Allegue et al. 2012).

High Pressurized and Multiple Stage AD To increase the efficiency of the AD process various research projects are designed to estimate different formations, i.e., single and multi-stage reactors. According to modern studies, the physical partition of the AD in two phases, i.e., acidogenesis or hydrolysis and methanogenesis or acetogenesis in separate reactors helps to elevate the organic matter decomposition into methane. The configuration of multiple bioreactors plays an important role to increase efficiency and process stability (Yu et al. 2017). Blonskaja et al. studied that the use of a two-stage scheme gives high growth of methanogens which respond in high gas production (Blonskaja et al. 2003). Similarly, Kim et al. referred that by using the four stages anaerobic digestion system the digestion activity enhanced rather than the single-stage Scheme (Kim et al. 2011). Furthermore, Nasr et al. suggested that the two-stage technology enriched the efficiency and performance of the process (Nasr et al. 2012). A recent technique is developed which works at high pressure (100 bars) and it gives the methane content about 95%. Previous studies also showed that working at high pressure (up to 90 bars) affect the microbial processes and provide enriched methane. However further analysis is required to find the microbial pressure-dependent techniques (Lindeboom et al. 2011).

24.7 Challenges, Approaches, and Enhancement Techniques

24.7.1 A Gap between Biotech and Commercialization Research

Lignocellulosic biomass, i.e., forestry residues, municipal wastes, and crop wastes are the high potential and sustainable feedstocks for the production of biofuels worldwide. The production of biogas from lignocellulose requires further research efforts for developments. It is due to the technical problems and lack of understanding of reactor operations involved in the process. The complications of the AD process and the threat to the technologies' strength are the notable problems (Himmel et al. 2007; Weber et al. 2010). To identify the research-biotech gaps, it is important to evaluate the impacts on economical, technical, and ecological barriers. For example, to reduce the cost, it is compulsory to determine the critical stages (e.g., use of enzymes or the investment on multi-stage AD systems) which affect the economy impressively. Once these standards are analyzed, they will help to indicate the costs, benefits, and research issues for improvement (Lynd et al. 2005). The finding of economically sustainable pretreatment processes has been identified as the major hurdle for the commercialization of biofuels (Philbrook et al. 2013). The amount and type of biocatalyst and microorganisms used for degradation affect the process stability and conversion rate but their cost is very high. So recent research initiatives have pay attention to the improvement of biocatalysts or microorganisms with better characteristics, low production cost, and wider applications. Recent studies also suggested the combination of high pressure and multi-stage technologies. These technologies will improve process efficiency (Blanch 2012; Banerjee et al. 2010). The research gap and scheme for the bio-industry are displayed in Fig. 24.13.

24.7.2 Biogas Future in a Green/Circular Economy

One of the renewable energy sources is biogas, and it does not generate CO₂. However, CO₂ is absorbed from the atmosphere during the biochemical process in AD and it is released with energy. When the CO₂ and minor constituents are taking away, then 100% methane is obtained. This is a zero-carbon source that is compatible with any ancillary natural gas that makes it a perfect fuel (Bharathiraja et al. 2018). Biogas has many industrial, household applications and gradually is finding as a vehicular fuel. Many efforts have been made to enhance the methane content through the optimization of techniques (i.e., pretreatment and multi-stage AD system). Various new technologies are used to improve biogas production but the challenges are still present. These challenges are (1) hydrolysis as the rate-limiting step, (2) lignocellulosic biomass particle size, lignin content, and crystallinity of cellulose. The enzyme pretreatment method helps to increase the lignocellulosic digestibility. In recent times, the biogas generates from SOWs may satisfy nearly 20% of the total natural gas. Extensive research is in progress to diversify the technological advancements and low-cost energy sources. Although to complement

