Chapter 3
A Comprehensive Review on Microbial Technology for Biogas Production

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Abstract Biogas, an alternative to fossil fuels, is a blend which consists predominantly of CH4 and CO2 used for transportation and collective heat as well as power (CHP) generation. The factors affecting biogas manufacture are characteristics of substrate (especially C/N and VSS/TSS ratios), concentration of substrate in feed, process temperature, retention time, working pressure, and pH of feed. Biogas is produced by anaerobic digestion, in which biopolymers are transformed to biogas in the nonappearance of O2. This digestion process is essentially anaerobic which contains four major steps. These are hydrolysis of polymer, acidogenesis, acetogenesis, as well as methanogenesis. Hydrolysis involves the breakdown of biopolymers to its monomers with the help of water. Acidogenesis involves the formation of acids, which are essentially volatile, from the monomers. Acetogenesis produces acetates and acetic acid from various volatile acids. Finally, acetates and acetic acid are converted to methane and carbon dioxide during methanogenesis. Anaerobic digestion takes place in the presence of co-culture containing hydrolytic, acidogenic, acetogenic, and methanogenic organisms. In this chapter, a comprehensive review on the development of hydrolytic, acidogenic, acetogenic, and methanogenic organisms for biogas production is presented.

Keywords Biogas · Anaerobic digestion · Hydrolytic organisms · Acidogenic organisms · Acetogenic organisms · Methanogenic organisms

3.1 Introduction

The demand of energy increases because of urbanization and industrialization. An alternate source of producing energy is required to come across the demand as well as reduce the necessity of the fossil fuels (York 2012). Biogas, a combination of

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carbon dioxide and methane in the molar ratio of 1:2, is a gaseous fuel cast-off for transportation as well as combined heat as well as power (CHP) generation (Emerson 2008). Biogas can be also used as a precursor to produce valuable biochemicals. It is manufactured through a sequence of different chemical reactions collectively called as anaerobic digestion (Sivamani et al. 2018). Anaerobic digestion converts substrate to biogas as well as digestate, which can be used as a replacement for chemical fertilizers, that enhances the sustainability of environment, energy security, as well as social economy (Ganguly et al. 2006). Figure 3.1 shows the detailed flowchart for biogas production process.

Anaerobic digestion is a complicated method that requires strong basic knowledge on biochemistry, microbiology, and process engineering (Ali Shah et al. 2014). It involves a group of microbes such as hydrolytic, acidogenic, acetogenic, as well as methanogenic organisms with different growth requirements as well as metabolic capacities. The nutritional requirements of each group of microbe should be complete for their growth as well as efficient biogas production (Schnürer 2016). The factors affecting biogas production are characteristics of substrate (especially C/N ratio and VSS/TSS ratio), concentration of substrate in feed, process temperature, retention time, working pressure, as well as pH of feed. Substrate characteristics are one of the essential parameters in biogas production because its nutrients provide sufficient growth factors (Westerholm and Schnürer 2019). Pure substrates or co-substrates which are selected for biogas production based on C/N as well as VSS/TSS ratios are used to deliver favorable conditions for microbial growth as well as biogas generation (Khan 2019). However, additives are essential to support the metabolic activity of microorganisms as well as avoid process damage.

In addition to the nutritional factors, non-nutritional parameters such as concentration of substrate in feed, process temperature, retention time, working pressure, as well as pH of feed should be optimized to achieve maximum biogas yield with minimum inhibition. Thus, numerous aspects are to be considered to obtain sufficient metabolic activity as well as higher gas production (Banerjee and Sirkar 2012). The process becomes complicated because of the interaction between nutritional and non-nutritional parameters (van Ommen et al. 2009). Figure 3.2 illustrates the digestion process (anaerobic) life cycle.

Table 3.1 shows the sequence of steps in anaerobic digestion process. This is a biochemical as well as microbial process comprising hydrolysis of the complex nutrient, acidogenesis of the converted biomass, acetogenesis of the remaining product, as well as methanogenesis. Hydrolysis contains the breakdown of biopolymers to its monomers in the occurrence of water (Thirugnanasambandham et al. 2014). Acidogenesis involves the formation of volatile acids from the monomers (Karichappan et al. 2014). Acetogenesis produces acetates as well as acetic acid from various volatile acids (Thirugnanasambandham et al. 2016). Finally, acetates as well as acetic acid are converted to methane as well as carbon dioxide during methanogenesis (Sivamani et al. 2020).

Methanogens are a type of biocatalysts which will supply the energy in the form of methane (Enzmann et al. 2018). There are a diverse group of methanogens which have a potential ability to supply energy. Methane is considered to be the alternative

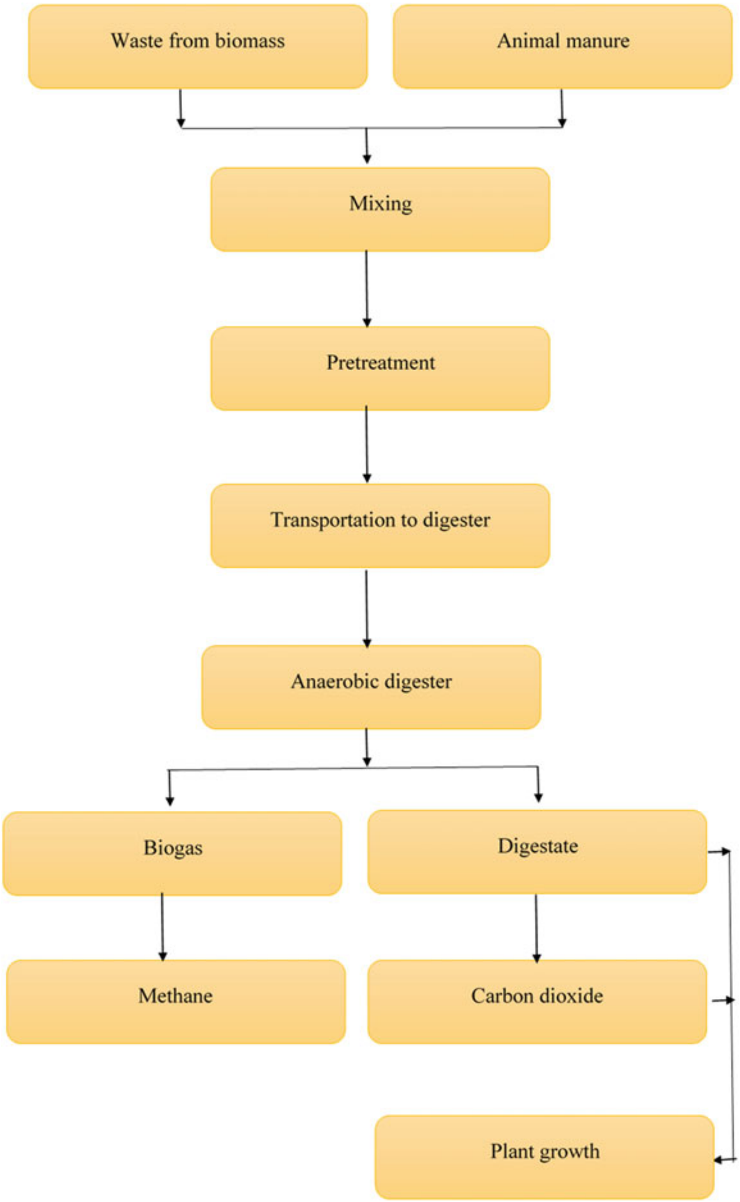

Fig. 3.1 Detailed flowchart for biogas production process

as well as replacement of the fossil fuel in the future (Olah 2005). Methanogens are converting biomass in the form of carbon dioxide as well as methane in the nonappearance of oxygen (Vavilin et al. 2008). Novel presentation of methanogens, for example, electromethanogenesis, is in the developing stage, yet many findings

