Chapter 6
Recycling of Multiple Organic Solid Wastes into Biogas via Anaerobic Digestion

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Abstract The accumulation of solid organic wastes (SOW) has reached critical levels globally and therefore, sustainable management of wastes is the key to minimize the risks to human health, avoid depletion of natural resources, reduce environmental burden and maintain the ecological balance. SOWs mainly include food waste, animal manure, waste activated sludge, yard waste, and agricultural waste. Anaerobic digestion (AD) is one of the most viable and popular technologies for recycling the organic fraction of solid wastes for the production of renewable energy in the form of biogas that can be crucial in meeting the world's ever-increasing energy demands. Employing sophisticated treatment techniques for the diverse organic fractions present in solid wastes enable proper waste management as well as add value to the economy. Detailed knowledge about the physical properties of these SOWs to determine suitable operating conditions as well as research on the genetic engineering of microbes involved in the AD process are needed to produce biogas efficiently. This chapter summarizes the science underlying the anaerobic digestion process, different feedstock types, the diverse array of microorganisms involved, process variables crucial for AD efficiency, industrial scope of the

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different reactor modes, and the optimization and pretreatment methods to improve process efficiency.

Keywords Renewable energy · Solid organic wastes · Anaerobic digestion models · Resource recovery · Pretreatment

6.1 Introduction

The energy crisis in the twenty-first century caused by global population swelling and the development of industries has reached an unprecedented level. Currently, fossil fuels are the main source of world energy which are non-renewable and cause environmental pollution that has triggered scientists' motivation to look for renewable, clean sources [1]. Besides, the world is currently witnessing a tremendous increase in the production of solid wastes. A major quantity of the generated solid wastes is organic by nature, and they originate from the municipal, industrial and agricultural sectors. Municipal solid wastes (MSW) are one of the most common organic solid wastes and it is projected that by 2025, the annual MSW production could reach 2.2 billion tonnes [2]. Agricultural wastes are another class of biodegradable wastes that are generated during livestock and food production which can be utilized for biogas production, thereby contributing to the economics of agriculture. However, in many countries, a major percentage of the organic wastes end up in landfills or are disposed off in water bodies, resulting in serious soil and water pollution, which can affect human health and hygiene. Hence, using appropriate processing methods to convert biodegradable organic wastes into biofuel such as biogas is of utmost importance to allow energy recovery and prevent adverse environmental effects. Anaerobic digestion (AD) is a simple yet powerful process that can be used to overcome the challenge posed by organic wastes to the environment. AD is primarily used to convert organic wastes into gaseous biofuel, biogas (biogas is a mixture of methane, carbon dioxide, and other trace gases). Biogas produced in rural areas is mainly used for cooking and heating homes. Additionally, the biogas produced in large scale plants can be used for steam generation in boilers or combined heat and power (CHP) generation in power station or heat engines. Through anaerobic digestion as the organic wastes which is usually released to the environment or landfills is diverted for biogas production it helps in the fight against greenhouse gas emissions by reducing methane and nitrous oxide emissions from landfills. Furthermore, AD process can be used to promote soil fertility by using digestates as a nutrient rich material for the production of compost and organic fertilizer [3].

Although organic wastes appear in solid form, they contain up to 90% moisture. This restricts the application of thermo-chemical treatment such as incineration for energy recovery as the process would end up requiring excessive heat to overcome the high-water percentage, making it energy intensive. AD overcomes this limitation by allowing the controlled release of energy from the chemical bonds present in the organic compounds that makeup the wastes. Therefore, AD has become a prevailing choice for the sustainable treatment of organic wastes having high moisture content. It involves the microbial degradation of organic feedstocks through a series of anaerobic stages to produce methane-rich biogas for renewable energy production and use.