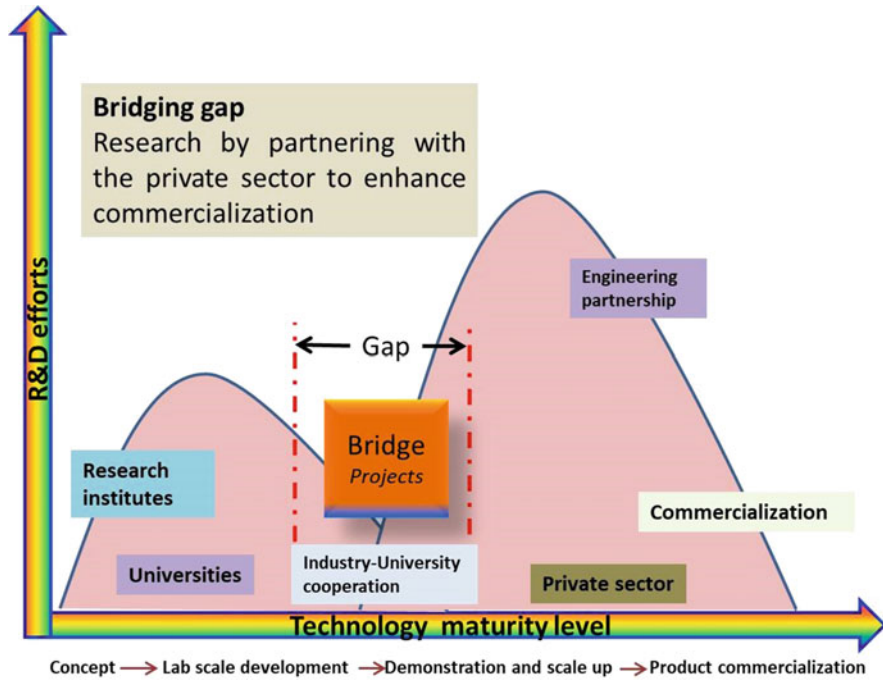


Fig. 24.13 Research gap and scheme for bio-industry (Bharathiraja et al. 2018)

the existing and developing technology, there is a sustainable management scheme for the future (Christy et al. 2014).

24.7.3 Pretreatment Techniques to Enhance Biogas Production

The treatment of solid organic wastes (SOWs) mainly agriculture waste and yard waste is very essential to expose cellulose and hemicellulose for bacterial attacks and enzymatic hydrolysis (Hu et al. 2015; Ravindran and Jaiswal 2016). Pretreatment has been classified into three main types as shown in Fig. 24.14.

Chemical Pretreatment

Chemical pretreatment of SOWs uses ionic liquids, alkalis, oxidizing agents and strong acids, etc. The reactions involved in this pretreatment are electrochemical, hydrolysis and oxidation reactions, etc. Some chemical pretreatment methods are shown in Table 24.3. It received more attention compared to physical pretreatment owing to its sound performance in enhancing methane yield. It increases organic waste's surface area and lowers the cellulose crystallinity and degree of polymerization. Despite a larger enhancement in biogas synthesis, but only alkali hydrolysis has found its practical application in the industry particularly for SOWs containing low

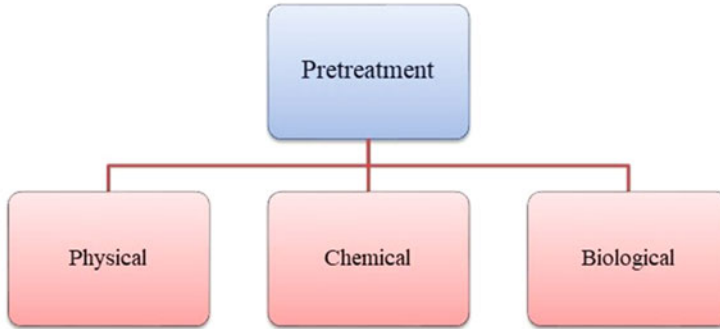


Fig. 24.14 Classification of pretreatment

lignin content (Shah et al. 2015). Whereas the main disadvantages of NaOH pretreatment are Na^+ ions that not only inhibit methane formation but also cause detrimental environmental impacts like soil salinization as well as water pollution (Zheng et al. 2014).

Modern research is trying to find eco-friendly chemicals for the maximum biogas yield.

Physical Pretreatment

The physical treatment technique does not use microorganisms or chemicals. It is used for the anaerobic conversion of SOWs to biogas as shown in Table 24.4.

Table 24.4 shows that the highest yield of biogas is produced as a result of hydrodynamic pressure homogenization (HPH) treatment of SOWs. The HPH is an environmentally friendly technique that produces a high quantity of CH_3 without any chemical in a very short time at room temperature. The lignocellulosic networks of biomass are destroyed in this technique due to sudden expansion (Yusaf and Al-Juboori 2014; Fang et al. 2015). It is also used in pharmaceutical and food industries for cell distraction and food emulsification consequently (Zhang et al. 2013c). Another physical method such as milling or comminution not only decreased the degree of polymerization and crystallinity of cellulose but also increased the surface area of feedstock by decreasing its particle size. The ultrasonic process uses high-frequency waves to obliterate the complex polymerization network in SOWs that facilitate enzymatic degradation efficiency (Ormaechea et al. 2017). Microwave is an irradiation technique that generates intense heating by applying an electromagnetic field to water comprising substances.