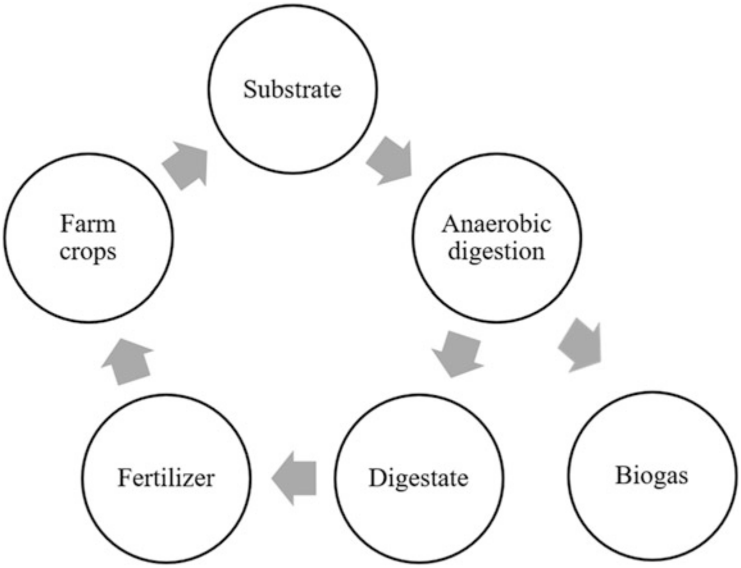

Fig. 3.2 Digestion process (anaerobic) life cycle

Table 3.1 Steps in anaerobic digestion process

S. No.	Step	Process and reaction
1.	Hydrolysis	Breaking down of complex to simpler molecules in the presence of water Carbohydrates/lipids/proteins + water → sugars/fatty acids/amino acids
2.	Acidogenesis	Conversion of simpler molecules to volatile acids Sugars/fatty acids/amino acids → volatile acids
3.	Acetogenesis	Production of acetates as well as acetic acid from volatile acids Volatile acids → acetates as well as acetic acid
4.	Methanogenesis	Biogas generation from acetates and acetic acid Acetates and acetic acid → biogas

are underway on methanogens (Blasco-Gómez et al. 2017), and various such features about the characterization of strain as well as simple genetic tool developments are still going to proliferate (Voegeli et al. 2009). Table 3.2 shows the sources of methanogenic microorganisms.

Table 3.2 Sources of methanogens

Source	Methanogen
Termite hindgut	*Methanobrevibacter arboriphilus* *Methanobacterium bryantii*
Wet wood of trees	*Methanobrevibacter arboriphilus*
Rumen of cow	*Methanobrevibacter ruminantium* *Methanomicrobium mobile*
Protozoa	*Methanobacterium formicicum*
Cecum of horse	*Methanobrevibacter* sp.
Anaerobic oceans	*Methanogenium cariaci*
Large intestine of human	*Methanobrevibacter smithii*
Hydrothermal vent	*Methanopyrus kandleri*
Landfills	*Methanobacterium bryantii* *Methanosarcina barkeri*
Sewage sludge digester	*Methanobacterium formicicum* *Methanobacterium thermoautotrophicum*

3.2 Hydrolytic Organisms

Figure 3.3 shows the sequential phases of anaerobic digestion process. Güllert et al. (2016) adapted farming biogas reactors for the production of methane from plants using a variety of microbes in the absence of oxygen. When assessed between natural and artificial schemes, biogas fermenters are inadequate in their capability of hydrolysis. The causes are not understood for the same. They showed that a representative commercial biogas reaction system added by way of chicken manure, manure of cow, as well as maize silage has shown comparatively lesser conversion in hydrolysis reactions against herbivores' feces samples. Also, they provided evidence that on average, 2.5 genes encoding cellulolytic GHs/Mbp were identified in the biogas fermenter compared to 3.8 in the elephant feces and 3.2 in the cow rumen data sets. Coding of genes for cellulose-degrading GH enzyme ratio associated with the *Bacteroidetes* versus the *Firmicutes* was 1:2.8. Besides, RNA sequencing data designated that more copied sequencing of cellulases in the biogas reactor were quadrapulated when associated with the *Firmicutes* equated to the *Bacteroidetes*, whereas a same spreading of these types of enzymes was seen in the case of the sample of excreta of elephant. The results indicated that a bacterial population has comparatively reduced association with the *Bacteroidetes* phylum and, to a certain level, *Fibrobacteres* is affiliated with a reduced activity of projected lignin- as well as cellulose-degrading enzymatic constituents in biogas reactors. This change may be ascribed to an incomplete coding of genes for cellulose-degrading bacterial GH enzymatic constituents which are associated with the *Bacteroidetes* as well as the *Fibrobacteres.* The fractional lack of these genetic constructions infers a possibly essential constraint in this biogas reactor with respect to the starting time of biomass hydrolysis. The results predicted that enhancing the participants of

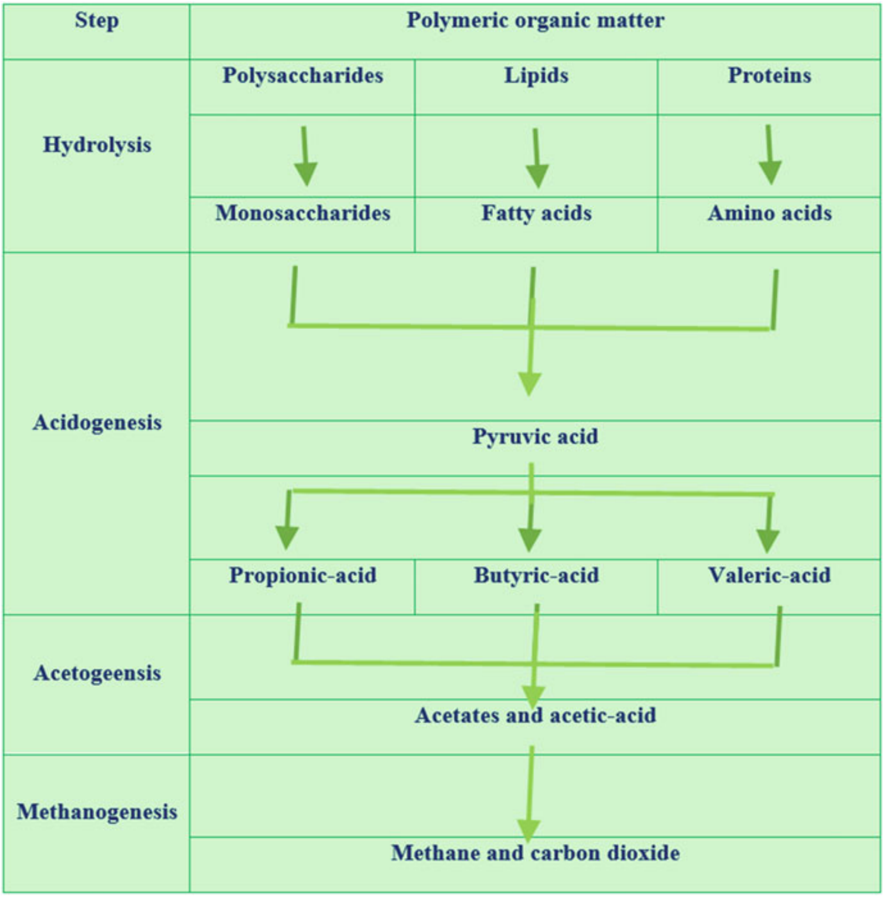

Fig. 3.3 Sequential steps of anaerobic digestion

Fibrobacteres as well as *Bacteroidetes* in biogas reactors will more probably effect in an enhanced efficiency of hydrolysis.

