



# Role of Microorganisms in Biogas Production from Animal Waste and Slurries

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## Abstract

Energy supply and waste management are two of the great challenges that humanity as a whole faces. The world's energy supply is mainly dependent on fossil fuels whose combustion leads to excessive carbon dioxide emission, which, when released into the atmosphere in greater concentration, causes global warming. Moreover, the amount of solid waste produced is increasing and is expected to grow rapidly in the next decades. Therefore, to meet these challenges in the future, it is necessary to use life-cycling technology as a robust tool capable of combatting environmental waste into energy. It is becoming apparent that the majority of organic waste from various agricultural and industrial sources can be converted by microorganisms into biofuels. These biofuels provide renewable energy sources that could significantly lower greenhouse gas emissions and ensure sustainable waste management. The concept of bioenergy production from waste has developed significantly over the last few decades. Biogas is among the gaseous biofuels produced by the anaerobic digestion of organic material, and recently, its production from animal waste such as cow dung is an economically viable way to reduce environmental pollution and provide an opportunity for effective waste management and production of valuable products. Biogas consists of mainly methane (CH<sub>4</sub>), carbon dioxide (CO<sub>2</sub>), and small amounts of hydrogen sulfide (H<sub>2</sub>S). This chapter focuses on the production of

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biogas from animal wastes. The chapter will provide an overview of the concept of biogas production, microorganisms used in the production of biogas, the anaerobic digestion process, and the anaerobic digester. The chapter will also attempt to highlight the key stages involved in biogas production (hydrolysis, acidogenesis, acetogenesis, and methanogenesis), and the benefits of biogas. Details of factors influencing the production of biogas are also discussed.

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**Keywords**

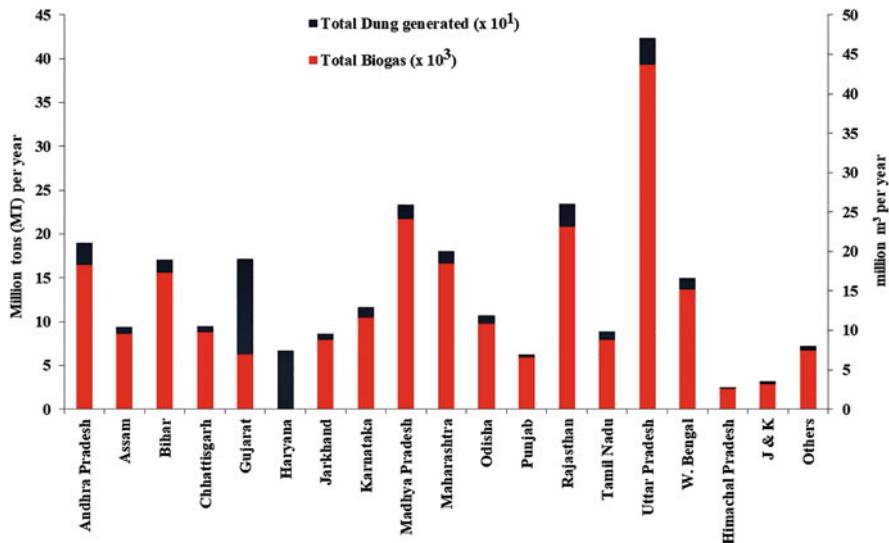
Global warming · Biofuels · Anaerobic digestion · Environmental pollution · Biogas

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## 8.1 Introduction

The present world energy supply is largely dependent on fossil-origin fuel such as petroleum, coal, and natural gas, etc. They are the ossified remains or impressions of dead plants and animals, which have been preserved in the Earth's crust for millions of years. Utilization of such resources converts carbon stored for millions of years into carbon dioxide (CO<sub>2</sub>), and its release into the atmosphere in greater concentrations causes global warming. For this reason, fossil fuels are non-renewable energy sources. One of the main threats to society today is the continuous increase in organic waste production. Therefore the task of waste management and inadequate energy supply are two of the enormous problems that are increasingly threatening the life of many people (Onwuliri et al. 2013). Sustainable management of waste as well as avoiding and reducing waste have become major priorities, representing a significant part of the public efforts to reduce pollution and greenhouse gas emissions and mitigate global climate changes.

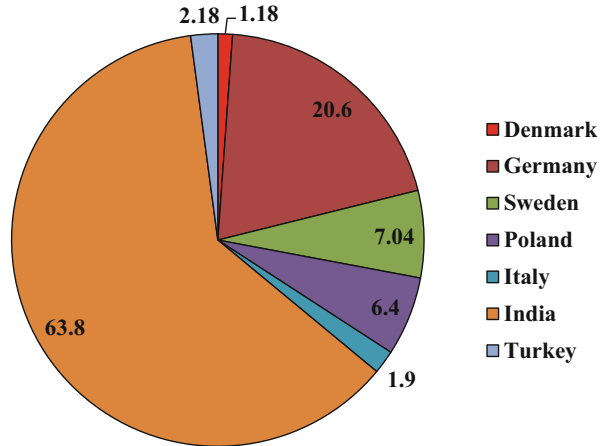
The decrease in the production of non-renewable energy sources along with the climate change problem has driven the search for renewable and more environmentally friendly energy sources as an alternative to fossil fuels which allow for sustainable development, as the system seems auspicious to achieve sustainable energy production without destructing our environment (Chojnacka et al. 2015). It is therefore important to implement a renewable energy system to replace fossil fuels. Research has shown that biogas is one such alternative energy source, particularly for the rural community (Raja and Wazir 2017). In contrast to fossil fuels, biogas is renewable energy as it is produced from biomass. Biogas will not only upgrade energy stability but also make a significant influence on the conservation of natural resources and environmental protection. It will increase the security of the energy supply, reduce dependency on fossil fuels and help to ensure sustainable development. Govarathanan et al. (2022) reviewed critically various research works and suggested that utilizing lignocellulosic (LC) biomass generates biogas at a high rate and also nanotechnology intervention was found to be very effective in biogas production (Yadav et al. 2020).



**Fig. 8.1** Quantifiable sources of livestock dung (MT per year) and potential for biogas generation (million m<sup>3</sup> per year) in India. (Adapted from Kaur et al. 2017)

Biogas is a promising renewable alternative to natural gas with similar applications. It is typically a mixture of different gases which primarily comprises methane (CH<sub>4</sub>), carbon dioxide (CO<sub>2</sub>), a small amount of hydrogen sulfide (H<sub>2</sub>S), moisture (H<sub>2</sub>O), and a few other gases formed in the absence of oxygen due to the breakdown of organic material (Liaquat et al. 2017). Nearly any organic waste materials can be biologically degraded and transformed into biogas and other energy-rich organic compounds by the process of anaerobic digestion, thereby enabling sustainable waste management (Goswami et al. 2016). Production of biogas through anaerobic digestion of animal waste converts these wastes into renewable energy. Biogas production from animal waste is an economically feasible way to reduce environmental pollution and produce valuable products, i.e., methane (Pampillón-gonzález et al. 2017). It is a very important renewable source of energy produced from organic materials like cattle dung, human waste, and different types of biomass. Therefore, biogas is a renewable energy source as it is wholly energy self-sustenance technology, independent of any fossil fuel, and reduces greenhouse gas emissions into the environment. State-wise generation of animal dung and the tentative theorized estimate of this untapped source for biogas production in India are shown in Fig. 8.1. The annual production of dung is estimated to be approximately 2600 million tons (MT), which is enormous in terms of volume, making it an important untapped energy source. The total dung generated which is mentioned in Fig. 8.1 comprises of large animal dung, small animal dung, pig dung, and poultry dung. Total potential biogas production from all dung sources was calculated in terms of annual yield measured in million m<sup>3</sup> per year.

**Fig. 8.2** Biogas potential of different countries (in billion m<sup>3</sup>). (Source: Karaca 2018)



When the biogas potentials of some other countries are examined, it is seen that India has good potential (Fig. 8.2).

## 8.2 Anaerobic Digestion and Biogas Production

Anaerobic digestion (AD) is a natural biological process whereby organic matter is decomposed and transformed by microorganisms into biogas in the absence of oxygen (Fedailaine et al. 2015). During the process, microorganisms digest plant and/or animal material in sealed containers, producing biogas. The process occurs in an anaerobic environment (oxygen-free environment) through the activities of a diverse group of microorganisms that break down the organic material and produce methane (CH<sub>4</sub>), carbon dioxide (CO<sub>2</sub>) in a gaseous form known as biogas, and other nutrient-rich compounds (Kythreoutou et al. 2014). It is a complex process that involves two stages. At the initial phase of the process, degradation is executed by fast-growing, acid-forming microbes (acidogenic), where protein, carbohydrate, lipids, cellulose, and hemicellulose in the waste are hydrolyzed and metabolized into organic acids and volatile fatty acids (VFAs), along with carbon dioxide and hydrogen gases. At this stage, the decomposing products have noticeable, disagreeable, effusive odors from the organic acids, H<sub>2</sub>S, and other metabolic products (Liaquat et al. 2017). In the second phase of the process, most of the organic acids and other intermediary products of the earlier phases of the process are metabolized by methanogenic microorganisms, thereby producing biogas as the end-product, which comprises a mixture of different gases, as shown in Table 8.1.

Biogas production through anaerobic digestion (AD) is an environmentally friendly technology for bioenergy production utilizing the increasing amounts of organic waste produced worldwide. A wide range of waste streams, including industrial waste, domestic waste, human excreta, municipal wastewater, agricultural waste, animal waste as well as plant residues, can be treated with this technology. It

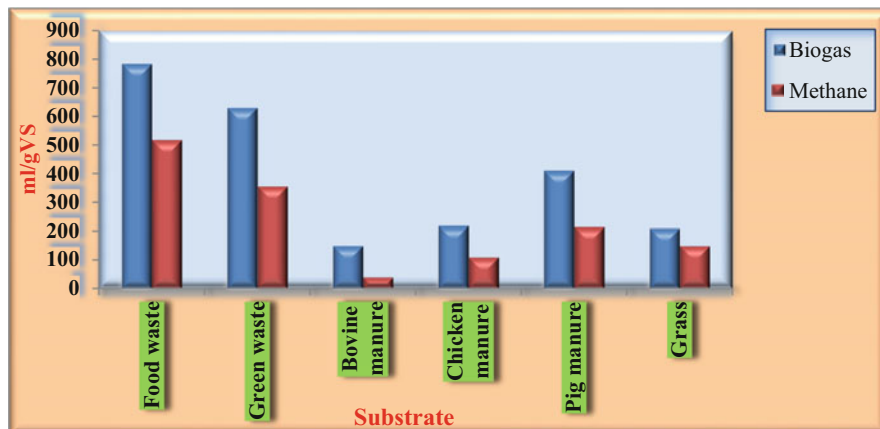
**Table 8.1** Typical percentage composition of biogas (Liaquat et al. 2017; Schnurer and Jarvis 2009)

S. no.	Biogases	Formula	Percentage %
1.	Methane	CH <sub>4</sub>	50–75
2.	Carbon dioxide	CO <sub>2</sub>	25–50
3.	Nitrogen	N <sub>2</sub>	0–10
4.	Oxygen	O <sub>2</sub>	0–2
5.	Hydrogen	H <sub>2</sub>	0–1
6.	Hydrogen sulphide	H <sub>2</sub> S	Traces
7.	Water vapor	H <sub>2</sub> O	Traces
8.	Ammonia	NH <sub>3</sub>	0–0.05

is an effective process to convert animal waste into profitable by-products as well as reduce the pollution of air, water, and soil caused by these wastes. The organic material in animal waste is easily decomposable, so a lot of microorganisms thrive in it. These microbes are mostly anaerobic and thus ideally suited to decompose the organic material in an anaerobic digester and produce biogas (Pampillón-González et al. 2017). The production of biogas through this process proffers significant benefits over other systems of bioenergy production and many other waste treatment processes. The major product of this process, i.e., the biogas, is a renewable energy source, while the by-product, i.e., the digester residue, can be used as a biofertilizer because of its high nutrient content available (Horváth et al. 2016). Biogas production is influenced by the amount of organic material and the number of anaerobic bacteria that degrade the organic material (Hidayati et al. 2018). Therefore, the quantity and quality of the biogas appear to be controlled by the type of biomass being digested and the microbial inoculum fed into the biogas plant. Biogas can be generated from nearly all types of biomass; nevertheless, animal waste and slurries represent one of the largest resources. Animal waste and slurries from cows, pigs, sheep, goats, and poultry have been estimated as among the major waste streams for biogas production, which, if left unprocessed or inadequately managed, may become a major environmental problem because of nutrient leaching (N, P), ammonia evaporation, and pathogen contamination. Among animal waste, it has been reported that pig manure produces a high yield of biogas and methane compared to other animal waste, as shown in Fig. 8.3 (Enzmann et al. 2018; Verma et al. 2018).