The steam explosion method consumes efficiently wheat straw as a substrate to increase the yield of biogas production. It is a commercial-scale process, but it yields a smaller amount of methane than HPH. It is a favorable choice for more industrial installation due to its number of benefits, for example, low energy input, commercially available tools, and low pollution tendency (Bauer et al. 2010; Forgács et al. 2012).

Table 24.3 Some chemical pretreatment approaches for SOWs

Reaction type	Conditions	Chemicals	Substrates	CH ₃ % yield (enhanced)	References
Ionic liquid treatment	1-15 h, 50–55% W/W NMNO, 120–130 °C	(NMNO) N-methyl-morpholine-N-oxide	Birchwood, oil palm bunch, rice straw, softwood spruce, etc.	47–1200%	Goshadrou et al. (2013)
Oxidation	3 h, pH 7–9, H ₂ O ₂ /COD: 0.05–0.25, 20 ± 2 °C	H ₂ O ₂	Olive mill waste	> 1000 °C (77% COD reduction)	Siciliano et al. (2016), Travaini et al. (2016)
	15-120 min, 100–200 °C, 200 rpm, 6-12 bar	O ₂ (air)	Distilleries effluents	280% (biogas)	Travaini et al. (2016)
	1 h, gas flow rate 12 L/min, 0.6–1% O ₃	O ₃	Wheat straw	45%	Padoley et al. (2012)
Acid hydrolysis	60 min, 50% (V/V) an organic solvent, 190 °C	CH ₃ COOH	Forest residue	500%	(Monlau et al. (2013))
	1–5% (W/W), 170 °C	H ₂ SO ₄	Sunflower oil	48%	Kabir et al. (2015)
Electrochemical treatment	2 h, 0.5 M, 110 °C,	Na ₂ CO ₃	Rice straw	125%	Dehghani et al. (2015)
	40 min, 2 cm electrode	Hypochlorite	Waste activated sludge (WAS)	63.40%	Iskander et al. (2016)
Alkali hydrolysis	10-240 min, 0.2–1% (W/W), 50–121 °C	Sodium hydroxide	Sugarcane bagasse, wheat straw, YW	30–78% or 250%	Bolado-Rodríguez et al. (2016)
	2.0% ca (OH) ₂ and 0.5% KOH,	Ca(OH) ₂ or KOH	Corn Stover	77%	Li et al. (2015a)
	10 bar, 0–30.8%	NH ₄ OH	Wheat straw	56% (biogas)	Li et al. (2015b)

(continued)

Table 24.3 (continued)

Reaction type	Conditions	Chemicals	Substrates	CH ₃ % yield (enhanced)	References
	(W/V), 6–48 h, 20–80 °C				
	72 h, 8–10% (W/W), 25 °C	Calcium hydroxide	Rice straw	34.3–36.7% (biogas)	Gu et al. (2015)

Table 24.4 Physical treatment of Solid Organic Wastes to produce biogas

Substrate	Conditions	Methods	CH ₃ % yield (enhanced)	References
Wheat straw	60 minutes, 140 °C	Steam explosion	4–30%	Bauer et al. (2010)
Wheat straw	6–33 mm particle length	Milling or comminution	11–13%	Dumas et al. (2015)
Wheat straw and waste activated sludge	96 KJ/kg sludge specific energy	Microwave	20–28%	Jackowiak et al. (2011)
Organic residues and waste activated sludge	96–3380 KJ/kg total waste specific energy	Ultrasonic	27–71%	Cesaro et al. (2014)
Yard waste and wheat straw (aqueous suspension containing 5% wt.)	170 °C, 20 minutes	Expansion	41%	Kuttner et al. (2015)
Wheat straw	2300–2700 rpm rotor speed	Hydrodynamic cavitation	144%	Patil et al. (2016)
Yard waste	10 MPa	High-pressure homogenization	250%	Jin et al. (2015)
Yard waste Sewage sludge	0.5–15 h 70–121 °C	Thermal treatment	20–88%	Ruffino et al. (2015)

The major drawback of hydrothermal waste pretreatment is the high temperature required to heat liquid water present in the waste substrate. Globally, it is an effective advantageous technique compared to both chemical and biological pretreatments.