Based on the total solid content of the waste and the percentage of moisture present, the AD can be classified as either liquid-state AD (solid content $<15\%$) or solid-state AD (solid content $>15\%$) [4]. Liquid state AD, also called as wet AD, is primarily used to treat substrates with high moisture content, such as waste activated sludge and animal manures. However, the large water content in this process significantly lowers the volumetric methane productivity as well as creating the problem of generating a large amount of digestate as waste product [5]. On the other hand, solid-state AD (dry AD) involves digestion of feedstocks with high organic loading and minimal water content. Solid-state AD is generally preferred for digestion of the organic fraction derived from municipal solid waste and agricultural wastes, and often results in a high volumetric methane productivity. Moreover, the heating-energy requirement and wastewater generation are also reduced in the solid-state AD. However, due to inadequate mass transfer, solid-state AD has disadvantages such as longer retention time, high cost, and a tendency to accumulate inhibitors [6]. Thus, the major focus of this chapter is on the different methods of AD of organic wastes and how matching the treatment process to the selected type of waste can help in the maximization of the biogas production for renewable energy generation. This chapter also deals with the different microbial conditions and species required for facilitating the different stages of AD, synthetic biology approaches for engineering strains towards AD as well as models available to better understand the molecular processes. Also, the primary conditions such as organic loading rate (OLR, a definition for OLR is provided in Sect. 6.4.5), biogas production rates, and the influencing environmental conditions like temperature, pH, alkalinity, etc., have been discussed that contribute significantly towards the successful design and operations of the treatment process. In addition, this chapter also highlights emerging technologies like solid-state AD and the different processes available for large-scale AD.

6.2 Feedstocks for AD

Solid wastes are broadly grouped into three categories of municipal, industrial, and agricultural-based on their source. Considering that, municipal solid waste generation will reach 6.1 million metric tonnes/day in 2025 [7], consolidated solid waste management approaches such as AD are required to create a pollution-free environment. However, a more rigorous classification of AD feedstocks is necessary to better manage the wastes and to optimize the operating conditions of AD. More specifically, solid organic wastes are also classified into agricultural wastes (AW), animal manure (AM), waste activated sludge (WAS), yard waste (YW), and food

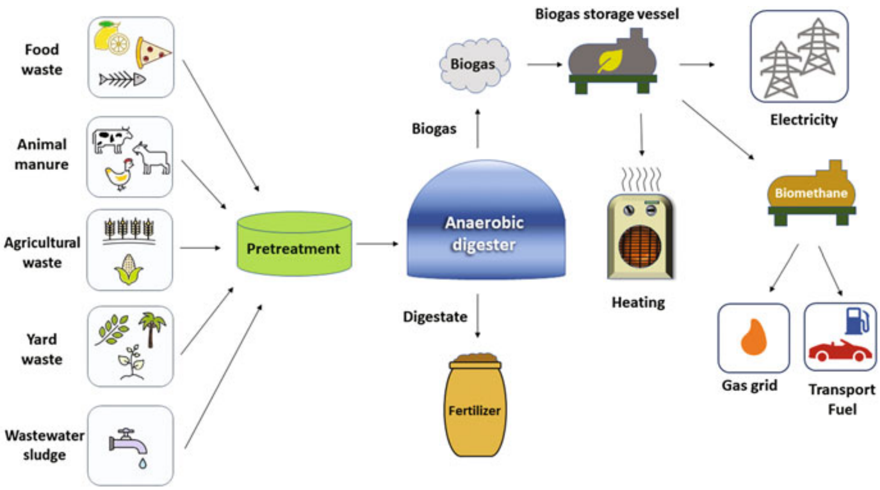

Fig. 6.1 Schematic depicting the different types of organic solid wastes fed into anaerobic digester and the common applications of the products generated via AD

waste (FW). Figure 6.1 shows the classification of solid wastes fed into AD and the applications of the products generated via AD.

6.2.1 Agricultural Waste

As the name indicates, residues of agriculture such as corn stover, rice straw, etc., can be used as the feedstock for the AD [8]. It has been estimated that around 90.7 million dry tonnes of primary crop residues are projected to be collected in the US out of which 75% is corn stover [9]. These wastes are composed of cellulose, hemicellulose, and lignin, which are hard to be broken down by natural enzymes and consumed by bacteria, and therefore, AD of these materials without pretreatment would not be effective. For instance, the corn stover silage is composed of 35% cellulose, 25.2% hemicellulose, and 4.3% lignin in which using a biological pretreatment (fungal) can increase methane yield by 23% [10]. Another strategy to improve the anaerobic digestion of agricultural wastes is to reduce their particle size. For example, Menardo et al. [11], adopted physical pretreatment to reduce the barley straw’s size from 5.0 cm to 0.5 cm and this improved the methane yield by 54.2%. In the same work, thermal pretreatment at 120 °C on barley and wheat straw increased methane production by 40.8% and 64.3%, respectively. Table 6.1 provides the composition of various lignocellulosic feedstocks found in agricultural wastes.