The purpose of using anaerobic digestion is usually related to waste management and energy production. The remaining digestate is an added benefit, which creates additional value. Hence, the practice of anaerobic digestion can assure appropriate waste management, production of biofertilizers, and improved environmental impact and sustainability (Luo et al. 2013). Anaerobic digestion (AD) technology is widely used in the treatment of organic wastes to achieve the reduction of the wastes with the simultaneous production of biogas, the technology allows the treatment of high organic loading wastes to reduce their volume and load while recovering biogas, which can be used to produce heat, electricity, and or upgraded to be biofuels for automotive vehicles (Awe et al. 2017; Madakka et al. 2020).

The anaerobic digestion technology has gained considerable momentum over a few years and it is considered a valuable technology for the production of renewable



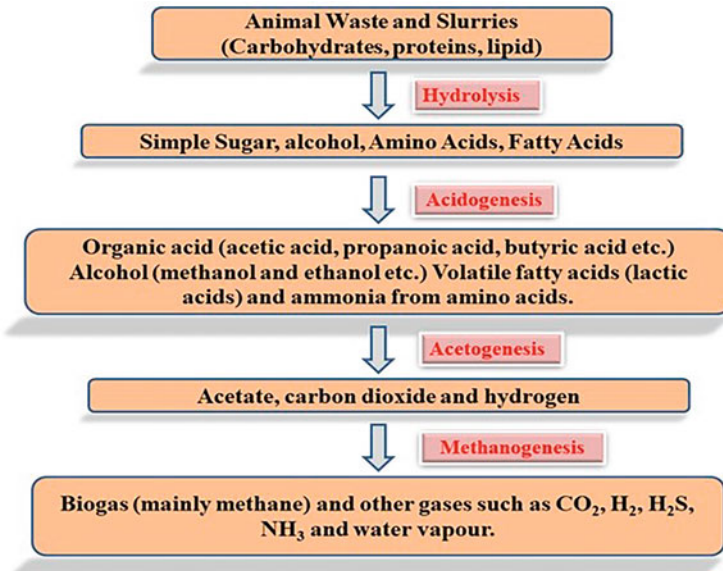
**Fig. 8.3** Biogas and methane contents of some organic waste in milliliter per gram volatile solids (mL/gVS) (Heo et al. 2003)

energy and offers a way to mitigate problems related to low access to energy (Anukam et al. 2019; Náthia-Neves et al. 2018).

The systems have undergone various modifications in the last decades to increase the efficiency of the process. An important milestone was the development of a new reactor design, i.e., the up-flow anaerobic sludge blanket (UASB) reactor, containing a well-settleable methanogenic sludge due to the formation of a dense sludge bed. Another technology making it possible to retain active biomass within the system was the application of membrane bioreactors (MBRs), which can also be utilized for the parting of inhibitory substances, which otherwise would negatively disturb the biological process (Mainardis et al. 2020). Additionally, advances in molecular biology techniques could provide scientists and students with a valuable tool to understand the complex microbiological processes involved in the anaerobic digestion of organic materials. By the application of these techniques, it would be possible to regulate and control the process and discover disturbances much earlier than using traditional process parameters for monitoring the process.

### 8.3 Stages of Biogas Production by the Anaerobic Digestion Process

Biogas production through anaerobic digestion (AD) of organic materials is the combinative activity of various microbial populations carried out by several different groups of bacteria and fungi such as hydrolyzing, acidifying, acetogenic, and methanogenic microbes, which in the final stage produce biogas mainly methane ( $\text{CH}_4$ ) and carbon dioxide ( $\text{CO}_2$ ) (Heeg et al. 2014). The production of biogas is usually carried out in four biological and chemical stages, i.e., hydrolysis, acidogenesis, acetogenesis, and methanogenesis. These four main stages account



**Fig. 8.4** Key steps of biogas production

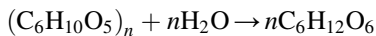
for the production of biogas from different organic matter as it takes place in an anaerobic reactor (Fig. 8.4). In the single-stage batch reactor, all wastes are loaded simultaneously, and all four processes are allowed to occur in the same reactor sequentially; the compost is then emptied at the end of a given retention period or cessation of biogas production (Kwietniewska and Tys 2014).

### 8.3.1 Hydrolysis

Hydrolysis is the first step in biogas production. In this step, the complex organic matter (polymers), that is, proteins, carbohydrates, and lipids (fats) are broken down and transformed into simple and smaller water-soluble compounds such as amino acids, fatty acids, and simple sugars, which in turn can be utilized by acidogenic bacteria (Chandra et al. 2012). During the hydrolysis process, hydrolytic bacteria present in the reactor secrete extracellular enzymes that convert complex organic substrates containing carbohydrates, lipids, and proteins into sugars, long-chain fatty acids, and amino acids, respectively (Li et al. 2011). However, certain substrates, such as lignin, cellulose, and hemicellulose, may find it difficult to degrade, and can be inaccessible to microbes due to their complex structures; enzymes are often added to enhance the hydrolysis of these carbohydrates (Lin et al. 2010).

From a chemical perspective, hydrolysis refers to the cleavage of chemical bonds by the addition of water. Cations and anions react with water molecules, altering pH

in the process to create a cleavage of H–O bonds. The reaction associated with this step is given below:



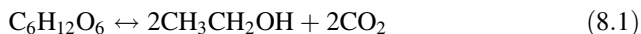
From the reaction, the hydrolysis of cellulose ( $C_6H_{10}O_5$ ) via the addition of water ( $H_2O$ ) to form glucose ( $C_6H_{12}O_6$ ) as the primary product and gives off  $H_2$ . The reaction is catalyzed by homogeneous or heterogeneous acids to produce glucose ( $C_6H_{12}O_6$ ) (Zupancic and Grilc 2007). Hydrolysis is the slowest step of biogas production, especially when solid waste substrates are used. The process rate depends on factors such as pH, particle size, enzyme production, diffusion, and enzyme adsorption on waste particles that are exposed to the degradation process. The magnification of the hydrolysis process increases the performance of digestion (Yu et al. 2016). Biological, chemical, and mechanical pre-treatments, or a combination of these can be used to accelerate hydrolysis, because they can cause lysis or disintegration of the substrate and allow the release of intracellular matter, allowing greater accessibility of anaerobic microorganisms, thus reducing the retention time in the digester (Ferrer et al. 2008).

### 8.3.2 Acidogenesis

This is the second stage of biogas production, where the products of the hydrolysis (water organic monomers of sugars and amino acids) are further broken down and converted mostly into several organic acids (acetic acid, propionic acid, butyric acid, succinic acid, pentanoic, etc.), VFAs (lactic acid), alcohols (methanol, ethanol), and ammonia (from amino acids) (Christy et al. 2014). Acidogenesis is usually the fastest step of biogas production and occurs due to the action of acidogenic fermentative microorganisms. With the rapidity of this stage, it is important to note that while the production of VFAs creates direct precursors for the final stage of methanogenesis, VFA acidification is widely reported to be a cause of digester failure (Akuzawa et al. 2011).

The exact compounds to be formed depend on the substrate and process conditions, as well as the microorganisms available. Studies have shown that volatile fatty acid concentrations can vary significantly for digesters operating at different pH, with different studies presenting seemingly contradictory results (Huang et al. 2015). The important acid in this stage is  $CH_3COOH$ , and it is the most significant organic acid used as a substrate by  $CH_4$ -forming microorganisms. Whereas the production of volatile fatty acids (VFAs) is increased when the process pH is greater than 5, the production of ethanol ( $C_2H_5OH$ ) is favored by a low pH value of less than 5 with the reaction process coming to a halt at a  $pH < 4$  (Bajpai 2017). Eqs. (8.1)–(8.3) present the reaction sequence that summarizes the acidogenic stage of biogas production (Barua and Dhar 2017).





Acetates,  $\text{CO}_2$ , and  $\text{H}_2$  pass through the basic pathway of transformation, while other products of acidogenesis play an insignificant role. As a consequence of these transformations, the new products may be directly used by methanogenic microbes as substrates and energy sources. This stage is very significant because it links the phase of fermentation with the phase of production of methane. Thus, more acid is produced to form elements of methanogens that generate methane gas (Ntaikou et al. 2010).

### 8.3.3 Acetogenesis

Acetogenesis is the third stage of biogas production. It is the process where acetogens produce acetate (a derivative of acetic acid) utilizing carbon and energy sources. In this phase, acetogenic microbes convert the compounds generated during the acidogenic phase, producing hydrogen, carbon dioxide, and acetate (Chandra et al. 2012). Acetogenic microbes digest the biomass to an extent from which, methanogens utilize it as a substrate to produce biogas (methane). This stage explains the efficiency of the production of biogas as, in the process of acetate reduction, more than 70% of  $\text{CH}_4$  is generated. Subsequently, acetate is the main intermediate product of the process of methane production (Gkamarazi 2015).

The stage involves coordination between the oxidizing microbes and the methanogenic microbes that are active in the next phase of the methane-producing process (Heeg et al. 2014). The reaction associated with this stage of AD is represented by Eqs. (8.4)–(8.6) (Anukam et al. 2019).



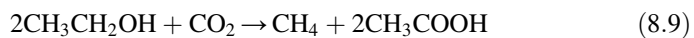
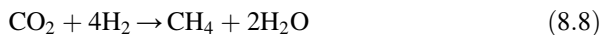
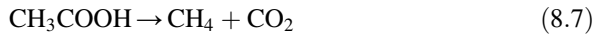
### 8.3.4 Methanogenesis

Methanogenesis is the final stage of the biogas production process. In this process, methanogens generate biogas from the end products of acetogenesis which consists mainly of methane ( $\text{CH}_4$ ) and carbon dioxide ( $\text{CO}_2$ ), but also comprises some other gaseous “impurities” such as hydrogen sulphide ( $\text{H}_2\text{S}$ ) (easily detectable by its smell

of rotten eggs), nitrogen, oxygen, and hydrogen (Chojnacka et al. 2015). The actual process of methanogenesis is very complex and needs explicit substrates and cofactors, the major substrates used are acetate, carbon dioxide, H<sub>2</sub>, formic acid, methanol, methylamine, and dimethyl sulfide. But two substrates, carbon dioxide and acetate, are the most commonly used (Costa and Leigh 2014). The pathway which precedes methane production exclusively depends on the methanogenic microbes and the availability of the substrate that favors the degradation process. Generally, there are six pathways of methanogenesis, each converting a different substrate into methane gas. The three major pathways are:

1. Hydrogenotrophic methanogenesis (production of methane by the reduction of H<sub>2</sub>/CO<sub>2</sub>)
2. Acetotrophic methanogenesis (production of methane by acetate decarboxylation)
3. Methylotrophic methanogenesis (production of methane by removal of the carboxyl group of methyl alcohols, methyl amines, etc.), (Slonczewski and Foster 2013)

The acetotrophic pathway is the main pathway of methane production in the anaerobic digestion process as 70% of the total methane generated during the process is through this pathway (Merlino et al. 2013), and the most commonly used pathway is hydrogenotrophic methanogenesis, which transforms carbon dioxide into methane by reduction of H<sub>2</sub>/CO<sub>2</sub> (Slonczewski and Foster 2013). The reaction equation representing the condition taking place in the methanogenesis step is represented by the following (Ostrem 2004):



The first Eq. (8.7) shows the conversion of CH<sub>3</sub>COOH into CH<sub>4</sub> and CO<sub>2</sub>. The CO<sub>2</sub> formed is reduced to CH<sub>4</sub> through H<sub>2</sub> gas in the second Eq. (8.8) and, lastly, Eq. (8.9), shows the production of CH<sub>4</sub> by decarboxylation of CH<sub>3</sub>CH<sub>2</sub>OH.

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## 8.4 Anaerobic Digesters

Anaerobic digesters are vessels in which a biochemical process is carried out and involve organisms or biologically active substances derived from such organisms.

Three basic types of digesters that have been executed in developing nations are floating-drum digester, fixed-dome digester, and tubular digester, all of which are wet digestion systems worked uninterruptedly under mesophilic conditions. These three types are easy to handle, low-cost, built with nearby available material, do not

have numerous moving parts and are thus less predisposed to failure. An additional digester type, the garage-type digester, which is worked as a dry digestion system in batch-mode, is considered another potential biogas technology suitable for low- and middle-income countries. Although this technology is being tested in some African countries like Ghana by converting a used shipping container, it is not yet ready for the commercial market as no viable low-cost design exists that has been successfully tested at full-scale (Vögeli et al. 2014).