Biological Pretreatment

Generally, biological pretreatment uses fungal species or biological agents to produce biodegradable enzymes that help in SOWs degradation (Yıldırım et al. 2017). The main advantages of this technique are described in the following:

1. Minimum input energy due to its low operational cost.
2. Environmental friendly.
3. No expensive consumption of chemical.

The objective of this method is to remove lignin with fewer carbohydrates that can be obtained from the SOWs (Zhang et al. 2014). Some common types of this method such as fungal, sludge, bacterial, and enzymatic pretreatment are shown in Table 24.5. Among these types, bacterial and fungal pretreatment improves both biodegradable efficiency and biogas conversion of corn straw (Zhong et al. 2011). This has not been applied effectively on a large scale due to the slow microbial growth rate and enzymatic reaction rate (Shah et al. 2015). Enzymatic treatment improves only 13–19% biogas yield.

More suitable SOWs substrates for chemical, physical and the combination of both of these techniques are agriculture and yard waste. However, simple physical process such as milling, animal manure, and food waste are preferred to reduce their particle size. The physical process breaks down capably large granules of WAS into smaller particles.

Table 24.5 Different categories of biological pretreatment for SOWs

Category of biological pretreatment	Substrates	Active constituent	Conditions	Biogas % yield (enhanced)	References
Fungi	Tall wheatgrass; Miscanthus	<i>Ceriporiopsis ubvermispota</i> and <i>Flammulina velutipes</i>	4 weeks and 28 °C	120%	Lalak et al. (2016)
The liquid fraction of digestate pretreatment	Corn Stover	Mixed microorganisms	3 days, 17.6% of TS content, 20 ± 1 °C	70%	Wei et al. (2015)
Bacteria	Corn straw and organic sludge	<i>Thermophilic aerobic</i> bacteria	pH 5.0–8.5, 20 °C, or 60–70 °C 0.01% (W/W) dose of microbial agent for 15 days	30–150%	Zhong et al. (2011)
Enzymatic pretreatment	Spent hops and sugar beet pulp	Xylanase, endoglucanase and pectinase	24 h, 0.03–0.75FUP/g enzymatic dose, 50 °C	13–19%	Zięmiński and Kowalska-Wentel (2015); Passos et al. (2016); Kiran et al. (2015)

24.7.4 Genetic Engineering

Recently, genetic engineering plays an important role to improve biogas yield by either integrating particular DNA fragments or manipulating specific genes into desirable species (Lim et al. 2018; Han et al. 2017). A yeast strain was genetically engineered in 2010 to generate its own enzymes for cellulose digestion. Nowadays, 205 Eubacterial and 21 Archaeal genomes have been sequenced. Almost 80% of genomes of Archaeobacteria are methanogens that were insulated from sludge as well as from other anaerobic environments. In the same way, many acidogenic bacterial genomes are sequenced too. The genome of *Methanobacterium thermoautotrophicum H* (thermophilic bacteria) is fully sequenced which was segregated from municipal solid waste (Zhu et al. 2017; Kougias et al. 2017).

24.7.5 Bioaugmentation

As it is discussed earlier the AD process requires microorganisms for each step. The disturbance in microorganism balance may cause bioreactor instability and lead to inhibition of methane production (Christy et al. 2014). This disturbance is due to various inhibitory factors, e.g., high level of sulfate, ammonia, phosphate, and metal ions. Some other parameters, i.e., pH variation, temperature, and resistance of feedstock also became the reason to decrease AD efficiency (Mao et al. 2015; Divya et al. 2015b). So to overcome these limitations, bioaugmentation as an alternate strategy might be used. Bioaugmentation is the addition of efficient stress-resistant microscopic species into a community of bacteriological to improve the efficacy of methane production (Lebeau et al. 2008). Some of the bioaugmentation examples are as follows:

Upgrading of Hydrolysis, Acetogenesis, and Acidogenesis In AD the very first phase is hydrolysis in which the feedstock is converted into simpler compounds. Cellulose, lignin, and hemicellulose containing substrates are among the most commonly used substrates. Though the major drawback of feedstock is the hydrolysis resistance to produce desirable products for fermentation. Different pretreatment techniques are used to improve hydrolysis but they have their limitations, i.e., partial hydrolysis and high cost (Carlsson et al. 2012). To overcome these problems various microorganisms are added that enhance the hydrolysis process due to their greater ability to break molecules (Mshandete et al. 2005). Coll and Weiss used a hemicellulolytic microbes group on the sludge obtained from the maize silage digesting plant. The outcomes displayed a 53% increase in methane production as compare to non-bioaugmented culture (Weiß et al. 2010). Similarly, Zhang and Coll suggested a pretreatment method for cassava residue. To achieve these thermophilic microorganisms enriched with cellulose and hemicellulose were used for the pretreatment of cassava residues. The outcomes showed a 97% growth in methane production (Zhong et al. 2011).