Song and Clarke (2009) investigated the hydrolytic capacity of cellulose through a diverse culture augmented with waste material, used for landfill in a continuous type of reactor operating at longer retention times to permit methanogen conditions. Equilibrium hydrolysis chemostat studies with methanogenic conditions are very poorly reported. Continuous process of digestion was investigated in a 1.2 L digestion reactor fed by a 1.1% (w/v) suspension of cellulose of 50 μm in sterile leaching residue extracted from a 210 L digestion reactor cast-off in a combined metropolitan solid waste material. The unsterilized leaching residue was cast off as an inoculum. Steady as well as fast hydrolytic environments were recognized at retention times of 5, 3.5, as well as 2.5 d with a hydrolytic rate having a first order of 0.44 ± 0.06 d-1 as well as higher concentration of methane produced ranging from 56 to 64% of soluble cellulose on the basis of COD. The yield of biomass was in the range of 30–36% of soluble COD cellulose, which is more than three times than that detected in the culture of fermentation process. This is accredited to the variety of the microbial populace that completely converts COD solubilized to methane gas, as evidenced by VFA yields of volatile fatty acid which is lesser than 8% on the basis of COD.

Cirne et al. (2007) understood the role of the varied inhabitants of microbes accountable for the biological degradation of organic compound to form methane as well as carbon dioxide. They conducted research to develop information about the relationships between bacteriological populations and the hydrolytic as well as restrictive phase of two-stage production of biogas from energy-producing crops. Bacterial groups as well as process performance (as determined by fluorescent hybridization of in situ manner) were studied within two distinct two-stage sugar beet as well as grass/clover digestion. Bacteriological populations established in the hydrolysis stage of anaerobic digestion of beet as well as grass/clover exhibited few connections, with the hydrolytic dynamical behavior being comparable. In both cases, the solubility of organic material was speedy during the first 11 days as well as was escorted by a gathering of lactate as well as volatile fatty acids (AGV). Among days 11 and 15, the lactate as well as VFA concentrations reduced, as did the dissolution rate. For both cases, Archaea began to give the impression in the hydrolysis stage between days 11 and 15, and the bacterial count reduced. The main cluster of bacteria identified in the fraction for beet leachate was *Alphaproteobacteria*, while for the substrate grass or clover, it was *Firmicutes*. The number of microbes that join the probes precisely pointing microorganisms with cellulolytic activity was greater in the digestion of grass than in the digestion of beet. The current investigation certified the general bacteriological cluster identification involved as well as the determination of a marked transformation in the bacterial populace when the hydrolytic rate for all of the inspected substrates became limiting. The study results can be seen as a first step in developing approaches to additionally boost the hydrolytic capacity as well as finally intensify the methane manufacture as well as yields of reactor-based digestion of these substrates.

Strong et al. (2011) assessed the breaking down of larger molecules in municipal biosolids by hydrolysis at high temperatures (145 or 160 °C) as well as wet-type oxidation (225 °C) followed by natural degeneration via anaerobic digestion (AD) which is essentially mesophilic at 35 °C. Wet oxidation (WO) destroyed more than 93% of the VSS, while thermal hydrolysis (TH) at 140 and 165 °C destroyed 9% and 22%, respectively. Sequential HHT-AD resulted in the breakdown of half of VSS. The ultimate biochemical methane production potential (BMP) of the HHT-AD from the HHT at 142 and 166 °C enhanced by 13–15% comparative to the sample. Production of biogas from destruction of matter by the WO was 54% of the controlling yields as well as solely ascribable to dissolved organic carbon in the fraction of liquid, denoting that the WO broke down entirely possible carbon compound from the heavy fraction. Analysis of samples at different points throughout the BMP shows that the development of methanogen inhibits not only the hydrolysis of solid but also the kinetic obstruction of the digestion process.

Valladão et al. (2007) examined a group of hydrolases with 21.4 μg lipase action which was formed by the important fungus *Penicillium restrictumin* fermentation of solid inoculum and wastewater and solid waste from the *Orbignya oleifera* oil manufacturing unit (babassu). Enzyme-based hydrolytic process and anaerobic biodegradation examinations were carried out in effluents from poultry slaughter-houses with different fat as well as oil contents (155–1250 mg per L) as well as enzyme concentrations of fixed pool (0.1–1.0% weight/volume). The improved efficacy of anaerobic management on the crude runoff was attained when 0.1% of the enzyme group concentration was cast off in the case of the pre-hydrolytic phase by 1250 mg of fat as well as oil (elimination of the COD efficiency) of 86% vs 54% and methane production of 178 mL versus 38 mL after 5 days.

Sangali and Brandelli (2000) characterized bacteria that deplete feathers isolated from waste from the poultry product manufacturing unit. A *Vibrio* sp. kr2 strain that produced a high keratinolytic action was isolated when developed in natural quill broth. The bacteria cultivated to an optimal range at pH 6.1 and 35 °C, where the extreme spring break action was also detected. Production of keratinase was comparable at 26 and 32 °C, while the extreme solvable protein concentration was reached at 32 °C. A drop in disulfide bridges was also detected, which increased with the time of growth. The keratinase of the kr2 strain was energetic as substrates in Ala-Ala-p-nitroanilide, benzoyl-arginine-p-nitroanilide, azocasein, as well as azokeratin. The constituents of amino acid in the feather hydrolysate were found as well as showed resemblances to that described for lysate of feather, raw feathers, and feather meal. A different innovative bacterium was sequestered and categorized as well as exhibited higher keratinolytic action. Full feather breakdown was attained in the course of farming. The kr2 strain shows prospective for use in biotechnological processes involving keratin hydrolysis.

Joshua et al. (2014) emphasized the sequential role of each microorganism as well as enzymes in the biological digester to identify each one by the role it plays, which is a way to promote more research in the production of biogas, where the isolation of these enzymes as well as microorganisms and its artificial production will help to produce more production per digester when it is artificially introduced. Biogas is a combination of gaseous mixture (containing methane 50–75% and carbon dioxide 25–50%, while nitrogen 0–10%, hydrogen sulfide 0–3%, and hydrogen 0–2%) made by anaerobic digestion (fermentation). The consecutive enzyme-based degradation of organic matter (biomass) in the biodigester is carried out in four essential as well as main steps, namely, hydrolysis, acidogenesis, acetogenesis, as well as methanogenesis. The microorganism and enzymes show an acute role in the production of biogas, which is generally not used to increase the yield per digester, commercializing the production as well as sales of biogas.

Gopinath et al. (2014) carried out to isolate different bacterial species from cow manure as well as to build four different bacterial consortia to analyze their biogas production efficiency. Microorganisms show a crucial role in the processing of organic material as well as the return of chemical compound in the active cycle. In these decomposers, they are operative in dismantling organic complex compound through successive decomposition as well as release of energy. Biogas is one of those processes that occur without presence of oxygen and involves different groups of microbes in the disintegration of organic complex and the release of methane gas. To obtain biogas with a higher concentration of methane, it is significant to generate as well as retain the appropriate bacterial consortia within the digester. Biogas manufacture was performed in a batch reactor in pilot scale for 30 days with poultry feces as substrate as well as four different bacterial consortia in four separate digesters. Different hydrolytic enzymes, volatile fatty acids, and biogas production were measured in an interval of 10 days. From the preceding study, it was established that consortia that contain many methanogenic bacteria produced the highest production of biogas with methane 79.45%.