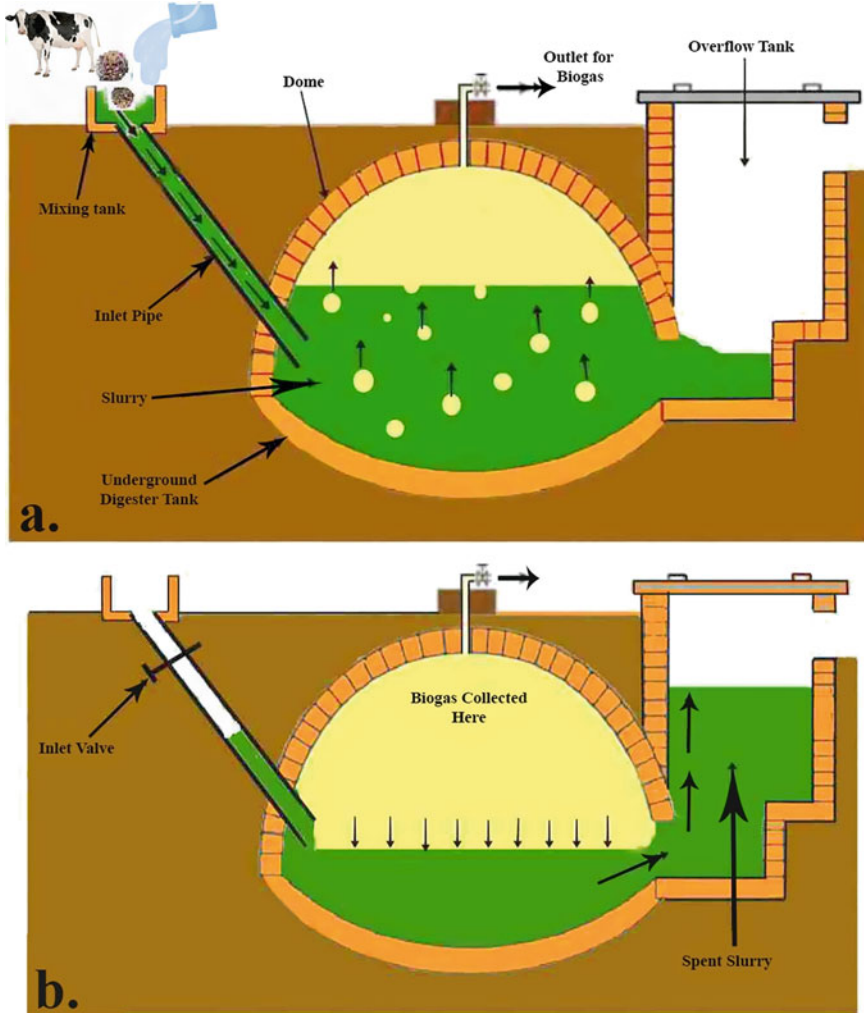
### 8.4.1 Fixed-Dome Digester

A fixed-dome plant is invented of a closed, dome-shaped digester with a fixed, feedstock inlet, a firm gas-holder, and the compensation tank or overflow tank. A schematic diagram is shown in Fig. 8.5. The digester stored the gas produced in the upper part of the reactor. The digestate was pushed into the compensation tank by the high pressure generated by gas produced in the digester with a closed outlet gas valve. The gas pressure falls and a relative amount of slurry flows back into the digester from the tank of compensation, once the gas valve is open for gas utilization. Given this design, gas pressure varies always, depending on gas production and use. Usually, such a plant is constructed underground, protecting the digester from low temperatures during cold seasons and at night. The internal pressure in the digester, which is normally 0.1–0.15, bar balances the surrounding soil up to the top of the gas-filled space (Werner et al. 1989).

Fixed-dome plants are only suggested for situations where experienced biogas technicians with specific technical skills in construction are available to ensure a gas-tight construction. In general, fixed-dome plants are characterized by modest initial cost and long operational life (about 15–20 years), since no moving or corroding parts are required. Though with time, the masonry building may become liable and spongy to cracking, resulting in gas leakages. Porosity may be counteracted with the use of special sealants; however, cracking often causes permanent leaks. The fluctuating gas pressure in this digester type might confound gas utilization (Nzila et al. 2012).

There are numerous designs of the fixed-dome digester such as the Chinese fixed-dome plant, the Indian *Deenbandhu*, or the CAMARTEC model developed in Tanzania. Fixed dome digester can be constructed in different sizes, typically ranging from 6 to 16 m<sup>3</sup>.

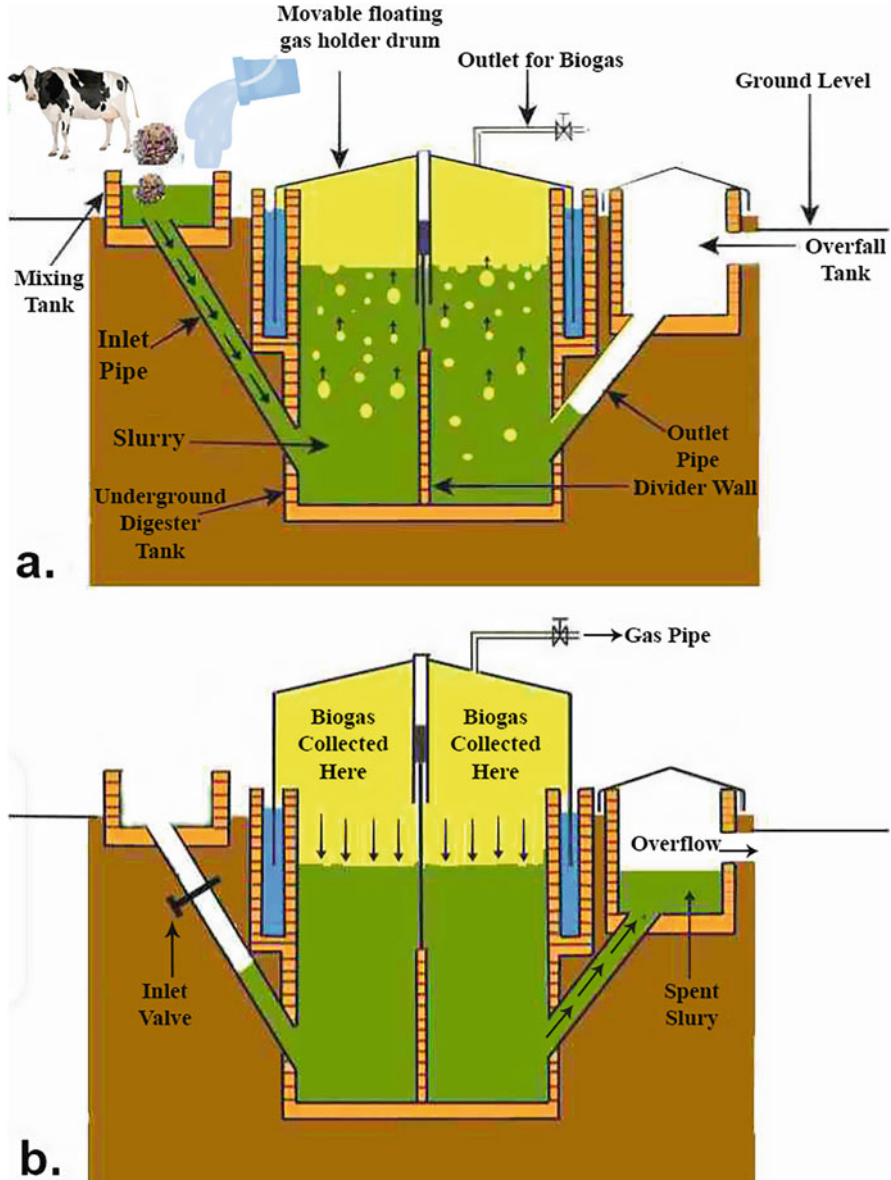
Nevertheless, the principle design elements of all fixed-dome digesters are the same. Generally, the fixed-dome digester type was classically used for cow dung-fed systems, but it is also appropriate for treating other waste types such as kitchen waste. Sometimes, toilets are also connected to the digester to treat the human waste product, which does not create significant problems.



**Fig. 8.5** Scheme of the fixed-dome digester; (a) production and collection of biogas, (b) digestate pushed into the overflow tank by the high pressure generated by gas

### 8.4.2 Floating-Drum Digester

A floating-drum biogas plant contains a cylinder-shaped digester and a movable, floating gasholder (drum). The digester is mostly built underground (see Fig. 8.6), while the floating gasholder is above the ground. Smaller domestic-scale systems usually are above ground. The reactor part of the digester is typically made with bricks, concrete, or quarry-stone masonry and then plastered. The gas-holder is typically prepared from metal and is covered with synthetic paints, oil paints, or bitumen paints to protect it against corrosion. Conversely, it is important to ensure



**Fig. 8.6** Scheme of the floating-drum digester; (a) production and collection of biogas, (b) digestate pushed into the overflow tank by the high pressure generated by gas

sustained use by regular de-rusting, and the cover coating should be re-applied annually. A well-maintained metal gas-holder can be expected to last between eight to twelve (8–12) years in a dry climate or 3–5 years in humid areas. A proper

alternative to standard grades of steel is fiberglass-reinforced plastic or galvanized sheet metal (Nzila et al. 2012).

The generated gas collects in the gas drum, which falls or rises again, depending on the volume of gas produced and used. The drum level thus contains a valuable visual indicator of the quantity of gas available. The gas is provided at moderately constant pressure, which is contingent on the weight of the drum. Additional weights can be added on top of the gasholder, to increase gas pressure. Braces can be welded onto the inside of the drum which then helps to break up the scum layer when the drum is rotated (Vögeli et al. 2014).

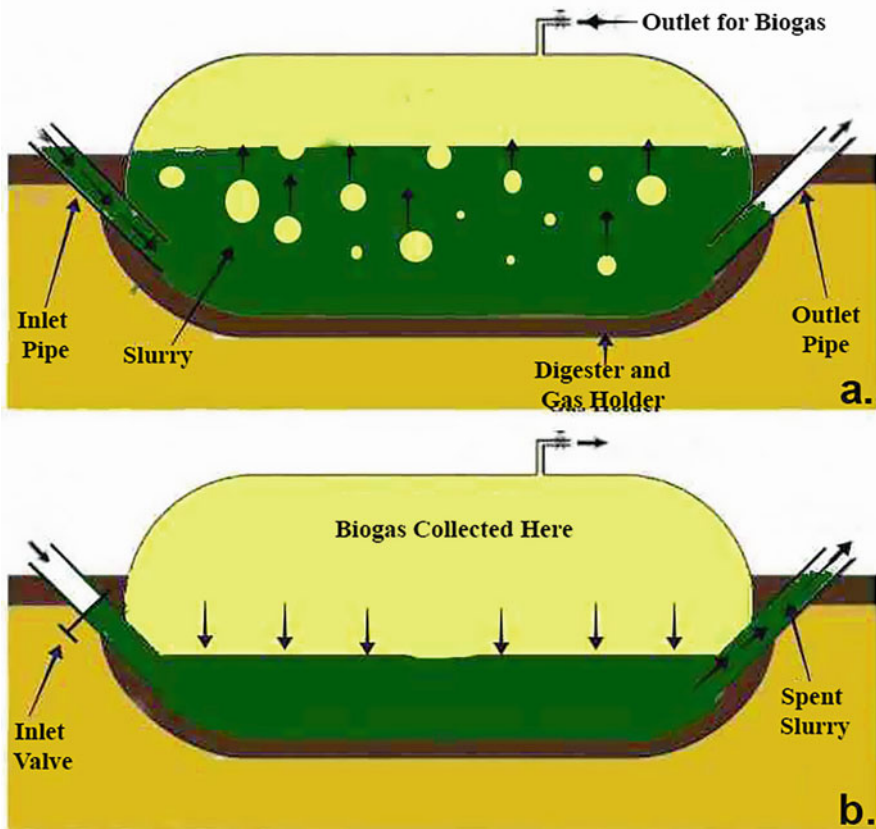
The gasholder is either a specifically constructed separate water jacket or floats directly on the fermenting slurry which reduces methane leakage, as shown in Fig. 8.6. A guiding frame constructed inside of the gas drum is an additional measure to prevent the tilting of the drum when it rises (see guide pole in Fig. 8.6). The design size of floating-drum plants is springy, with bioreactor sizes usually ranging between 1 and 50 m<sup>3</sup> (Vögeli et al. 2014).

### 8.4.3 Tubular Digester

A tubular biogas plant comprises a longitudinal-shaped heat-sealed, rubber bag (balloon) or weather-resistant plastic that serves as a digester and gas holder in one. The upper part of the balloon stores the gas produced. The outlet and inlet are attached straight to the skin of the balloon. No short-circuiting takes place as a result of the longitudinal shape, but since tubular digesters naturally have no stirring device, active mixing is incomplete and digestate flows through the reactor in a plug-flow manner. The pressure of the gas can be increased by placing weights on the balloon while taking care not to damage it. Figure 8.7 shows a schematic diagram of a typical tubular digester (Vögeli et al. 2014).