Role of H₂ in the AD The hydrogen produced in acetogenesis is used for the reduction of CO₂. Generally, methanogenesis does not carry out at a low H₂ level. So the high level of H₂ leads to enhancing methane production (Pap et al. 2015). Through bioaugmentation, the thermophilic *Caldicellulosiruptor saccharolyticus* is used to convert hemicelluloses, cellulose, and pectin to acetate, H₂, and CO₂ (Bagi et al. 2007). In 2010 it is evaluated that the *C. saccharolyticus* species uses cellulose to produce H₂ (Herbel et al. 2010). Similarly, the bioaugmentation *Acetobacteroides hydrogenigenes* on corn straw and biogas slurry give a high yield of acetate and H₂. The outcomes showed a 23% increase in methane production (Zhang et al. 2015). It is evident from literature that the concentration of H₂ smaller than 10⁻⁴ is thermodynamically unfavorable for methane production. On the other hand, the high concentration of H₂ (>10⁻⁸) acts as an inhibitory factor to hydrogenotroph. So it is very important to maintain a suitable concentration of H₂ to produce CH₄ (Kovacs et al. 2004).

Overcoming Ammonium Inhibition The obtainability of nitrogen is persistent with the cell growth which is obtained from nitrogenous matter. In aqueous solution, the inorganic nitrogen is present in the form of NH₃ and NH₄⁺. It is showed that the high concentration of ammonia inhibited the AD process because nitrogen is diffused into cells, causing potassium deficiency and proton imbalance (Chen et al. 2008). High temperature and high pH values produce free NH₃ in a higher concentration that increases toxicity. To overcome the toxicity of ammonia various methods have been studied, i.e., addition of NH₃ binding ions, high C/N ratios and low temperature of digester that reduces NH₃ toxicity (Nielsen and Angelidaki 2008). Fotidis and Coll suggested the bioaugmentation with an archaea species, i.e., hydrogenotroph *Methanoculleus bourgensis* can tolerate high ammonia levels (Fotidis et al. 2014).

Overcoming Low Temperature To enhance the AD process temperature is another significant parameter. Generally, by increasing the temperature the metabolic rate also increases which leads to high methane production. For example, when the mesophilic microorganisms are revealed to low temperature, the overall yield of biogas decreased (Appels et al. 2008). However, at low temperatures when the AD process is operating, the bioaugmentation with psychrophilic species increases the methane production. Consequently, the decrease of methane production due to low temperature can be overcome by using microorganisms that work more effectively at low temperatures (Akila and Chandra 2010).

Overcoming O₂ Produced Toxicity The O₂ present in the reactor leads to the amassing of H₂ by decreasing methanogens as a result methane production decreases. Under these conditions, exogenous methanogens accumulation helps to restore methane yield. Schauer-Gimenez and Coll used a group of H₂ amassing methanogens for bioaugmentation of the bioreactor. The outcomes showed a 60% increase in methane production (Schauer-Gimenez et al. 2010).

24.8 Conclusion

The energy crisis has been increasing day by day and the resources of renewable energy would be enough to meet the 50% global energy needs by 2050. So, biogas production attains a strategic location in the global market. The stability and performance of AD to produce biogas are primarily dependent on various groups of microscopic organisms and in turn, their functions and networks are influenced by operational parameters as well as properties of substrates. The anaerobic waste treatment process is an efficient technique to lessen the mass of the organic waste. Microbes play a very important role in the biochemical process of biogas production. In this era, it is necessary to implement the better acceptance technologies such as biotechnological advancements and investigations are needed to discover the effective feedstocks, effectiveness, and competency of the microbes and substrates for pretreatment. In recent times, the obtainability of efficient and genetically modified microbes, preparation of enzymes that are substrate-specific, microbial growth understanding, and cost reduction would be a challenge for scientists. However, the multi-stage digester designs, biological pretreatment techniques, genetic engineering, and bioaugmentation are the outstanding options used for the sustainable development of AD performance in biogas generation.

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