Dioha et al. (2013) investigated the effect of numerous parameters such as concentration of suspension, pH humidity, temperature, total solids, and the carbon/nitrogen ratio on the production of biogas. The nitrogen as well as carbon content of different biogas feed stocks was calculated by typical procedures, and the capacity of biogas manufactured by the substrates was determined by the help of the cylinder. The outcomes indicate that the C/N ratio influences the capacity of the biogas produced. Biogas manufacture is governed largely on the selection of raw material as well as the C/N ratio.

Neshat et al. (2017) presented an assessment on the co-digestion of manure of animal and lignocellulosic raw material for the manufacture of biogas which is essentially an anaerobic process. Quite a few co-fermentation investigates of these wastes of organic materials are designated as well as evaluated. Extending the influence of various parameters including hydraulic retention time (HRT), temperature, organic loading rate (OLR), pH, C/N ratio, volatile fatty acid concentration (VFA), and alkalinity on the steadiness and performance of the co-digestion procedure deliberated, it is conferred the effect of numerous basic treatment approaches, including chemical, physical, as well as biological pre-treatments, on the supply of a well-organized substrate for co-digestion which is essentially anaerobic and consequently the improvement of the production of biogas.

Table 3.3 summarizes the literature on hydrolytic organisms. This also reveals from this research that the intermediates and the main factors may slow down the process and even can stop the process also. This type of digestion process is biotechnologically versatile to transform the complex organic material into the valuable form biogas. Manure anaerobic digestion makes the utmost of the process, since it allows the concurrent production of biological energy, the manufacture of adaptation of soil which is nutrient-rich, the control of odors, and the reduction of greenhouse gas emissions; therefore, it fits in with agriculture performers which is essentially climate-friendly. Despite the listed benefits, the probability of compost for biogas manufacture is not essentially fully exploited due to the little as well as unbalanced carbon and nitrogen (C/N) ratio in animal dung. To meet anaerobic digestion supplies as well as to recompense for carbon shortage in compost, additional carbon-rich material must be processed together with compost to develop its features for anaerobic digestion. Lignocellulosic biomass deposits display potential for this.

Table 3.3 Summary of literature on hydrolytic organisms

References	Significant findings from hydrolytic organisms
Güllert et al. (2016)	Enhancing the participation of *Fibrobacteres* as well as *Bacteroidetes* in biogas reactors will more probably effect in an enhanced efficiency of hydrolysis
Song and Clarke (2009)	Hydrolytic capacity of cellulose through a diverse culture augmented with waste material in a continuous reactor operating at longer retention times enhances methane yield
Cirne et al. (2007)	Hydrolytic capacity as well as final intensification of methane manufacturing improved yields biogas
Strong et al. (2011)	Development of the methanogens inhibits not only the hydrolysis of solid but also the kinetic obstruction of the digestion process
Valladão et al. (2007)	The improved efficacy of anaerobic management on the crude runoff was attained
Sangali and Brandelli (2000)	The kr2 strain shows prospective for use in biotechnological processes for biogas production
Joshua et al. (2014)	The microorganisms and enzymes increase the yield of biogas per digester
Gopinath et al. (2014)	Consortia containing many methanogenic bacteria produced the highest production of biogas with methane 79.45%
Dioha et al. (2013)	Biogas manufacture is governed largely on the selection of raw material as well as the C/N ratio
Neshat et al. (2017)	The effect of numerous basic treatment approaches improved the gasification of biomass

3.3 Acidogenic and Acetogenic Organisms

Choi (2020) investigated the effect of acidic rice bran broth of fermentation process (RFFB), tap water (TFFB), or the by-product constituents of fresh fish (FB) on the decrease of slurry as well as biogas manufacture in a co-digestion procedure which is essentially anaerobic. The acidogenical fermentation of FB with the indigenous rice bran constituents was quicker and provided supplementary VFA than tap process water and municipal supply water. The decreased efficiency for the oxygen consumption of chemicals, VS, as well as total amount of solids was maximum at RFFB. The kinetic parameter λ (d), which signifies the delay phase length, was shorter with RFFB (1.093 d) as well as higher in sewage municipal and domestic sludge (8.87 d). As the quantity of VS is weighed down and the necessity for chemical oxygen increases, the quantity of biogas accumulated also increases. The quantity of methane made and the recovery of energy were higher at the RFFB (5.72 kWh). The anaerobic joint fermentation of FFB as well as municipal sewage sludge has enabled the reduction of sludge as well as recovery of the energy through the use of scrap waste by way of an organic carbon source. Figure 3.4 shows the products formed during acidogenesis and acetogenesis processes.

Coelho et al. (2020) examined the potential evaluation as well as kinetic modelling of CA production with milk wastewater as a substrate. The work should also evaluate the possible manufacture of CA from milk-derived wastewater coming

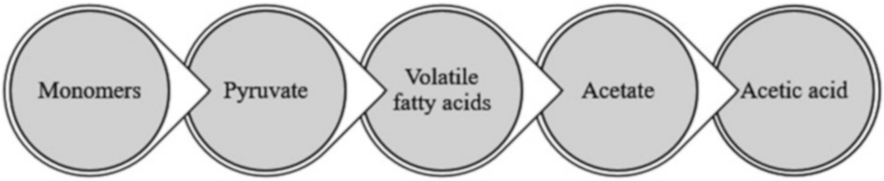

Fig. 3.4 Products formed during acidogenesis and acetogenesis processes

from the dairy wastewater (DW) as well as implement a modelling of the kinetic parameters of the method. The experimentations were carried out in quadruple batch type of reactors (volume is in the range of 250 mL) with a microbial seed material from an upflow anaerobic sludge blanket (UASB) stirrer at 0.6 ± 0.05 g COD g VSS-1. To prevent methanogenic reaction, 1/20% chloroform (v/v) was injected inside the working reactors. Investigations have shown that DW is undergone into the fermentation steps easily on behalf of acidogenic microorganisms since it shows larger short-chain CA creation rates in the initial 2 days of the experimentation. Small concentrations of middle-chain CA point out that protein and fats were not the chief constituents of the source of carbon for the fermentation of DW. The product attained was 0.67 mg CA mg CODA-1, which corresponds to 0.83 mg CODCA mg CODA-1. Investigation of the kinetic model reveals the fact that the first-order model of the exponential phase can be easily described. It is also reveals that the Fitzhugh models are suitable for the simulation of the carboxylic acid production. After all, DW appears to be an encouraging and favorable substrate for the study on the carbon platform.

Li et al. (2020) carried out tests to produce biogas from silage of the straw of corn (CSS) as one of the principal solid organic wastes. The goal of the team was to scrutinize the probability and the optimum control approach for the anaerobic digestion of CSS (EA). Four leach bed reactors (LBR) were functioned at diverse pH standards. The extreme concentration of volatile fatty acids (VFA) of 19.33 g/L was attained at pH 8.1 with vinegar as well as propionic acids as the leading VFA. Later bacteriological analyses showed that the plentiful bacteria were *Proteobacteria*, *Firmicutes*, as well as *Bacteroidetes*. The UASB is integrated as a methane conversion reactor in the case of the LBR. The organic compound load (OLR) might touch to 8.0 g COD/l • d if converted effectively into AGV. Acetotrophic methanoates as well as hydrogenotrophic methanobacteria have acted a significant character in the process of methanogenesis. Throughout the procedure, the outcomes exhibited that a yield of methane which is 144.4 mL CH4/g volatile solid (VS) was attained. Two-phase OLR controls and pH were possible for the manufacture of gaseous methane from CSS.