The advantage of these digesters is that they can be constructed at a low cost by standardized prefabrication. Furthermore, because of the shallow below-ground installation, they can be used in places with a high groundwater table. The plastic balloon though is quite liable to mechanical damage and has a comparatively short life span of 2–5 years (Nzila et al. 2012).

To prevent damage to and deterioration of the balloon, it is also very important to protect the bag from direct solar radiation with a roof. Moreover, a wire-mesh fence protects against damage by animals. This system can be modified for it to work at different altitudes and climates. For example, on the Bolivian Altiplano in west-central South America (more than 4000 m above sea level), biodigesters are surrounded in a polyethylene greenhouse, supported by two lateral adobe walls along the whole length of the shallow trench. A layer of 20 cm of insulating material (e.g., dry cereal straw and natural grass) can be used to decrease heat loss through the walls of the trench. The lateral walls accumulate the heat so that with freezing temperatures during wintertime nights, the digester remains operational of its high thermal inertia. Also, dark pipes are installed to pre-heat the water used for mixing the substrate before entering the balloon (Martí-Herrero 2008).



**Fig. 8.7** Scheme of tubular digester; (a) production and collection of biogas, (b) spent slurry pushed into the outlet pipe by the high pressure generated by gas

## 8.5 Microbes Involved in Biogas Production

Microbiology of anaerobic transformation of biological wastes is a method that involves numerous different kinds of microbes, such as hydrolytic, acid-forming, acetogenic, and methanogenic bacteria which produce  $\text{CO}_2$  and  $\text{CH}_4$  as the by-products of the digestion process. Each organic waste accounts for the degradation of a different type of compound.

### 8.5.1 Microbes Involved in Hydrolysis and Acidogenesis

The hydrolytic and acidogenic phases may be combined in the anaerobic acidogenic bacteria. The most commonly found acidogenic bacteria in digesters include species

of *Butyrivibrio*, *Propionibacterium*, *Selenomonas*, *Lactobacillus*, *Clostridium*, *Bacteroides*, *Bifidobacterium*, *Eubacterium*, *Ruminococcus*, *Acetivibrio*, *Peptostreptococcus*, *Peptococcus*, *Streptococcus*, and members of the *Enterobacteriaceae*. In mesophilic sewage sludges, there are usually between  $10^8$  and  $10^9$  hydrolytic bacteria per milliliter (Borja 2011; Kashyap et al. 2019).

### 8.5.2 Acetogenic Bacteria

Acetogenetic species can be subdivided into those that do reduce protons to hydrogen obligately and those that are not obligately proton-reducing, that is, hydrogen-producing species during acetogenesis. The first group has a wide range, comprising the homoacetogens and species that may direct their metabolisms to proton reduction in the presence of a sufficient hydrogen-removing system. Homoacetogenic species are known in the genera *Acetobacterium*, *Acetogenium*, *Acetoanaerobium*, *Butyribacterium*, *Eubacterium*, *Clostridium*, and *Pelobacter* (Borja 2011).

In environments with sufficient  $H_2$  sinks, such as anaerobic digesters, many of the acidogenic bacteria direct their metabolism to acetogenesis. This facultative change in metabolism has been demonstrated in defined methanogenic co-cultures degrading alcohols, pyruvate, lactate, fructose, glucose, cellobiose, and cellulose. Obligately proton-reducing acetogenic microbes can only be grown in a sufficiently electron-removing environment, for example, in monoxenic culture with a hydrogen-removing or formate-removing species. The mixed culture concerning this type of “mutualistic” interaction is a culture containing the acetogenic bacteria and a hydrogen-removing bacterium such as a methanogen. *Desulfovibrio* spp. is obligatory proton-reducing acetogens when metabolizing ethanol or lactate in the absence of sulfate, and can be cultivated in mutualistic co-culture with methanogens. Some of the acetogens and their metabolizing substrate have been depicted in Table 8.2. The relative significance of formate and hydrogen in interspecies electron transfer essentials is to be established in different digesters and under different operating conditions (Borja 2011).

**Table 8.2** Acetogenic bacteria (Schiel-Bengelsdorf and Dürre 2012)

S. no.	Microbes	Metabolize/degrade [organic waste carbon chain acid ( $C_5$ ) to acetate ( $C_2$ )]
1.	<i>Methanobacterium suboxydans</i>	Pentanoic acid ( $C_5$ ) to propionic acid ( $C_3$ )
2.	<i>Methanobacterium propionicum</i>	Propionic acid ( $C_3$ ) to acetate ( $C_2$ )
3.	<i>Syntrophobacter wolinii</i>	Propionic acid ( $C_3$ ) to acetate ( $C_2$ )
4.	<i>Syntrophomonas wolfei</i>	Butyrate
5.	<i>Syntrophusbus wellii</i>	Benzoate
6.	<i>Desulfovibrio</i> spp.	Ethanol or lactate



### 8.5.3 Methanogens

Methanogenic microbes are present in sewage sludges at populations of up to  $10^8$  per milliliter and contribute up to 10% of the volatile solids. They are a morphologically diverse group of archaeobacteria unified by their capability to derive energy from methanogenesis. A limited range of substrates are utilized by the methanogens,  $H_2 + CO_2$  and acetate being the most important substrates in AD. Most methanogenic microbes utilize  $H_2$  and  $CO_2$ , but species of only two genera, *Methanothrix* and *Methanosarcina*, can produce methane from acetic acid. The species of methanogens that most commonly use  $H_2$  and  $CO_2$  as substrate and those that use acetate found in anaerobic digesters are described in Table 8.3.

Alternatively, hydrolysis is claimed to be rate-limiting when the biological waste contains much insoluble material (e.g., cellulosic compounds). Though, in the AD of soluble substrates, either methanogenesis from acetate or acetogenesis is considered to be rate-limiting. Under certain conditions, the rate of acetogenesis is controlled by the  $H_2$ -utilizing methanogens and so methanogenesis by either the acetate- or  $H_2$ -utilizing methanogens can be rate-limiting to the Anaerobic Digestion process (Schiel-Bengelsdorf and Dürre 2012).

**Table 8.3** Methanogens that most commonly use  $H_2$  and  $CO_2$  as well as acetate as a substrate are found in anaerobic digesters (Schiel-Bengelsdorf and Dürre 2012)

S. no.	Methanogens that use			
	$H_2$ and $CO_2$ as a substrate		Acetate as substrate	
	Genus	Species	Genus	Species
1.	<i>Methanobacterium</i>	<i>Bryantii</i> , <i>formicum</i> , <i>wolfei</i> , <i>thermoautotrophicum</i> , <i>uliginosum</i> , <i>thermoalcaliphilum</i> , <i>thermoaggregans</i>	<i>Methanosarcina</i>	<i>Barkeri</i> , <i>mazei</i> , <i>acetivorans</i>
2.	<i>Methanobrevibacter</i>	<i>Arboriphilus</i> , <i>ruminantium</i> , <i>smithii</i>	<i>Methanothrix</i>	<i>Soehngenii</i> , <i>concilii</i>
3.	<i>Methanothermus</i>	<i>Fervidus</i>		
4.	<i>Methanococcus</i>	<i>Maripaludis</i> , <i>deltae</i> , <i>vannielii</i> , <i>voltae</i> , <i>jannaschii</i> , <i>halophilus</i> , <i>thermolithotrophicus</i> , <i>frisius</i>		
5.	<i>Methanomicrobium</i>	<i>Mobile</i> , <i>paynteri</i>		
6.	<i>Methanogenium</i>	<i>Cariaci</i> , <i>marisnigri</i> , <i>olentangyi</i> , <i>tatii</i> , <i>aggregans</i> , <i>thermophilicum</i> , <i>bourgense</i>		
7.	<i>Methanospirillum</i>	<i>Hungatei</i>		
8.	<i>Methanoplanus</i>	<i>Limicola</i>		

### 8.5.3.1 Characteristics of the Methanogen Families, Substrates for Methanogenesis; Digester Input, and % of Biogas Produced

The two families of methanogenic microbes are Methanobacteriaceae and Methanothermaceae which are closely related. These methanogens have cell walls composed in part of pseudomurein (Kandler and König 1985). The Methanothermaceae also contain an additional surface layer composed of protein on their cell wall. The family of Methanothermaceae contains one genus, Methanothermus, and both species are extremely thermophilic bacilli with temperature optima of 83–88 °C. Like in many of the Methanobacteriaceae, the only substrate for methanogenesis is  $H_2 + CO_2$ . The family of Methanobacteriaceae contains two genera composed of mesophilic as well as thermophilic species. These genera, Methanobacterium and Methanobrevibacter, are bacilli that utilize either  $H_2 + CO_2$  alone or  $H_2 + CO_2$  and formate as substrates for methanogenesis (Miller and Wolin 1983).

Some of the most important and most distinctive features of all six families of methanogenic species, substrates for methanogenesis; digester input and % of biogas produced are summarized in Table 8.4 below:

**Table 8.4** Some characteristics of the methanogen families, substrates for methanogenesis; digester input, and % of biogas produced (Rosenberg et al. 2014)

S. no	Family	Characteristics	Substrates for methanogenesis
1	Methanobacteriaceae	Long or short rods, mostly Gram-positive; contain pseudomurein; nonmotile; GC content, 23–61 mol%	$H_2 + CO_2$ , formate, or alcohols But Cocci, utilize only $H_2 +$ methanol
2	Methanothermaceae	Rods; Gram-positive; contain pseudomurein; nonmotile; extreme thermophiles; GC content, 33–34 mol%	$H_2 + CO_2$
3	Methanococcaceae	Irregular cocci, Gram-negative; motile; GC content, 29–34 mol %	$H_2 + CO_2$ , and formate
4	Methanomicrobiaceae	Rods, spirals, plates, or irregular cocci; Gram-negative; motile or nonmotile; GC content, 39–61 mol%	$H_2 + CO_2$ , frequently formate and sometimes alcohols
5	Methanocorpusculaceae	Small, irregular cocci; motile or nonmotile; GC content, 48–52 mol%	$H_2 + CO_2$ , formate, and sometimes alcohol
6	Methanosarcinaceae	Pseudosarcina, irregular cocci, sheathed rods; substrates for methanogenesis are Gram-positive or negative; frequently nonmotile; GC content, 36–52 mol	Sometimes $H_2 + CO_2$ , acetate, and methyl compounds; formate is never used

### 8.5.3.2 Cooperation of Microorganisms in the Methane Fermentation Process

The four different groups of microorganisms that are responsible for the conversions of complex organic compounds to biogas mainly  $\text{CH}_4$  and  $\text{CO}_2$  are presented in Table 8.5. These groups of microbes may be counted among secondary fermentation bacteria (syntrophic and acetogenic bacteria), primary fermentation bacteria, and two types of methanogens belonging to the domain Archaea. These microbes occur in the ordinary environment and fulfill various roles during the process of anaerobic degradation of wastes (Conrad 1999). Syntrophy is a form of association of two metabolically different groups of bacteria, which permits the degradation of various substrates (Demirel and Scherer 2008).

Cooperation of the population of microbes permits the synthesis of certain products which are then used by a different group of bacteria. The bacteria which are involved in the production of methane belong to the domain Archaea and exhibit symbiosis relationships with other populations of microbes. They may develop only when hydrogen is used by hydrogenotrophs. Such cooperation between microbes producing hydrogen and using hydrogen was defined as the interspecific transfer of hydrogen (Conrad 1999). Syntrophy between microorganisms producing and using hydrogen allows for the growth and activity of these bacteria.

## 8.6 Factors Affecting Biogas Production

Biogas production through the anaerobic digestion process is influenced by a large number of factors that can influence digestion efficiency and the potential of biogas production (Mathew et al. 2015). Biogas production can be significantly improved with statistical optimization and pretreatment techniques (Gopal et al. 2021). Some of these factors are discussed below.