Table 6.1 Composition of various lignocellulosic materials found in agricultural and yard wastes

No.	Biomass	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Ref.
1	Rice Straw	34.63	29.74	15.34	[24]
2	Wheat Straw	35.19	22.15	22.09	[25]
3	Sugarcane bagasse	46.21	20.86	22.67	[25]
4	Pinewood	44.50	28.00	26.80	[26]
5	Elmwood	46.40	26.30	26.20	[26]
6	Corn stover	42.62	22.99	12.75	[27]
7	Sunflower Stalks	34.00	20.80	29.70	[28]
8	Banana Waste	13.20	14.80	14.00	[29]

6.2.2 Animal Manure

Animal manure is a good source of organic matter that can act as a feedstock for biogas production. As the quality of different animal manure is subtly different from each other, the conditions at which the AD operates are different as well [12]. A study in 2011 showed that out of the total animal manure feedstock used for AD, about 13.62% is recovered as energy in the form of biogas, while the remaining 73.14% is present as digestate [13]. The digestate also has the value of being used as fertilizer [14]. Animal manure is considered as a complex waste which contains a high amount of nitrogen that might cause reactor failure due to ammonia (NH_3) inhibition [15]. In this regard, when the concentration of NH_3 in an AD process exceeds a threshold, process failure might happen. Both free NH_3 and ammonium ion (NH_4^+) can inhibit the process if their concentration surpass ~1800 $mg.L^{-1}$ in high-rate digesters [16, 17]. Various techniques for recovery from ammonia inhibition have been discussed by Yenigün and Demirel, such as periodic removal of supernatants, reduction of protein content in wastewater feedstock, adjustment of pH and C:N ratio, etc. [15].

6.2.3 Waste Activated Sludge

Sludge generated during the treatment of municipal wastewater is considered as waste activated sludge. Its disposal can account for up to 50% of the wastewater plant's operating costs and one of the most preferred methods for sludge disposal and recycling is AD [18]. The disposed sludge of wastewater treatment plant can also be used as the feedstock which has a low carbon-to-nitrogen (C/N) ratio due to the high amount of nitrogen present in this type of waste [19]. However, the optimal C/N ratio of anaerobic digestion is around 20–30 [20]. To counterbalance the nutrients and ammonia inhibition the sludge can be used in co-digestion along with other wastes such as agricultural wastes that have a high C/N ratio.

6.2.4 Yard Waste

Yard waste or garden waste mostly includes leaves falling from trees and bushes, grasses and other various parts of plants that are mostly aggregated in the green cities and areas which can be used as the raw material of AD [21]. Table 6.1 provides the composition of various lignocellulosic feedstocks found in yard wastes as well. Garden wastes also have the same problem and difficulties to be used for AD as agricultural wastes as they are also composed of lignin, cellulose and hemicellulose which need to use pretreatments. For instance, Dussadee et al. [22] conducted AD of Napier grass (*Pennisetum purpureum*) for biogas production with and without pretreatment. After AD, they obtained 164 L biogas per kilogram volatile solids (L/kg VS) without pretreatment, whereas by integrating AD with chemical pretreatment they obtained 179 L/kg VS. In another study by Panigrahi et al. [23], four different pretreatments of hot air oven, hot water bath, autoclave and microwave applied on yard wastes and they obtained approximately 10% increase in biochemical methane potential from 328.9 ± 15 mL/g VS (untreated after 45 days) to 364.5 ± 11 mL/g VS (after 26 days) using microwave pretreatment.

6.2.5 Food Waste

Food waste is another important organic solid waste that can be used to produce biogas via AD, which mostly contains uneaten or discarded food from houses, restaurants or even industrial sectors. A study conducted by European Union reported that 88 million tonnes of edible and non-edible food wastes were generated in 2012 [30], which is shocking as it is equal to 20% of the total food produced. These food wastes can be used to produce biogas and further in electricity which studies have shown that 9900 ton of corn silage can be replaced by 6600 ton of food waste to reduce the carbon footprint by 42% [31]. Food wastes also are used as co-digestion feedstock to balance the nutrients in an anaerobic digester and improve the biogas production of various feedstocks. Yong et al. [32], investigated the biogas production from food wastes as well as using it as a co-digestion with straw. They obtained 0.16 m^3 CH_4 /kg_{VS} from AD of food waste individually, while using it as co-digestion improved the methane production yield of straw by 149.7% confirming using food waste as a desirable feedstock for nutrient balancing in AD.

As mentioned earlier, characterizing the properties of these solid organic wastes can provide researchers the necessary knowledge to design optimal conditions for AD. Table 6.2 shows a literature review on the AD of various substrates for biogas production and their operational conditions.