Mukhuba et al. (2020) examined serious environmental problems such as emission of the greenhouse gas caused by the uncontrolled overproduction of fruit and vegetable waste. The team examined the connection among the construction of the bacteriological community as well as the production of biogas with mixed fruit as well as vegetable residues (MFVW) and cow dung as in the form of substrates.

Anaerobic digestion (EA) is gradually a widespread technique for treating food waste while producing biogas.

Agustini et al. (2020) investigated the possibility of using raw tannery wastewater as a substitute for the nutrient supply in the anaerobic co-fermentation of two solid tanneries with respect to energy efficacy, waste treatment efficacy, as well as economy. The results showed that the use of tannery wastewater as a nutrient source for the solid tannery waste AD was sufficient from the viewpoint that the three wastes were treated simultaneously. There was biogas production of only 1.9 ± 0.3 mL/VSS. However, the methane present in the biogas reached 33% at the beginning of the process, which shows that there is methanogenic activity and EA was founded. The cost analysis showed that wastewater treatment and solid waste disposal costs were reduced by 23% and 18% of electricity consumed as well as 11% and 8% of heat consumed, respectively.

Tongco et al. (2020) aimed to improvise the process of the basic sludge degeneration with the help of the lipase and protease enzyme, and the optimum ratio of these two enzymes is evaluated. Three types of the Korean WWT plant are used for the enzymatic hydrolysis of the basic sludge. Lipase as well as protease was separated from enzyme manufacturing secondary sludge microbes, which were taken at eight diverse fermentation places in Korea. The major degradation of the sludge by enzymatic hydrolysis was followed by the measurement of the decrease in the suspended volatile solids (VSS) of the suspension-enzymatic mixture at 41 °C and pH 7.1 for 72 h. The primary mud enzyme mixture from Ulsan treated with 1:3 lipase protease was optimal with a 33.3% reduction in VSS. Methane biochemical potential (BMP) assays for the optimum enzyme mixture were cast off to measure the possibility of the hydrolytic substrate for further degradation (VSS reduction). The significant decrease in VSS as well as the developed methane and biogas production treated with primary enzymes are related to the degradation of the polymer organic complex materials, which leads to effective use of microbes in the process of anaerobic digestion.

Ngan et al. (2020) examined the process of anaerobic digestion (EA) of the decomposition of organic substances by microbes in the absence of oxygen where biogas as well as the methane, a key source of renewable energy, is generated. The chapter also dealt with current research results on the generation of biogas from the co-digestion process which is essentially anaerobic, by mixing farming by-products, concentrating on rice straw and animal compost as substrates. The use of the biological suspension of the process of fermentation in marine culture activities as well as agronomic cultivation is also discussed. When using only a source of the organic material such as pure substrates, it is hard to raise the AD procedure for the unevenness of the nutrient, the deficiency of suitable bacteriological populations, as well as the impact of operating restrictions. Since rice straw is rich in cellulose, it must be pre-treated before being placed in the anaerobic fermenter. Table 3.4 summarizes the literature on acidogenic organisms.

Uma et al. (2020) examined anaerobic fermentation technology for converting organic substrates into biomethane potential. This study evaluates the common digestibility of food waste (FW) as well as pasture (SG) in different ratios as well

Table 3.4 Summary of literature on acidogenic organisms

References	Significant findings from acidogenic organisms
Choi (2020)	As the quantity of VS reduces and the COD increases, the quantity of biogas accumulated also increases
Coelho et al. (2020)	Dairy wastewater appears to be an encouraging and favorable substrate for the study on the carbon platform
Li et al. (2020)	A yield of methane is 144.4 mL CH_4/g volatile solid (VS)
Agustini et al. (2020)	Wastewater treatment and solid waste disposal costs were reduced by 23% and 18% of electricity consumed as well as 11% and 8% of heat consumed, respectively
Tongco et al. (2020)	Methane biochemical potential (BMP) assays for the optimum enzyme mixture were cast off to measure the possibility of the hydrolytic substrate for further degradation
Ngan et al. (2020)	The use of the biological suspension of the process of fermentation in marine culture activities as well as agronomic cultivation was explored
Uma et al. (2020)	The occurrence of sluggish and profligate decomposable organic materials contributes equally to the production of biomethane

as mixed temperatures. To respond to the assessment of the performance, the reaction of the volatile acid groups like valeric acid, propionic acid, butyric acid, as well as acetic acid, the pH value was coupled to the generation of biological methane. The highest methane yield observed was 266 mL/g VS in the mesophilic state and 235 mL/g VS in the thermophilic state. Methane performance reacts positively to dual digestion, which is established by the digestion performance index (DPI). In addition, the parameters of the process, namely, the concentrations of butyric acid as well as acetic acid, were in the range of 15–70% and 18–70% for the loads at 36 °C and 56 °C. SG showed the highest concentration of butyric acid as well as on the contrary the maximum created acetic acid by FW or SG. Although a lower inhibition of biomethane yield is observed at higher acid concentrations during the performance evaluation, the result showed that 1:1 co-digestion under mesophilic and thermophilic conditions resulted in better yield with FW as well as SG. The result showed that 1:1 co-digestion under mesophilic and thermophilic conditions resulted in better presentation with FW as well as SG. The study approves that the occurrence of sluggish and profligate decomposable organic materials contributes equally to the performance of biomethane.

Ghosh et al. (2020) assessed the possibility of simultaneous digestion of municipal sewage sludge (SS) in addition to organic portion of municipal solid waste (OPMSW) to improve the production of biogas. A biogas production of 585.2 mL biogas/g VS with the maximum methane composition of 69.6% was perceived with an optimal OFMSW:SS mass ratio (2:3). Fungi as well as bacteria have been shown to be primarily associated with the early phases of AD and hydrolysis. The hydrotrophic path was followed fewer, as evidenced by the decrease in the frequency of oxidants in synchrophic acetate.

Depraect et al. (2020) investigated a new three-stage process from tequila vinasse (tv) for cascading lactate, bihydrogen, as well as methane, focusing on achieving a great as well as steady biological hydrogen product rate (HPR) by using lactate in the form of precursor to bihydrogen. In the principal step, the adjusted working situations of a batch sequence reactor maintained a concentration of lactate 12.5 g/L, which corresponds to 88.9% of the entire organic acids created. In the second step, stimulating dark fermentation, which focused on lactate, which separates the creation of hydrogen starting the use of carbohydrates, was an actual method that allowed the steady creation of hydrogen with fewer than 10.5% HPR fluctuations with an extreme HPR of 12.2 l/Ld and a hydrogen production of 3.2 l/LTV. Finally, 1.6 L CH_4/L.d and 6.5 L CH_4/L_{TV} were obtained when feeding the biohydrogen fermentation effluent to a third methanogenic stage, yielding a global energy recovery of 267.5 kJ/L_{TV}.

Paulista et al. (2020) investigated the anaerobic digestion of raw glycerin by biodiesel production as a practicable way to produce methane. Ultrasound stimulates the hydrolysis of low-chain fatty acids as well as biodegrades microorganisms. In addition, *Escherichia coli* and *Aspergillus niger* produce lipases that can break down LCFA. The study aimed to increase the methane production of the ultrasound-assisted anaerobic digestion for the biodegradation of *A. niger*/*E. coli*. The effects of the various treatments were evaluated in a batch digester mixed with CG in the range from 0.2 to 3.3% (v/v). The optimum situations were reproduced in an upstream reactor to act out on a large measure. PMBR experimentations showed that the steps of biodegrading *A. niger* or ultrasound enhanced the yield of methane from 99% for 1.7% CG to 11% for 0.2% CG. Using a UASB digester, CG ultrasound resulted in 29% increase in the production of methane. *A. niger* achieved an average 77% increase in methane production was achieved using a preliminary CG biodegradation step, when operated at a loading rate of 2.9 kg COD m^{-3} day^{-1}.