### 8.6.1 Temperature

Temperature is a critical and very important parameter to take into consideration during biogas production. It is the principal environmental factor affecting biogas

**Table 8.5** Microbial cooperation in organic matter degradation (Zieminski and Frac 2012)

Microorganisms	Electron donor	Electron acceptor	Product	Reaction type
Fermentative bacteria	Organic carbon	Organic carbon	$\text{CO}_2$	Fermentation
Syntrophic bacteria	Organic carbon	Organic carbon	$\text{H}_2$	Acidogenesis
Acetogenic bacteria	Organic carbon/ $\text{H}_2$	$\text{CO}_2$	$\text{CH}_3\text{COOH}$	Acetogenesis
Methogenic bacteria	Organic carbon/ $\text{H}_2$	$\text{CO}_2$	$\text{CH}_4$	Methanogenesis

digester performance (Mata-Alvarez et al. 2014). It affects the physical and physicochemical properties of the compounds present in the digester and the kinetics and thermodynamics of the biological process (Kougias et al. 2013). Temperature causes significant effects on the microbial community, interfering with the stability of the process, microbial growth, substrate utilization rate, and biogas yield (Khalid et al. 2011). The rate of biological reactions is designated by temperature. Temperature is a significant parameter that quite often has to be scrutinized, specifically, when there is a variation in the weather. There are three temperature ranges for biogas production, which are psychrophilic temperatures: 10–20 °C with an optimum at 25 °C; mesophilic temperatures: 20–45 °C with an optimum at 35 °C; and thermophilic temperatures: 50–65 °C with an optimum at 55 °C (Kothari et al. 2014).

There are mainly two temperature ranges that provide optimum digestion conditions for the production of methane—the mesophilic and thermophilic ranges. The types of active microbial consortia at the two temperature conditions are quite dissimilar. The choice of temperature condition will be determined by the type of expected outcome. However, the temperature should be appropriate to the type of microorganisms used. Thermophilic temperatures' condition is commonly used in large-scale biodigester (Kwietniewska and Tys 2014). This temperature condition requires higher energy costs and may favor the acidification of the reactor by inhibiting biogas production (Mao et al. 2015). Silwadi et al. (2022) investigated the effect of temperature on the enhancement of biogas production by anaerobic digestion of three different animal droppings, namely, cow, camel, and chicken. They found that digestion of cow, camel, and chicken manure at 37 °C increased the production by 2.2-, 2.1-, and 1.3-fold, respectively, compared to that obtained at 25 °C. Hossain et al. (2022) studied various factors which influence biogas production and found that biogas production rate and cumulative biogas production were found to increase with a rise in temperature.

## 8.6.2 pH

pH is one of the major operational factors that affect biogas production. During anaerobic digestion, different optimal pH values are required at different stages of biogas production. Each microbe grows much better at a certain pH value range, and the uttermost growth of the microbes occurs at an optimum pH value (Montañés et al. 2015). The optimum pH range to achieve high biogas yield in the anaerobic digestion process lies in the range of 6.5–7.5. During anaerobic digestion, the processes of hydrolysis and acidogenesis occur at acidic pH levels (pH 5.5–6.5), as compared to the methanogenic phase (pH 6.5–8.2) (Khalid et al. 2011). Methanogens are sensitive to acidic situations. The growth of microbes and the yield of methane could harmfully be affected by this acidic condition (Arsova 2010).

pH is a very important factor in the anaerobic digestion process. It provides an overview of the effectiveness of the process (Mathew et al. 2015). The lower pH is an indication of the failure of the system or low buffering capability that can inhibit digestion. High pH can also limit the methanogenesis process. The optimal pH value

is of great significance, and to keep a constant pH level, buffers such as lime and calcium carbonate need to be added to the system. To retain a steady pH value within the system, the interaction between the VFAs and bicarbonate concentration is crucial (Liu et al. 2008).

### 8.6.3 Nutrients Requirements

The nutrient requirement is a key concern for the steady execution of biogas production processes (Mathew et al. 2015). As for any biological processes, where microorganisms are involved, both the nutrient required in large and small quantities (macro and micronutrients) should be provided to the microorganisms in the right proportion to be able to achieve efficient biogas production. The nutrients should be found in abundance in the digester as the shortage of any of them may inhibit the process (Mara and Horan 2003). Insufficient availability of nutrient concentration may lead to low biogas yields and process uncertainty (Lebuhn et al. 2008). The macro and micronutrients are essential for the continual performance of the biogas production process (Bruni et al. 2010). Fundamental macronutrients such as carbon (C), nitrogen (N), phosphorus (P), and sulfur (S) are necessary for microbial growth and therefore must be provided to ensure efficient and stable biogas production. Among micronutrients iron (Fe), nickel (Ni), cobalt (Co), molybdenum (Mo), and tungsten (W) are the most important ones (Zandvoort et al. 2006). The growth of methanogenic microbes is reliant on many of these ions, so it is essential for all methanogenic pathways and thus their availability is necessary for biogas production. However, the exact quantity of the required ions should be determined individually in each case because it depends on the microbial consortia and the substrate used (Jagadabhi 2011).

### 8.6.4 C/N Ratio

The carbon/nitrogen ratio plays an important role in the anaerobic digestion process. It is the ratio between the amount of carbon and nitrogen contained in organic matter. The relation between the measure of carbon and nitrogen in organic matter is described by the C/N ratio. It is an important parameter in estimating nutrient deficiency and ammonia inhibition (Hartmann and Ahring 2006). Carbon present in organic matter is of great importance for biogas production. Nitrogen deficit can result in an inadequate consumption of the carbon source, which may result in the decline of microbial growth and lastly lead to a decrease in the biogas yield (Resch et al. 2011). Nitrogen is used as a nutrient by the microorganisms responsible for anaerobic digestion. Nitrogen compounds from organic waste are converted into ammonia in the anaerobic digestion process which contributes to maintaining the pH of the system stable during the process (Khalid et al. 2011). The optimal C/N ratio for anaerobic digestion of organic waste ranged from 20 to 35 (Mathew et al. 2015). A large carbon/nitrogen ratio is a sign of fast ingestion of nitrogen by methanogens,

which then leads to lower biogas yield, but if the ratio is low, an accumulation of ammonia occurs and pH values then may exceed 8.5, such condition can negatively influence the activity methanogenic bacteria (Kothari et al. 2014). Therefore, an optimal C/N ratio must be maintained to ensure efficient biogas production.

### 8.6.5 Agitation

The purpose of mixing the substrate in an anaerobic digester is to mix the new material with digestate containing the microbes (Mao et al. 2015). It is not essential, but always advantageous. Agitation is done to make sure that the contact between substrate and microbes is close and hence results in an enhanced digestion rate of the substrate (Hajji et al. 2016). Agitation enhanced biogas production by about 62% compared to gas production without agitation and thus increase biogas yield (Cavinato et al. 2013). The agitation has the advantage of bringing a homogeneous environment and maintaining a uniform slurry, thereby preventing scum formation. Scum can result in blockage of the gas pipe or potentially lead to foaming over the digester, avoids temperature gradients within the digester, and agitation also prevents grit deposition. Inappropriate mixing can interrupt the contact of microbes to the substrate and decrease biogas production, hence slow, occasional, and harmonious mixing of slurry which enhances biogas production is preferred (Prasad 2012).

### 8.6.6 Water Content

Water is an important nutrient for microorganisms' life and activity. It is an essential component of the organic matter breakdown process since it acts as a solvent and contributes to the mass transfer and diffusion of microorganisms, allowing interaction between the surface of the substrate with microbes involved in the anaerobic digestion process (Bollon et al. 2011). Biogas production from organic matter breakdown requires aqueous environments with water activity higher than 0.91 (Kwietniewska and Tys 2014). The highest methane production occurs at 60–80% moisture as high levels of moisture facilitate the digestion process (Khalid et al. 2011). The movement of bacteria and the activity of extracellular enzymes are highly determined by water content in the digester. The optimum water content of 60–95% has to be maintained in the digester. Although, the optimum moisture content varies with the different input materials, chemical characteristics, and degradation rates (Demetriades 2009).

### 8.6.7 Hydraulic Retention Time (HRT)

Hydraulic Retention Time describes the average time period for which the organic material remains inside the digester or the time required for a complete breakdown of

organic matter. Hydraulic Retention Time (HRT) can be expressed by the equation below:

$$\text{HRT} = V/Q$$

Where  $V$  is the reactor volume ( $\text{m}^3$ ) and  $Q$  is the flow rate of the fresh substrate ( $\text{m}^3/\text{day}$ ) (Kothari et al. 2014). Maximum biogas production occurs at the optimal value of HRT. Underloading and overloading reduce biogas production (Dobre et al. 2014). VFA will accumulate if the retention period is less than the optimal value, which will cause severe fouling and result in reduced biogas production. And if the retention period is above the optimal value, the biogas component will not be utilized effectively, hence biogas production will be reduced (Chen et al. 2016). Hydraulic retention time depends on the temperature of the system and the substrate to be digested. Usually, the HRT for mesophilic temperature conditions ranges from 10 to 40 days, while for the thermophilic condition, the time is shorter, 14 days (Kothari et al. 2014). In conditions where the influent streams have large solids concentrations, extensive retention times are vital to maximize biogas production (Khanal 2009). Hydrogen-producing bacteria prefer short retention times. In contrast to methane-producing bacteria, short retention times lead to a decrease in methane production.

### 8.6.8 Redox Potential

The redox potential of a digester is another important factor that affects biogas production. It is a measure of the oxidizability or reducibility of its content. Biogas production only proceeds in an environment free of oxygen (an anaerobic environment). The optimal value of the redox potential of a reactor must be less than  $-330$  mv for efficient biogas production (Weinrich et al. 2018).

### 8.6.9 Ammonia

Ammonia is frequently described as one of the impeding substances in the biogas production process. Free ammonia or ammonium ions are produced by the breakdown of nitrogenous matter in the digester, commonly present in the form of proteins (Chandra et al. 2012). Microorganisms need some ammonia to form cellular protoplasm for growth and reproduction (Lin et al. 2011). A healthy system will have an ammonia concentration of around 200 mg/L to support the anaerobic growth of the bacteria, while the increase in concentrations of ammonia greater than 1500 mg/L will cause inhibitory effects. This inhibition will cause inequity and accrual of intermediate digestion products such as VFAs which can result in acidification of the reactor, which in turn may result in a reduction in methane production. However, the effects of ammonia inhibition can be lessened by dilution with water in extreme

ammonia overloads, or altering feedstock to adjust C/N ratio in lesser overloads (Kayhanian 1999).

### 8.6.10 Organic Loading Rate (OLR)

Organic Loading Rate (OLR) is the amount of substrate (biomass) fed into or loaded to a unit of volume of the reactor under a unit of time. It signifies the quantity of substrate introduced into the digester in a given time. Organic Loading Rate is typically expressed in terms of kg volatile solid per m<sup>3</sup> per day [kg VS (m<sup>3</sup> day)<sup>-1</sup>], and can be defined by the equation:

$$\text{OLR} = Q.VS/V$$

Where OLR is the organic loading rate (kg VS substrate/m<sup>3</sup> digester/day), [kg VS (m<sup>3</sup> day)<sup>-1</sup>],  $Q$  is the fresh substrate added daily (kg/day),  $V$  is the volume of the bioreactor (m<sup>3</sup>) and VS stands for volatile solids [kg VS (kg)<sup>-1</sup>] (Kothari et al. 2014).

Biogas production is highly influenced by the organic loading rate. The organic loading rate depends on the types of biomass fed into the digester. Underloading and overloading reduce biogas production (Babae and Shayegan 2011). If OLR is increased, the metabolic activity of microbes will be high and hence improve biogas yield. Very high overloading of OLR leads to a significant rise in VFAs and causes its accumulation, which may result in acidification, a decrease in pH and the production of biogas, and may eventually result in system failure (Chen et al. 2016). This in turn influences the biological activity of microbes that generate methane as their growth is inhibited below a pH of 6.6, thus reducing the production of methane, which is the major product of biogas. To optimize digester efficiency and maximize methane production, it is therefore very crucial to assess the suitable OLR for a particular substrate.

### 8.6.11 Volatile Fatty Acids

Volatile Fatty Acids (VFAs) are also an important factor that affects biogas production. VFAs are needed in small quantities as part of the intermediary step for metabolic pathways of methane production by methanogens (Xu et al. 2018). It is estimated that to have a stable process of anaerobic digestion for the production of biogas, the volatile fatty acids, concentration, particularly acetic acid, should be below 2 g/L (Jain and Mattiasson 1998). VFAs can accumulate in a reactor when methanogens cannot keep up with the rate of degradation in the earlier digestion stages, causing a drop in the pH, which in turn will inhibit methanogens, and finally result in biogas digester failure (Yang et al. 2015).



### 8.6.12 Particle Size

The particle size of the substrate also affects biogas production. For microbes to digest, the large particle size of a substrate is problematic and may also result in reactor blockage. Small particles have a large area for adsorption of the substrate and thus allow for increased microbial activity, thus increasing the production of biogas (Sreekrishnan et al. 2004).

### 8.6.13 Inocula

Biogas production is not possible without a sufficient quantity of microbes that support biogas production. Inoculating the digester with microorganisms is necessary for the anaerobic digestion process. Diluted cow dung (optimally 1:1 ratio with water) is an ideal inoculate. At the start-up phase of biogas production, the bacteria population needs to be progressively acclimatized to the feedstock. This can be attained by gradually increasing the everyday feeding load which permits time to attain a stable microbial population. Some of the effluents are collected and inoculated back into the reactor. It is a way of inoculating fresh manure with active microbes. This inoculation of fresh manure can increase biogas production by up to 30% (Budiyono et al. 2014).