Lamoh et al. (2020) worked on the application of the “waste-to-energy” (WtE) approach to achieve sustainability in the supply of renewable energies as well as the atmosphere. The goal of the team was to present a study on the performance of biogas creation through the anaerobic fermentation process of the wastewater coming from the palm oil plant (POME). Research has attempted to solve the problem associated with the low production of biogas from the anaerobic fermenter known to the industry as POME. Several published articles suggest that the enactment of the anaerobic reactor of continuous type based on the continuous stirred tank reactor (CSTR) is expressively poor and theoretically as well as economically unworkable (Banerjee and Biswas 2004; Carpenter et al. 2015). A two-stage CSTR with inoculum was used for the digestion of POME, which was enriched with the ratio of carbon/nitrogen (C/N) at diverse pH values. The operation temperature of this type of reactor is 35 °C with different input areas. The Design-Expert® is the traditional software which is cast off to regulate the variety as well as level of inputs as well as to control the quantity of investigational tests through various groupings of input dynamics. The results of this study show that optimal biogas manufacture at an important level (p-value <0.06) of the use of organic substances ($R^2 = 62.25\%$) was achieved in the process of digestion with the time-based rate of organic pollution of 5.1 g VSS/Ld, C/N of 30.6, and pH of 6.65. The results of this study would be beneficial in case of palm oil industry to optimize the making of biogas since POME like WtE. The innovation of this investigation is the usage of a C/N ($12 < C/N < 42$)-enriched inoculum made of banana peels in the POME substrate to produce biogas.

Vassalle et al. (2020) used upflow anaerobic sludge blanket (UASB) reactors to purify domestic wastewater and often need the treatment of the product stream. Few are recognized about the usage of higher-speed algae pools (HRAP) for post-treatment of wastewater from UASB reactors. The study was to estimate a UASB reactor, monitored by an HRAP, in the case of efficacy of wastewater management as well as biogas generation. The UASB reactor jointly preserved the fresh wastewater as well as the microalgae biomaterial in the HRAP that was recycled in the reactor. The same type of UASB reactor was used as a control, which only treated raw sewage. The results showed a total elimination of 66% COD and 60% N-NH4 in the scheme. In addition, the methane produced with microscopic algae increased by 25% from 155 to 210 L CH_4 kg^{-2} VS after simultaneous anaerobic digestion. An energy evaluation was carried out with positive energy stability after the yearly typical deduction ratio value of 2.10.

Botta et al. (2020) investigated the utilization of paper for volatile fatty acids (VFA) as well as hydrogen (H_2) using microbial community. In nature, serial dilutions were executed to achieve a non-methanogenic fermentation consortium that was used as an inoculum. A small volume of H_2 was detected under thermophilic conditions. There was a wide variety of microbes compared to the cleaned rumen fluid. To summarize, temperature affects the structure of the metabolic pathway, the microbial consortia, and the main by-products that arise from fermentative activity.

Huang et al. (2020) explored the possible consequence of a shock burden of the macrolide clarithromycin taking place in the methane manufacture from the digestion process essentially in the absence of oxygen. The experimental outcomes exhibited that the time-based rate of CH_4 production in the clarithromycin strain was significantly suppressed during the initial times of breakdown, but slowly increased afterward. However, the entire accumulated methane produced in the absence or presence of clarithromycin displayed insignificant change after digestion, and the maximum methane production rate increased, at 15.0 ± 0.5 mL/(g VSS • d), with a higher concentration of CLA of 0–2100 mg/kg TSS, from 22.4 ± 0.8 mL/g volatile suspended substances (VSS). Mechanism studies have shown that CLA negatively influences hydrolysis, acidogenesis, acetogenesis, homoacetogenesis, as well as the process of methanogenesis.

Zahedi et al. (2013) investigated the production of hydrogen (HP) from the solid fraction of organic municipal waste under thermophilic as well as acidogenic circumstances. The consequence of nine diverse percentages of the biological material load (from 10 to 230 g total volatile solid/l/d) and the hydraulic residence time (HRT) (from 11 to 0.25 d) was examined. Butyrate was usually the primary acidic compound formed. The biogas generated was free of methane as well as sulfur in entirely OLRs verified. The increase in OLR led to an upsurge in both the amount and the superiority of yield of hydrogen, with the exception of the extreme tested OLR (225 g TVS/l/d). The highest percentage of hydrogen was 56 (vol/vol) with an

Table 3.5 Summary of literature on acetogenic organisms

References	Significant findings from acetogenic organisms
Ghosh et al. (2020)	A biogas production of 585.2 mL biogas/g VS with the maximum methane composition of 69.6% was perceived
Depraect et al. (2020)	A new three-stage process for cascading lactate, hydrogen, as well as methane was studied from tequila vinasse (TV)
Paulista et al. (2020)	An energy improvement of 0.49 kW.h/d was achieved with a biogas quality of 73%, 0.573 m^3 CH_4/kg VS, and 0.435 m^3 CH_4/kg COD removal
Lamoh et al. (2020)	An optimal methane was yielded at the rate of organic loading of 5.1 g VSS/L.d, C/N of 30.6, and pH of 6.65
Vassalle et al. (2020)	The UASB reactor preserved the fresh wastewater as well as the microalgae growth
Botta et al. (2020)	Temperature affects the structure of the metabolic pathway, the microbial consortia, and the main by-products that arise from fermentative activity
Huang et al. (2020)	The accumulated methane produced in the absence or presence of clarithromycin displayed insignificant change
Zahedi et al. (2013)	The increase in OLR led to an upsurge in both the amount and the superiority of yield of gas

OLR of 115 g total volatile solid/l/d (HRT = 0.6 d). HP ranged from 0.1 to 5.6 L hydrogen/l/d. Nakasaki et al. (2020) characterized the microbial community and its role in digesting anaerobic lipids. Table 3.5 summarizes the literature on acetogenesis.

3.4 Methanogenic Organisms

Methanogens are the types of prokaryotic cells (Fig. 3.5). There are mainly five orders by which the methanogens are subdivided. These are Methanomicrobiales, Methanobacteriales, Methanococcales, Methanopyrales, and Methanosarcinales. Methanococcales and Methanosarcinales are responsible to convert acetate to methane which is identified as aceticlastic methanogenesis (Timmers et al. 2017). An appreciable investigation of the metagenomic structure of methanogens has shown that methanogen cannot be confined to the Euryarchaeota. Bathyarchaeota (Evans et al. 2015) and Verstraetearchaeota (Vanwonterghem et al. 2016) are the main two classes which are hypothesized recently.