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## 8.7 Benefits of Biogas Technology

The production and use of biogas from anaerobic digestion provide socioeconomic and environmental benefits to society as a whole as well as the farmers involved. The use of the internal biogas production value chain boosts local economic potential, protects rural jobs, and strengthens regional financial strength (Saidmamatov et al. 2021). It contributes to the growth of the economy and society and increases living standards. Renewable energy sources are gaining popularity, and there is widespread interest in them. Biogas demand is gradually increasing as more people build biogas plants to supply biogas (Jørgensen 2009).

### 8.7.1 Reducing the Production of Greenhouse Gas

The use of fossil fuels such as crude oil, lignite, hard coal, and natural gas converts carbon deposited in the Earth's crust for hundreds of millions of years and releases it into the atmosphere as carbon dioxide (CO<sub>2</sub>). Carbon dioxide (CO<sub>2</sub>) is one of the constituents of greenhouse gas (GHG), thus global warming has resulted as a consequence of an increase in the current carbon dioxide (CO<sub>2</sub>) concentration in the atmosphere. On the other hand, the crucial difference, as compared to fossil fuels, is that the carbon in biogas was recently extracted from the environment by the plants' photosynthetic behavior (Tsaurai 2018). Thus, in a very short period

(between one and several years), the carbon cycle of biogas is closed. The generation of biogas by anaerobic digester also decreases methane and nitrous oxide emissions from the dumping and usage of untreated animal manure as fertilizer (Khayal 2019).

### **8.7.2 Source for Renewable Energy**

The present worldwide energy supply is dependent on fossil sources (crude oil, natural gas, lignite, hard coal). These are fossilized remains of dead animals and plants, which have been exposed to heat and pressure in the Earth's crust over millions of years. Fossil fuels are non-renewable resources; reserves are depleting much faster than new ones are being formed; as a result, the world's economies rely on crude oil today (Khan et al. 2017). There is some discrepancy among scientists on how long this fossil resource will last. Peak oil production is defined as "the point in time at which the extreme rate of the worldwide production of crude oil is reached, after which the rate of production enters its terminal decline." According to researchers, peak oil production has already happened or it is estimated to happen within the next period of time (Li 2008). The introduction and production of renewable energy systems such as biogas from anaerobic digesters would strengthen the reliability of the national energy supply and minimize reliance on imported fuels (Alhassan et al. 2019).

### **8.7.3 Low Input of Water**

As compared to other biofuels, biogas has several benefits. One of the benefits is that the method of anaerobic digestion requires the least amount of process water. This is an incredibly significant feature related to the assumed lack of water in many parts of the world (Khayal 2019).

### **8.7.4 Contribution to the EU Environmental and Energy Goals**

One of the key goals of European energy and environmental policy is to tackle global warming. The European targets for the development of renewable energy, the elimination of GHG emissions, and the effective management of waste are focused on the willingness of the Member States of Europe to take adequate steps to achieve them. The production and use of anaerobic digestion biogas have the potential to simultaneously comply with all three targets (Bartolini et al. 2017).

### **8.7.5 Reduction of Waste**

The ability to turn waste material into a valuable resource by using it as a substrate for anaerobic digestion is one of the key benefits of the biogas production process.

The overproduction of organic waste from manufacturing, agriculture, and households is a major problem affecting many developed countries. The production of biogas is an excellent way of coping with highly stringent national and European regulations in this region and of using organic waste for the production of energy, followed by the recycling of the digested substrate as fertilizer (Rai et al. 2020). Anaerobic digestion will also lead to a reduction in waste volume and waste disposal costs (Bong et al. 2017).

### 8.7.6 As an Excellent Fertilizer

A biogas plant is not solely an energy supplier but depends on the institutional structures and farmers' practices involved in making energy available. The digested substrate, commonly called the digestive, is beneficial nitrogen, phosphorus, potassium, and micronutrient-rich soil fertilizer that can be added to fields using the normal liquid manure application equipment. Due to higher homogeneity and nutrient abundance, better C/N ratio, and substantially decreased odor, digestive fertilizer performance has increased compared with raw animal manure (Kolar et al. 2011). Unpaprom et al. (2021) performed biogas production of crushed water hyacinth (WH) combined with swine dung (SD). The digestate from the biogas fermenter was confirmed to be an efficient alternative fertilizer with high nutrients (nitrogen, phosphorus, potassium) and environmentally friendly compared to chemical fertilizer.

### 8.7.7 Flexibility of Using Different Feedstock

For the production of biogas, different types of the feedstock may be used: animal manure and slurries, crop residues, organic waste from dairy production, food, and agro-industries, wastewater sludge, the organic component of municipal solid waste, household and catering organic waste, as well as energy crop waste. Biogas can also be obtained from landfill sites with unique infrastructure. The ability to use "wet biomass" types as feedstock, all characterized by a moisture content greater than 70% (e.g., waste sludge, animal slurries, flotation sludge from food manufacturing, etc.), is a significant benefit in biogas production. A variety of energy crops (grains, maize, rapeseed) have been primarily used as feedstock for the production of biogas in countries such as Austria and Germany. In addition to energy crops, biogas and fertilizer may be produced using all types of agricultural residues, degraded crops, unfit for food, or arising from unfavorable growing and weather conditions (Brémond et al. 2020).

### 8.7.8 Reduced Odor and Flies

Liquid manure, animal dung, and certain organic wastes are sources of constant, undesirable odor and attract flies at the time of their application and storage. Anaerobic Digestion eliminates these odors by as much as 75–85%. The digestive produced is nearly odor-free and the residual odors of ammonia fade soon after the application of fertilizer (Paolini et al. 2018).

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## 8.8 Future Prospects of Biogas Technology

The increasing energy demand compels the exploration of different types of waste and the development of new technologies for bioenergy production. Consumption of fossil fuels has contributed to detrimental effects on the environment and society (Korbag et al. 2020). Biogas is recognized as one of the leading bioenergy to address the existing environmental and energy challenges being faced by the world. It is an alternative energy source produced through solid waste management by the action of several microbes (Uche et al. 2020). The utilization of animal waste such as cow dung, pig dung, poultry dung, sheep dung, horse dung, etc. as the substrate for the production of biogas can effectively alleviate the shortage of energy and protect against environmental pollution (Gemechu 2020). Biogas is commonly used for cooking, lighting, heating, and power production and if purified further, it can be used as vehicle fuel (Roubik and Mazancová 2020).

The quantitative yield of biogas per unit weight of the substrate used differs from one type of substrate to another depending on the composition as well as the nature of the substrate. The methane content of biogas is the valuable portion of the gas and determines its calorific value (Nsair et al. 2020). Among the animal wastes that are used as substrate for biogas production, it has been reported that poultry waste has the highest methane content approximately 70% (Laiq Ur Rehman et al. 2019). Therefore, keen attention should be drawn to the utilization of several other types of animal waste that could have the potential to provide high methane content than poultry waste. Also, the degradation of organic waste material requires a co-ordinated action of several groups of microbial consortia with different metabolic capabilities (Palaniveloo et al. 2020). Conventional methods in molecular biology could help to classify only the most abundant microbial inhabitants found in the digester. Therefore, novel molecular biological techniques should be adopted that could provide a valuable tool for an improved understanding of this complex microbiological process, which in turn could help improve and control the process fruitfully in the future.

Biogas upgrading technologies are constantly being improved for better performance, enhanced upgrading efficiency, and low cost so that the technology gets a broader implementation globally. The current advancement of biogas upgrade techniques is illustrated by some innovative developments such as hydrate separation, cryogenic separation hybrid process, biological method, membrane enrichment, in situ upgrading, supersonic and industrial lung, multistage, and

high-pressure anaerobic digestion, though evaluated at laboratory and trial level (Olumide et al. 2017). However, commercial-scale optimization and testing are needed for these technologies to prove the full potential for biogas upgrading. Thus, there are still urgent needs for the development of novel anaerobic digestion technologies such as the development of a new reactor design to improve the efficiency of the process, increase biogas production rate and provide enormous potential concerning feasibility and technological simplicity with high efficiency. Also, research on the development of novel packing materials that can intensify mass transfer between gas and liquid and relatively low-pressure drop should be given utmost attention. There is also a need for the development of several computer models to model the biochemical anaerobic digestion process and regulate the process effectively. Better process management is essential for the future as well. Advanced monitoring and control systems will form part of the new epoch in the future of biogas plants and significantly contribute to process optimization (Theuerl et al. 2019). Operational process parameters like temperature, pressure, and flow rate of the gas should be optimized to decrease the large quantities of water needed, the cost for biogas compression, and water pumping.

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## References

- Akuzawa M, Hori T, Haruta S, Ueno Y, Ishii M, Igarashi Y (2011) Distinctive responses of metabolically active microbiota to acidification in a thermophilic anaerobic digester. *Microb Ecol* 61(3):595–605. <https://doi.org/10.1007/s00248-010-9788-1>
- Alhassan KA, Abdullahi BT, Shah MM (2019) A review on biogas production as the alternative source of fuel. *J Appl Adv Res* 4(2):61–65. <https://doi.org/10.21839/jaar.2019.v4i2.266>
- Anukam A, Mohammadi A, Naqvi M, Granström K (2019) A review of the chemistry of anaerobic digestion: methods of accelerating and optimizing process efficiency. *Processes* 7(8):504. <https://doi.org/10.3390/pr7080504>
- Arsova L (2010) Anaerobic digestion of food waste: current status, problems and an alternative product. Department of Earth and Environmental Engineering Foundation of Engineering and Applied Science Columbia University, New York
- Awe OW, Zhao Y, Nzihou A, Minh DP, Lyczko N (2017) A review of biogas utilisation, purification and upgrading technologies. *Waste Biomass Valoriz* 8(2):267–283. <https://doi.org/10.1007/s12649-016-9826-4>
- Babae A, Shayegan J (2011) Effect of organic loading rates (OLR) on production of methane from anaerobic digestion of vegetables waste. In: Proceedings of the world renewable energy congress – Sweden, 8–13 May, 2011, Linköping, Sweden, vol 57, pp 411–417. <https://doi.org/10.3384/ecp11057411>
- Bajpai P (2017) Anaerobic technology in pulp and paper industry, 1st edn. Springer, Singapore, pp 1–6. [https://doi.org/10.1007/978-981-10-4130-3\\_1](https://doi.org/10.1007/978-981-10-4130-3_1)

- Bartolini F, Gava O, Brunori G (2017) Biogas and EU's 2020 targets: evidence from a regional case study in Italy. *Energy Policy* 109:510–519. <https://doi.org/10.1016/j.enpol.2017.07.039>
- Barua S, Dhar BR (2017) Advances towards understanding and engineering direct interspecies electron transfer in anaerobic digestion. *Bioresour Technol* 244(1):698–707. <https://doi.org/10.1016/j.biortech.2017.08.023>
- Bollon J, Le-hyarc R, Benbelkacem H, Buffiere P (2011) Development of a kinetic model for anaerobic dry digestion processes: focus on acetate degradation and moisture content. *Biochem Eng J* 56(3):212–218. <https://doi.org/10.1016/j.bej.2011.06.011>
- Bong CPC, Ho WS, Hashim H, Lim JS, Ho CS, Tan WSP, Lee CT (2017) Review on the renewable energy and solid waste management policies towards biogas development in Malaysia. *Renew Sust Energ Rev* 70:988–998. <https://doi.org/10.1016/j.rser.2016.12.004>
- Borja R (2011) Biogas production. In: Moo-Young M (ed) *Comprehensive biotechnology*, 2nd edn. Elsevier B.V., Amsterdam, pp 785–798
- Brémond U, Bertrandias A, Loisel D, Jimenez J, Steyer JP, Bernet N, Carrere H (2020) Assessment of fungal and thermo-alkaline post-treatments of solid digestate in a recirculation scheme to increase flexibility in feedstocks supply management of biogas plants. *Renew Energy* 149:641–651. <https://doi.org/10.1016/j.renene.2019.12.062>
- Bruni E, Jensen AP, Pedersen ES, Angelidaki I (2010) Anaerobic digestion of maize focusing on variety, harvest time and pretreatment. *Appl Energy* 87(7):2212–2217. <https://ideas.repec.org/a/eee/appene/v87y2010i7p2212-2217.html>
- Budiyono B, Widiarta IN, Johari S, Sunarso S (2014) Increasing biogas production rate from cattle manure using rumen fluid as inoculums. *Int J Sci Eng* 6(1):31–38. <https://doi.org/10.12777/ijse.6.1.31-38>
- Cavinato C, Bolzonella D, Pavan P, Fatone F, Cecchi F (2013) Mesophilic and thermophilic anaerobic co-digestion of waste activated sludge and source sorted biowaste in pilot-and full-scale reactors. *Renew Energy* 55:260–265. <https://doi.org/10.1016/j.renene.2012.12.044>
- Chandra R, Takeuchi H, Hasegawa T (2012) Methane production from lignocellulosic agricultural crop wastes: a review in context to second generation of biofuel production. *Renew Sust Energ Rev* 16(3):1462–1476. <https://doi.org/10.1016/j.rser.2011.11.035>
- Chen C, Guo W, Ngo HH, Lee DJ, Tung KL, Jin P, Wang J, Wu Y (2016) Challenges in biogas production from anaerobic membrane bioreactors. *Renew Energy* 98:120–134. <https://doi.org/10.1016/j.renene.2016.03.095>
- Chojnacka A, Szczęsny P, Błaszczyk MK, Zielenkiewicz U, Detman A, Salamon A, Sikora A (2015) Noteworthy facts about a methane-producing microbial community processing acidic effluent from sugar beet molasses fermentation. *PLoS One* 10(5):e0128008. <https://doi.org/10.1371/journal.pone.0128008>
- Christy PM, Gopinath LR, Divya D (2014) A review on anaerobic decomposition and enhancement of biogas production through enzymes and microorganisms. *Renew Sust Energ Rev* 34:167–173. <https://doi.org/10.1016/j.rser.2014.03.010>
- Conrad R (1999) Contribution of hydrogen to methane production and control of hydrogen concentrations in methanogenic soils and sediments. *FEMS Microbiol Ecol* 28(3):193–202. <https://doi.org/10.1111/j.1574-6941.1999.tb00575.x>
- Costa KC, Leigh JA (2014) Metabolic versatility in methanogens. *Curr Opin Biotechnol* 29:70–75. <https://doi.org/10.1016/j.copbio.2014.02.012>
- Demetriades P (2009) Thermal pre-treatment of cellulose rich biomass for biogas production. Department of Microbiology Swedish, University of Agricultural Sciences, Uppsala. <http://urn.kb.se/resolve?urn=urn:nbn:se:slu:epsilon-s-7884>
- Demirel B, Scherer P (2008) The roles of acetotrophic and hydrogenotrophic methanogens during anaerobic digestion of biomass to methane: a review. *Rev Environ Sci Biotechnol* 7(2): 173–190. <https://doi.org/10.1007/s11157-008-9131-1>
- Dobre P, Nicolae F, Matei F (2014) Main factors affecting biogas production—an overview. *Rom Biotechnol Lett* 19(3):9283–9296

- Enzmann F, Mayer F, Rother M, Holtmann D (2018) Methanogens: biochemical background and biotechnological applications. *AMB Exp* 8(1):1. <https://doi.org/10.1186/s13568-017-0531-x>
- Fedailaine M, Moussi K, Khitous M, Abada S, Saber M, Tirichine N (2015) Modeling of the anaerobic digestion of organic waste for biogas production. *Procedia Comput Sci* 52:730–737. <https://doi.org/10.1016/j.procs.2015.05.086>
- Ferrer I, Ponsá S, Vázquez F, Font X (2008) Increasing biogas production by thermal (70°C) sludge pre-treatment prior to thermophilic anaerobic digestion. *Biochem Eng J* 42(2):186–192. <https://doi.org/10.1016/j.bej.2008.06.020>
- Gemechu FK (2020) Evaluating the potential of domestic animal manure for biogas production in Ethiopia. *J Energy* 2020:8815484
- Gkamarazi N (2015) Implementing anaerobic digestion for municipal solid waste treatment: challenges and prospects. In: Proceedings of the 14th international conference on environmental science and technology, CEST, Rhodes, Greece, September 3–5, 2015
- Gopal LC, Govindarajan M, Kavipriya MR, Mahboob S, Al-Ghanim KA, Virik P, Ahmed Z, Al-Mulhm N, Senthilkumaran V, Shankar V (2021) Optimization strategies for improved biogas production by recycling of waste through response surface methodology and artificial neural network: sustainable energy perspective research. *J King Saud Univ Sci* 33(1):101241. <https://doi.org/10.1016/j.jksus.2020.101241>
- Goswami R, Chattopadhyay P, Shome A (2016) An overview of physico-chemical mechanisms of biogas production by microbial communities: a step towards sustainable waste management. *3 Biotech* 6(1):1–12. <https://doi.org/10.1007/s13205-016-0395-9>
- Govarthanan M, Manikandan S, Subbaiya R, Krishnan RY, Srinivasan S, Karmegam N, Kim W (2022) Emerging trends and nanotechnology advances for sustainable biogas production from lignocellulosic waste biomass: a critical review. *Fuel* 312:122928. <https://doi.org/10.1016/j.fuel.2021.122928>
- Hajji A, Rhachi M, Garoum M, Laaroussi N (2016) The effects of pH, temperature and agitation on biogas production under mesophilic regime. In: 2016 3rd international conference on renewable energies for developing countries (REDEC), July, 13–15, 2016, Zouk Mosbeh, Lebanon, pp 1–4. <https://doi.org/10.1109/Redec.2016.7577510>
- Hartmann H, Ahring BK (2006) Strategies for the anaerobic digestion of the organic fraction of municipal solid waste: an overview. *Water Sci Technol* 53(8):7–22. <https://doi.org/10.2166/wst.2006.231>
- Heeg K, Pohl M, Sontag M, Mumme J, Klocke M, Nettmann E (2014) Microbial communities involved in biogas production from wheat straw as the sole substrate within a two-phase solid-state anaerobic digestion. *Syst Appl Microbiol* 37(8):590–600. <https://doi.org/10.1016/j.syapm.2014.10.002>
- Heo NH, Park SC, Lee JS, Kang H (2003) Solubilization of waste activated sludge by alkaline pretreatment and biochemical methane potential (BMP) tests for anaerobic co-digestion of municipal organic waste. *Water Sci Technol* 48(8):211–219
- Hidayati YA, Kurnani TBA, Marlina ET, Rahmah KN, Harlia E, Joni IM (2018) The production of anaerobic bacteria and biogas from dairy cattle waste in various growth mediums. *AIP Conf Proc* 1927(1):030021. <https://doi.org/10.1063/1.5021214>
- Horváth IS, Tabatabaei M, Karimi K, Kumar R (2016) Recent updates on biogas production - a review. *Biofuel Res J* 10:394–402. <https://doi.org/10.18331/BRJ2016.3.2.4>
- Hossain MS, Karim TU, Onik MH, Kumar D, Rahman MA, Yousuf A, Uddin MR (2022) Impact of temperature, inoculum flow pattern, inoculum type, and their ratio on dry anaerobic digestion for biogas production. *Sci Rep* 12(1):6162. <https://doi.org/10.1038/s41598-022-10025-1>
- Huang W, Wang Z, Zhou Y, Ng WJ (2015) The role of hydrogenotrophic methanogens in an acidogenic reactor. *Chemosphere* 140:40–46. <https://doi.org/10.1016/j.chemosphere.2014.10.047>
- Jagadabhi PS (2011) Methods to enhance hydrolysis during one and two-stage anaerobic digestion of energy crops and crop residues. Department of Biological and Environmental Science, University of Jyväskylä, Jyväskylä

- Jain SR, Mattiasson B (1998) Acclimatization of methanogenic consortia for low pH biomethanation process. *Biotechnol Lett* 20(8):771–775. <https://doi.org/10.1023/B:BILE.0000015920.45724.29>
- Jørgensen PJ (2009) Biogas – green energy, 2nd edn. Digisource Danmark A/S, Aarhus
- Kandler O, König H (1985) Cell envelopes of archaeobacteria. In: Woese CR, Wolfe RS (eds) *The bacteria. A treatise on structure and function, Archaeobacteria*, vol VIII. Academic, New York
- Karaca C (2018) Determination of biogas production potential from animal manure and GHG emission abatement in Turkey. *Int J Agric Biol Eng* 11(3):205–210
- Kashyap BK, Solanki MK, Pandey AK, Prabha S, Kumar P, Kumari B (2019) *Bacillus* as plant growth promoting rhizobacteria (PGPR): a promising green agriculture technology. In: Ansari R, Mahmood I (eds) *Plant health under biotic stress*. Springer, Singapore, pp 219–236. [https://doi.org/10.1007/978-981-13-6040-4\\_11](https://doi.org/10.1007/978-981-13-6040-4_11). ISBN: 978-981-13-6040-4
- Kaur G, Brar YS, Kothari DP (2017) Potential of livestock generated biomass: untapped energy source in India. *Energies* 10(7):847. <https://doi.org/10.3390/en10070847>
- Kayhanian M (1999) Ammonia inhibition in high-solids biogasification: an overview and practical solutions. *Environ Technol (United Kingdom)* 20(4):355–365. <https://doi.org/10.1080/09593332008616828>
- Khalid A, Arshad M, Anjum M, Mahmood T, Dawson L (2011) The anaerobic digestion of solid organic waste. *Waste Manag* 31(8):1737–1744. <https://doi.org/10.1016/j.wasman.2011.03.021>
- Khan IU, Othman MHD, Hashim H, Matsuura T, Ismail AF, Rezaei-DashtArzhandi M, Azelee IW (2017) Biogas as a renewable energy fuel—a review of biogas upgrading, utilisation and storage. *Energy Convers Manag* 150:277–294. <https://doi.org/10.1016/j.enconman.2017.08.035>
- Khanal SK (2009) Bioenergy generation from residues of biofuel industries. In: Khanal SK (ed) *Anaerobic biotechnology for bioenergy production: principles and applications*. Wiley-Blackwell, Hoboken, pp 161–188. <https://doi.org/10.1002/9780813804545.ch8>
- Khayal OMES (2019) *Biogas plants technology: types of biogas plants, advantages and limitations*. LAP LAMBERT Academic Publishing, London
- Kolar L, Kuzel S, Peterka J, Borova-Batt J (2011) Utilisation of waste from digesters for biogas production. In: Bernardes MADS (ed) *Biofuel's engineering process technology*. IntechOpen, London. <https://doi.org/10.5772/17029>
- Korbag I, Omer SMS, Boghazala H, Abusasiyah MAA (2020) Recent advances of biogas production and future perspective. In: *Biogas-recent advances and integrated approaches*. IntechOpen, London
- Kothari R, Pandey AK, Kumar S, Tyagi VV, Tyagi SK (2014) Different aspects of dry anaerobic digestion for bio-energy: an overview. *Renew Sust Energ Rev* 39:174–195. <https://doi.org/10.1016/j.rser.2014.07.011>
- Kougias PG, Boe K, Angelidaki I (2013) Effect of organic loading rate and feedstock composition on foaming in manure-based biogas reactors. *Bioresour Technol* 144:1–7. <https://doi.org/10.1016/j.biortech.2013.06.028>
- Kwietniewska E, Tys J (2014) Process characteristics, inhibition factors and methane yields of anaerobic digestion process, with particular focus on microalgal biomass fermentation. *Renew Sust Energ Rev* 34:491–500. <https://doi.org/10.1016/j.rser.2014.03.041>
- Kythreotou N, Florides G, Tassou SA (2014) A review of simple to scientific models for anaerobic digestion. *Renew Energy* 71:701–714. <https://doi.org/10.1016/j.renene.2014.05.055>
- Laiq Ur Rehman M, Iqbal A, Chang C-C, Li W, Ju M (2019) Anaerobic digestion. *Water Environ Res* 91:1253–1271. <https://doi.org/10.1002/wer.1219>
- Lebuhn M, Liu F, Heuwinkel H, Gronauer A (2008) Biogas production from mono-digestion of maize silage-long-term process stability and requirements. *Water Sci Technol* 58(8): 1645–1651. <https://doi.org/10.2166/wst.2008.495>
- Li M (2008) Peak energy and the limits to China's economic growth: prospect of energy supply and economic growth from now to 2050. Working papers wp189. Political Economy Research Institute, University of Massachusetts, Amherst