Various groups of methanogens can originate from different types of anoxic atmosphere (Garcia et al. 2000). For example, salty lakes as well as thermal discharge line may be the possible habitat of the methanogen. Some type of the methanogenic bacteria may be attached to animals as well as plants and may be set up in the anthropological body. *Methanobacterium arbophilicum* is one such type of methanogen which can be isolated from the tissue of the moist wood which mostly originates from the stem of plants and consumes hydrogen which is generated from the degradation of cellulose as well as pectin by *Clostridium butyricum* for

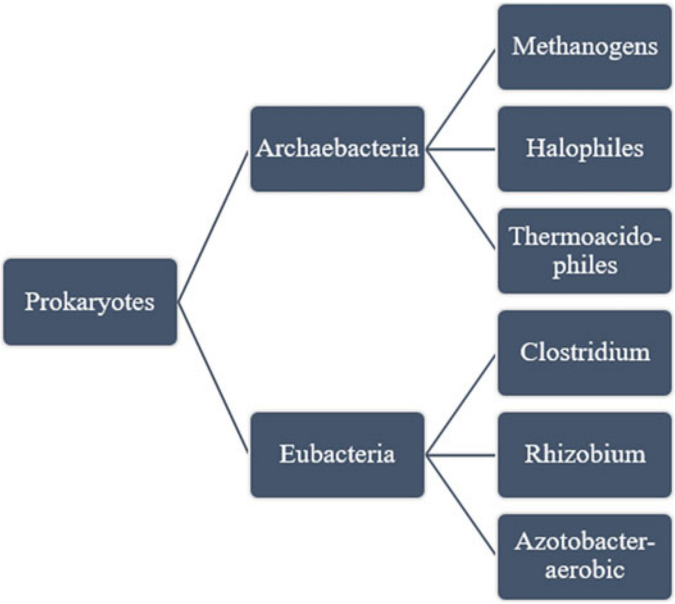

Fig. 3.5 Methanogens as a type of prokaryotes

methanogenesis (Schink et al. 1981). Also, various *Methanothermobacter* species may originate from the insect's GI tract especially in termites (Leadbetter and Breznak 1996).

Biogas plants or digesters as well as landfills are also the most common habitat of methanogenic bacteria. The community is also depending on the substrate and varies accordingly. In the case of biogas plant Due to the process of acetogenesis and fermentation, complex polymeric organic materials are hydrolyzed to amino acids as well as sugar, carbon dioxide and hydrogen is created for methanogenesis as substrate (Tumbula et al. 1997). In case of biogas plants, after hydrolytic activity of polymers, complex sugars as well as amino acids are produced through methanogenesis by acetate, H_2 as well as CO_2.

Figure 3.6 shows the percentage generation of various components during methanogenesis. Methanogenesis not only displays a wide range of information about their habitat, but it is morphologically also highly diversified. Also, it may vary in terms of pH, uniqueness, as well as temperature optimization. *Methanosphaera* or *Methanococcus* is in the group of coccoid which is short or long rod type. *Methanoplanus* which is a plate type shape and *Methanopyrus* which is rod type chain as well as *Methanospirillum* which is as per the name is spiral type belongs to the methanogenic group (Wang et al. 2017).

Differences in methanogenic bacteria are also found in diverse growth situations. Many methane-producing bacteria can be sustained in a mesophilic temperature

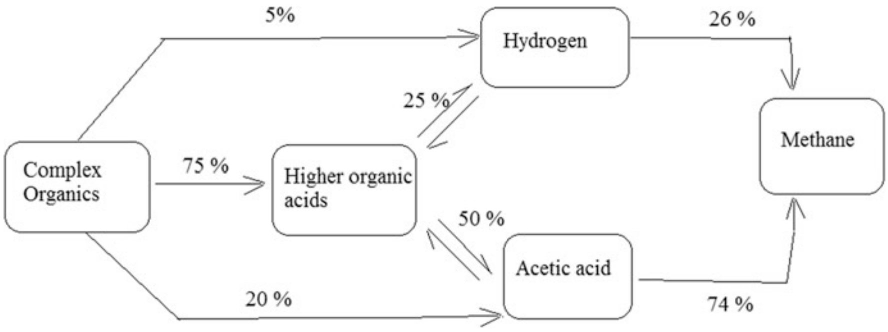

Fig. 3.6 Percentage generation of different components in methanogenesis

range. Most of the methanococci group such as *Methanobacterium* and *Methanosarcina* are of the same category. Hyperthermophilic as well as thermophilic methanogenic bacteria are also not rare. *M. jannaschii* and *Methanothermobacter* are proliferating in the range of 74–84 °C. Even some hyperthermophilic methanogen like *M. kandleri* can tolerate about 105 °C (Ward et al. 2008).

Temperature as well as high concentration of salt can also be significant parameters for methane-producing bacteria. A few methanogenic bacteria have survived as well as produced colonies in salty lakes as well as ponds which are considered to be hard environment for them due to the high concentration of the salt. These types of methanogens are protecting themselves by the salting-out mechanism and minimize the loss of water from their cell. Usually, the water is permeating through the cell boundary, and due to the higher concentration of salt present outside of the body, the water may permeate outside through the cell causing its death (Weiland 2010). Although most methanogens are optimally elevated in the vicinity of neutral pH, some, which are halophilic or halotolerant, also show conversion with alkaline pH.

Usually methanogenic bacteria can be separated into two categories as per the procedure of the conservation of the energy. Cytochromes are presents in one group of methanogenic bacteria and in the other group of methanogenic bacteria, cytochromes are absent (Mayer and Müller 2014; Thauer et al. 2008). Cytochrome is present in most of the methanogenic bacteria in which they have a coenzyme which creates a gradient of positive sodium ion across the cell membrane. *M. barkeri* or *M. mazei* is of this category which cheats this type of gradient of positive sodium ion across the cell membrane.

When a reactor is equipped with electrodes containing methanogenic bacteria, the methane gas is produced by the concerted action of methanogen across the reactor. The external voltage supplied to the electrode is used to electrolyze the water in the anode. In this case, due to the transfer of the electron in the anode, the water is fragmented in proton as well as oxygen ion. The generated extra electron is transported into the anode which usually happens in the microbial fuel cells. To date, most research of electromethogenesis have been conducted by mixed cultures,

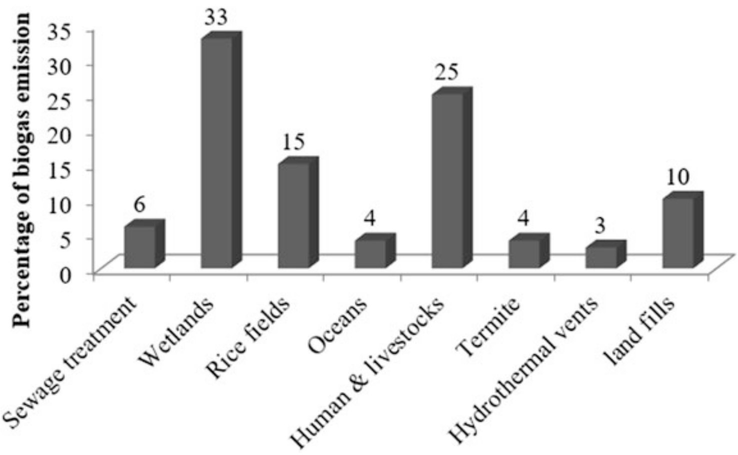

Fig. 3.7 Percentage atmospheric emission of biogas in the form of methane from different sources

such as from microbial fuel cells, biogas plants, or wastewater treatment plants (Ward et al. 2008).