- Li Y, Park SY, Zhu J (2011) Solid-state anaerobic digestion for methane production from organic waste. *Renew Sust Energ Rev* 15(1):821–826. <https://ideas.repec.org/a/eee/rensus/v15y2011i1p821-826.html>
- Liaquat R, Jamal A, Tauseef I, Qureshi Z, Farooq U, Imran M, Ali MI (2017) Characterizing bacterial consortia from an anaerobic digester treating organic waste for biogas production. *Pol J Environ Stud* 26(2):709–716. <https://doi.org/10.15244/pjoes/59332>
- Lin L, Yan R, Liu Y, Jiang W (2010) In-depth investigation of enzymatic hydrolysis of biomass wastes based on three major components: cellulose, hemicellulose and lignin. *Bioresour Technol* 101(21):8217–8223. <https://doi.org/10.1016/j.biortech.2010.05.084>
- Lin J, Zuo J, Gan L, Li P, Liu F, Wang K, Chen L, Gan H (2011) Effects of mixture ratio on anaerobic co-digestion with fruit and vegetable waste and food waste of China. *J Environ Sci* 23(8):1403–1408. [https://doi.org/10.1016/S1001-0742\(10\)60572-4](https://doi.org/10.1016/S1001-0742(10)60572-4)
- Liu C, Yuan X, Zeng G, Li W, Li J (2008) Prediction of methane yield at optimum pH for anaerobic digestion of organic fraction of municipal solid waste. *Bioresour Technol* 99(4):882–888. <https://doi.org/10.1016/j.biortech.2007.01.013>
- Luo G, Wang W, Angelidaki I (2013) Anaerobic digestion for simultaneous sewage sludge treatment and CO biomethanation: process performance and microbial ecology. *Environ Sci Technol* 47(18):10685–10693. <https://doi.org/10.1021/es401018d>
- Madacka M, Rajesh N, Jayaraju N, Lakshmana B, Kumaraswamy HH, Kashyap BK (2020) Eco-friendly microbial biofuel production from waste. In: Kashyap BK, Solanki MK, Kamboj DV, Pandey AK (eds) *Waste to energy: prospects and applications*. Springer, Singapore. [https://doi.org/10.1007/978-981-33-4347-4\\_4](https://doi.org/10.1007/978-981-33-4347-4_4)
- Mainardis M, Buttazzoni M, Goi D (2020) Up-flow anaerobic sludge blanket (UASB) technology for energy recovery: a review on state-of-the-art and recent technological advances. *Bioengineering* 7(2):43
- Mao C, Feng Y, Wang X, Ren G (2015) Review on research achievements of biogas from anaerobic digestion. *Renew Sust Energ Rev* 45:540–555. <https://doi.org/10.1016/j.rser.2015.02.032>
- Mara D, Horan N (2003) *Handbook of water and wastewater microbiology*. Elsevier, Amsterdam
- Martí-Herrero J (2008) Low cost biodigesters to produce biogas and natural fertilizer from organic waste. *IDEASS Latin America*. <https://www.ideassonline.org/innovations/brochureView.php?id=76>
- Mata-Alvarez J, Dosta J, Romero-Güiza MS, Fonoll X, Peces M, Astals S (2014) A critical review on anaerobic co-digestion achievements between 2010 and 2013. *Renew Sust Energ Rev* 36: 412–427. <https://doi.org/10.1016/j.rser.2014.04.039>
- Mathew AK, Bhui I, Banerjee SN, Goswami R, Chakraborty AK, Shome A, Balachandran S, Chaudhury S (2015) Biogas production from locally available aquatic weeds of Santiniketan through anaerobic digestion. *Clean Techn Environ Policy* 17(6):1681–1688. <https://doi.org/10.1007/s10098-014-0877-6>
- Merlino G, Rizzi A, Schievano A, Tenca A, Scaglia B, Oberti R, Adani F, Daffonchio D (2013) Microbial community structure and dynamics in two-stage vs single-stage thermophilic anaerobic digestion of mixed swine slurry and market bio-waste. *Water Res* 47(6):1983–1995. <https://doi.org/10.1016/j.watres.2013.01.007>
- Miller TL, Wolin MJ (1983) Oxidation of hydrogen and reduction of methanol to methane is the sole energy source for a methanogen isolated from human feces. *J Bacteriol* 153(2):1051–1055
- Montañés R, Solera R, Pérez M (2015) Anaerobic co-digestion of sewage sludge and sugar beet pulp lixiviation in batch reactors: effect of temperature. *Bioresour Technol* 180:177–184. <https://doi.org/10.1016/j.biortech.2014.12.056>
- Náthia-Neves G, Berni M, Dragone G, Mussatto SI, Forster-Carneiro T (2018) Anaerobic digestion process: technological aspects and recent developments. *Int J Environ Sci Technol* 15:2033–2046. <https://doi.org/10.1007/s13762-018-1682-2>
- Nsair A, Onen Cinar S, Alassali A, Abu Qdais H, Kuchta K (2020) Operational parameters of biogas plants: a review and evaluation study. *Energies* 13(15):3761

- Ntaikou I, Antonopoulou G, Lyberatos G (2010) Biohydrogen production from biomass and wastes via dark fermentation: a review. *Waste Biomass Valoriz* 1(1):21–39. <https://doi.org/10.1007/s12649-009-9001-2>
- Nzila C, Dewulf J, Spanjers H, Tuigong D, Kiriamiti H, Van Langenhove H (2012) Multi criteria sustainability assessment of biogas production in Kenya. *Appl Energy* 93:496–506. <https://doi.org/10.1016/j.apenergy.2011.12.020>
- Olumide WA, Yaqian Z, Ange N, Doan PM, Nathalie L (2017) A review of biogas utilisation. Purification and upgrading technologies. *Waste Biomass Valoriz* 8:267–283
- Onwuliri FC, Onyimba IA, Nwaukwu IA (2013) Generation of biogas from cow dung. *Bioremediat Biodegrad* 18:2–4. <https://doi.org/10.4172/2155-6199.S18-002>
- Ostrem K (2004) Greening waste: anaerobic digestion for treating the organic fraction of municipal solid wastes. The Fu Foundation of School of Engineering and Applied Science, Columbia University, New York
- Palaniveloo K, Amran MA, Norhashim NA, Mohamad-Fauzi N, Peng-Hui F, Hui-Wen L et al (2020) Food waste composting and microbial community structure profiling. *Processes* 8(6):723
- Pampillón-González L, Ortiz-Cornejo NL, Luna-Guido M, Dendooven L, Navarro-Noya YE (2017) Archaeal and bacterial community structure in an anaerobic digestion reactor (lagoon type) used for biogas production at a pig farm. *J Mol Microbiol Biotechnol* 27(5):306–317. <https://doi.org/10.1159/000479108>
- Paolini V, Petracchini F, Segreto M, Tomassetti L, Naja N, Cecinato A (2018) Environmental impact of biogas: a short review of current knowledge. *J Environ Sci Health A* 53(10):899–906. <https://doi.org/10.1080/10934529.2018.1459076>
- Prasad RD (2012) Empirical study on factors affecting biogas production. *Int Sch Res Notices* 2012:1–7. <https://doi.org/10.5402/2012/136959>
- Rai S, Solanki MK, Anal AKD, Sagar A, Solanki AC, Kashyap BK, Pandey AK (2020) Emerging frontiers of microbes as agro-waste recycler. In: Kashyap BK, Solanki MK, Kamboj DV, Pandey AK (eds) *Waste to energy: prospects and applications*. Springer, Singapore. [https://doi.org/10.1007/978-981-33-4347-4\\_1](https://doi.org/10.1007/978-981-33-4347-4_1)
- Raja IA, Wazir S (2017) Biogas production: the fundamental processes. *Univers J Eng Sci* 5(2): 29–37. <https://doi.org/10.13189/ujes.2017.050202>
- Resch C, Wörl A, Waltenberger R, Braun R, Kirchmayr R (2011) Enhancement options for the utilisation of nitrogen rich animal by-products in anaerobic digestion. *Bioresour Technol* 102(3):2503–2510. <https://doi.org/10.1016/j.biortech.2010.11.044>
- Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F (2014) *The prokaryotes*, 4th edn. Springer, Berlin, pp 298–306
- Roubik H, Mazancová J (2020) Suitability of small-scale biogas systems based on livestock manure for the rural areas of Sumatra. *Environ Dev* 33:100505
- Saidmamatov O, Rudenko I, Baier U, Khodjanizayov E (2021) Challenges and solutions for biogas production from agriculture waste in the Aral Sea Basin. *Processes* 9(2):199. <https://doi.org/10.3390/pr9020199>
- Schiel-Bengelsdorf B, Dürre P (2012) Pathway engineering and synthetic biology using acetogens. *FEBS Lett* 586(15):2191–2198
- Schnurer A, Jarvis A (2009) *Microbiological handbook for biogas plant*. Swedish Waste Management, Swedish Gas Centre, Malmö, pp 1–74
- Silwadi M, Mousa H, AL-Hajji BY, AL-Wahaibi SS, AL-Harrasi ZZ (2022) Enhancing biogas production by anaerobic digestion of animal manure. *Int J Green Energy* 20:257. <https://doi.org/10.1080/15435075.2022.2038608>
- Slonczewski JL, Foster JW (2013) *Microbiology: an evolving science: third international student edition*. WW Norton & Company, New York
- Sreekrishnan TR, Kohli S, Rana V (2004) Enhancement of biogas production from solid substrates using different techniques—a review. *Bioresour Technol* 95(1):1–10. <https://doi.org/10.1016/j.biortech.2004.02.010>

- Theuerl S, Heiermann C, Heiermann M, Grundmann P, Landwehr N, Kreidenweis U, Prochnow A (2019) The future agricultural biogas plant in Germany: a vision. *Energies* 12(3):396. <https://doi.org/10.03390/en12030396>
- Tsaurai K (2018) Greenhouse gas emissions and economic growth in Africa: does financial development play any moderating role? *Int J Energy Econ Policy* 8(6):267
- Uche AM, Emmanuel OT, Paul OU, Olawale A, Frank KB, Rita OO, Martin OS (2020) Design and construction of fixed dome digester for biogas production using cow dung and water hyacinth. *Afr J Environ Sci Technol* 14(1):15–25
- Unpaprom Y, Pimpimol T, Whangchai K, Ramaraj R (2021) Sustainability assessment of water hyacinth with swine dung for biogas production, methane enhancement, and biofertilizer. *Biomass Convers Biorefinery* 11:849–860. <https://doi.org/10.1007/s13399-020-00850-7>
- Verma P, Vasudevan V, Kashyap BK, Samsudeen TI, Meghvansi MK, Singh L, Kamboj DV (2018) Direct lysis glass milk method of genomic DNA extraction reveals greater archaeal diversity in anaerobic biogas digester slurry as assessed through denaturing gradient gel electrophoresis. *J Exp Biol Agric Sci* 6(2):315–323. [https://doi.org/10.18006/2018.6\(2\).315.323](https://doi.org/10.18006/2018.6(2).315.323)
- Vögeli Y, Lohri CR, Gallardo A, Diener S, Zurbrugg C (2014) Anaerobic digestion of biowaste in developing countries: practical information and case studies. Swiss Federal Institute of Aquatic Science and Technology (Eawag), Dübendorf
- Weinrich S, Schäfer F, Bochmann G, Liebetrau J (2018) Value of batch tests for biogas potential analysis; method comparison and challenges of substrate and efficiency evaluation of biogas plants. In: Murphy JD (ed), IEA bioenergy task , vol 37
- Werner U, Stöhr U, Hees N (1989) Biogas plants in animal husbandry. A practical guide. Springer, Berlin
- Xu F, Li Y, Ge X, Yang L, Li Y (2018) Anaerobic digestion of food waste—challenges and opportunities. *Bioresour Technol* 247:1047–1058. <https://doi.org/10.1016/j.biortech.2017.09.020>
- Yadav KK, Patil PB, Kumaraswamy HH, Kashyap BK (2020) Ligninolytic microbes and their role in effluent management of pulp and paper industry. In: Kashyap BK, Solanki MK, Kamboj DV, Pandey AK (eds) Waste to energy: prospects and applications. Springer, Singapore. [https://doi.org/10.1007/978-981-33-4347-4\\_13](https://doi.org/10.1007/978-981-33-4347-4_13)
- Yang L, Xu F, Ge X, Li Y (2015) Challenges and strategies for solid-state anaerobic digestion of lignocellulosic biomass. *Renew Sust Energ Rev* 44:824–834. <https://doi.org/10.1016/j.rser.2015.01.002>
- Yu H, Wang Z, Wu Z, Zhu C (2016) Enhanced waste activated sludge digestion using a submerged anaerobic dynamic membrane bioreactor: performance, sludge characteristics and microbial community. *Sci Rep* 6(1):1–10. <https://doi.org/10.1038/srep20111>
- Zandvoort MH, Van Hullebusch ED, Feroso FG, Lens PNL (2006) Trace metals in anaerobic granular sludge reactors: bioavailability and dosing strategies. *Eng Life Sci* 6(3):293–301. <https://doi.org/10.1002/elsc.200620129>
- Zieminski K, Frac M (2012) Methane fermentation process as anaerobic digestion of biomass: transformations, stages and microorganisms. *Afr J Biotechnol* 11:4127–4139
- Zupancic GD, Grlic V (2007) Anaerobic treatment and biogas production from organic waste. In: Management of organic waste. IntechOpen, London. <https://doi.org/10.5772/32756>