Methanogen is a very assorted cluster of bacteria, and most of the group can exist in extreme environment like in high pH as well as in high osmotic pressure and higher as well as lower temperatures. So the advancement and optimization of the industrial processes require the involvement of the methanogen (Valentine et al. 2000). Generation of biogas in the form of methane as well as carbon dioxide from the organic waste or substrate is the principal application of the methanogenic bacteria. In this recent decade, the production of biogas is holding a leading role, and 30% of the energy is produced by this method in entire Europe. The biomethanation process or the anaerobic digestion process is a four-stage process. The first step is the hydrolysis. In this process, the organic materials in various complex forms such as polysaccharides, proteins, lipids, etc. are hydrolyzed by the enzymatic action of hydrolytic bacteria to produce a monomer of various organic compounds such as sugar, amino acid, long- as well as short-chain fatty acids, etc. which is again consumed by bacteria. The second step is called acidogenesis. In this procedure, the hydrolytic combinations are fermented as well as oxidized to produce different fermented products like ethanol, formate, hydrogen, carbon dioxide, propionate, acetate, etc.; the third step is the acetogenetic step. In this procedure, the fermented yields are further oxidized to produce mainly carbon dioxide as well as acetate. Hydrogen is also generated during this process. The last step is methanogenesis. In this step, methanogenic bacteria are responsible in converting carbon dioxide as well as hydrogen into methane gas (McInerney et al. 2008).

Figure 3.7 shows the percentage atmospheric emission of biogas enriched with methane from various sources. Sewage treatment by means of anaerobic digestion process not only yields biogas in the form of methane but also delivers uncontaminated water. The use of methanogen transforms organic material into biogas and decreases the quantity of sludge and reduces its pathogen concentration, and generally less amount of bioenergy is required than aerobic digestion processes. Upflow anaerobic sludge blanket (UASB) reactor is cast off mainly in the anaerobic wastewater treatment process. In this process, there are two openings. The upper one is used to discharge the cleaned water, and the lower one is used to send the raw wastewater into the reactor. A sludge blanket is formed inside the reactor which is acting as a filter again for the treatment of upcoming wastewater, which is then discharged or removed from the reactor. In this blanket, the methanogens are converting organic materials into the stable product in the form of biogas (Sarkar and Banerjee 2013).

Agricultural wastes are the main biologically degradable waste to get biogas in the form of methane as well as carbon dioxide. It also consists of poultry, pig, and cattle waste as well as slurry and manure coming from animals. The anaerobic digestion of these types of waste not only decreases the pollution load as well as generates biogas in the form of methane, but it decreases the concentration of pathogen and smell and enhances the quality of the manure used as a fertilizer (Sahlström 2003). It is observed that in many agricultural fields like those of maize silage as well as sugar beet, simultaneously the biogas plant can also run (Demirel and Scherer 2008; Lebuhn et al. 2008).

Among the available technologies, anaerobic digestion presents a number of relevant advantages. Firstly, this process reduces the chemical oxygen demand (COD) of the waste to produce valuable energy (methane). Secondly, it has been experimentally demonstrated that this process is particularly well adapted for concentrated wastes such as agricultural (e.g., plant residues, animal wastes, etc.) and food industry wastewater. In addition, it is able to operate under severe conditions, i.e., high-strength effluents as well as short hydraulic retention times. Finally, anaerobic digestion is also often used as sludge treatment for the stabilization of primary as well as secondary sludge. Only a few research works have been reported for the production of methane-rich biogas using industrial wastes (Banerjee and Biswas 2004).

Several methanogenic strains have also been shown to produce hydrogen (Valentine et al. 2000; Gieg et al. 2008). This can happen when the amount of hydrogen is very low (around below nano-molar), so that methanogenic bacteria is about to start producing metabolic hydrogen instead of taking in hydrogen. It has been proven that formate and possibly other metabolites, not methane, may be the source of H_2. It is not seen in the case of reverse methanogenesis (Valentine et al. 2000; Lupa et al. 2008).

In current decades, tools for the production of genetically modified methanogen have been developed, which leads to open a novel arena of research. At the initial stage, the production of methanogenic microbes can be improved. As an example, modification of the strain *M. maripaludis* to create geraniol is possible in place of biogas from the formate or carbon dioxide and hydrogen (Liu et al. 2016).

About 70% of the petroleum is well stored in the field if natural extraction procedure is implemented. The residual oil present in the oil field is converted in the form of biogas by the concerted action of the methanogenic bacteria. The used strain is generated from the sediment of the intermediate layer, and maybe a high concentration of the petroleum product is present. The remaining oil has been shown to be converted to natural gas by a methanogenic consortium that was associated with the oil field (Jiang et al. 2014). The consortium used was derived from satellite sediments and can be enriched with crude oil. *Bacteroidetes*, *Clostridiales*, *Methanosaeta* sp., etc. are this type of methanogen.

Archaeologists are still struggling to gather enough evidence before reaching the final conclusions about the effectiveness of citrophic sulfate-reducing bacteria. Methane collected from the coal bed is the general methane source. Around 50% of this methane gas is generated by methanogenic bacteria present in the environment. Responsible aromatic constituents inside the coal bed are used as a substrate for this production (Mayumi et al. 2016). In this regard, it reveals that *Methermicoccus shengliensis* species can generate 11 microliter of methane gas from 1 g of coal. This methane gas is already consumed by various manufacturing units. It is also predicted by the researcher that this strain may be used for the production of the methane from other various sources.

Almost 82% of the world's industry waste is polluted by the metallic as well as organic pollutants. Statistics collected from both anaerobic and aerobic schemes prove that biological degradation of the organic matter can be decreased by the toxic nature of metal. Failure to consider metallic organic availability instead of total metals probably leads to metallic organic availability leading to substantial variability in the reporting of resistive densities of metals that affect the amount of metallic organic presence. Metals usually affect biodegradation. Latest methods to enhance biodegradation in the presence of metals include a reduction in the bioavailability of metals and the use of metal-resistant bacteria, additives of the treatment process, and soil minerals. Some metal is used as a catalyst in this biomethanation process. For example, iron in the form of ion if present in the biomethanation process accelerates the process. One of the theories behind it is it increases the activity of the methanogen by changing the electrons from the metals (Carpenter et al. 2015). It is also observed that the presence of hydrogen in the system can enhance the production of biogas. A methanogenic bioelectrochemical system (BES) is introduced and works on the simultaneous combination action of these two theories to enhance the biogas production. In this system, the current is passed through the system by means of the electrode connected with the system. Here, the bacteria can either consume the produced hydrogen at the cathode or directly gain the electron from the anode (Geppert et al. 2016). The effects of different metals on the production of biogas in the form of methane were studied by a few scholars (Carpenter et al. 2015; Geppert et al. 2016). It has been found that molybdenum, magnesium, cobalt, calcium, iron, as well as nickel separately as well as in grouping have enhanced the production of biogas in the form of methane and this is responsible for the increasing methanogenic bacteria in the reactor.

The shape, size, as well as material of construction of the membrane and electrode and the strength of the current that passed through the electrodes highly affect the electromethanogenesis action (Babanova et al. 2017; Krieg et al. 2014; Ribot-Llobet et al. 2013; Siegert et al. 2014). It is also observed that the favorable conditions for the production of the microbes and growth do not maintain a strong relationship with electron transfer (Blasco-Gómez et al. 2017).

Electrochemical methanogenesis is currently applied in a lab-scale. To achieve a commercial scientific process, concepts related to the scale-up and control of process characteristics and reactor balancing are required to develop. In this case and to further advance in bioelectrochemical applications, it may be necessary to produce methanogen with higher electronic adoption rates for the equipment.

3.5 Conclusion

A comprehensive review on the development of hydrolytic, acidogenic, acetogenic, as well as methanogenic organisms for biogas production was presented with more emphasis on methanogens. Methanogens are fascinating as well as attractive organisms, both biologically and technically. Studies in previous years have made it clear that the characteristics of this unique group are not fully understood. In contemporary years, biomethanation technology has been selected as a striking choice in view of the twin assistances of controlling environmental contamination as well as gathering nationwide energy requirements. This procedure has developed a technology of increasing importance. Therefore, the anaerobic digestion industry has been considered as the most beneficial and convenient method for waste treatment.

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