Table 6.2 A literature review on the AD of various feedstocks and their biogas/methane production

No.	Feedstock	System	OLR	Pre-treatment	pH	Biogas composition	Biogas/methane production	Ref.
1	Napier grass (*Pennisetum purpureum*)	Batch		Alkali	7.9	Methane 63.5% CO_2 30.1%	179.38 L biogas kg^{-1} VS	[22]
2	Municipal solid waste	Semi-continuous	6 kg VS m^{-3} day^{-1}	Mechanical(combination of a shear shredder, rotary cutter and wet macerator)	7.3	Methane 59%	0.54 STP m^3 biogas kg^{-1} VS_{added}	[33]
3	Municipal solid waste	Semi-continuous	6 kg VSm^{-3} day^{-1}	Mechanical(a combination of a shear shredder, rotary cutter and wet macerator)	8.4	Methane 59%	0.48 STP m^3 biogas kg^{-1} VS_{added}	[33]
4	Landfill leachate	Batch		Pulsed electric field	8.25–8.54		155.61–220.06 mL biogas g^{-1} VS	[34]
5	Fruit/Vegetable	Batch		Pulsed electric field	3.81–3.92		804.51–868.80 mL biogas g^{-1} VS	[34]
6	Organic fraction of municipal solid waste (OFMSW)	Batch		Sonication time 30 min Specific energy 7200 kJ/kg TS Power density 0.6 W/mL			455 mL biogas g^{-1} VS	[35]
7	Municipal solid waste			High-pressure extruding with 40 MPa pressure	6.96–7.64		674 mL CH_4 g^{-1}VS	[36]
8	Rice straw	Batch		Citric acid	7		197.86 mL biogas $g^{-1}VS_{total}$	[24]
9	Corn stover silage			Fungal (*Phanerochaete chrysosporium*)	4.3		265.1 mL CH_4 g^{-1}	[10]
10	Deinking sludge	Batch			7.3		160 mL_N[a] CH_4 g^{-1} DM	[37]
11	Sludge	Batch		–	7–7.5		64.8 mL biogas g^{-1} VS	[38]

(continued)

Table 6.2 (continued)

No.	Feedstock	System	OLR	Pre-treatment	pH	Biogas composition	Biogas/methane production	Ref.
12	*Scenedesmus* spp. (microalgae)	Continuous	0.3–0.4 g COD L^{-1} d^{-1}	Rumen microorganisms	6–7		214 mL CH_4 g^{-1} COD In	[39]
13	*Chlamydomonas reinhardtii* strain CC-1690		2	Thermal pretreatment			464 mL_N[a] CH_4 g^{-1} VS day^{-1}	[40]
14	Egeria densa	Semi-continuous		–			231 mL CH_4 g^{-1} VS	[41]
15	Municipal solid waste	Batch		–		70% CH_4	0.560 m^3 biogas kg^{-1} VS	[42]
16	Chicken manure	Batch	5.3 kg VS/m^3/d	–	7.7–8.4		0.18 m^3 CH_4 kg^{-1} VSFed	[43]
17	Chicken manure	Batch	1.8 kg VS/m^3/d	–	7.7–8.4		0.35 m^3 CH_4 kg^{-1} VSFed	[43]
18	Activated sludge	Batch		Alkali	9.5		282 mL CH_4 g^{-1} VSS	[44]
19	Corn Straw	Batch		Mechanochemical	7.0		239 mL CH_4 g^{-1}-TS	[45]
20	Paper wastes	Batch		–	7.5		243–316 mL CH_4 g^{-1}-VS	[46]
21	Food wastes	Batch		Microwave 2.45 GHz 1000 W	8.0		783.15 mL CH_4 g^{-1}-VS	[47]
22	Lipid wastes	Batch		Ultrasound 20 kHz 500 W	8.0		927.97 mL CH_4 g^{-1}-VS	[47]

[a]N refers to normal or normalized volume of a gas which is the gas volume at 0 ° C and 760 millimeters pressure

6.3 Microbial Communities in AD

6.3.1 Microbial Communities Involved in the Four Stages of AD

The AD process involves a series of biochemical reactions catalyzed by microbial communities and is grouped into four stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis as shown in Fig. 6.2 [48]. Hydrolysis is the first stage in which the high molecular weight complex organic polymers such as starch, cellulose, lipids, etc. are hydrolyzed into smaller chains/molecules. The breakdown of complex substrate is catalyzed by hydrolases (amylases, proteases, and lipases) produced by hydrolytic bacteria such as *Bifidobacterium* and *Bacteroides* [49]. Following hydrolysis, acidogenesis takes place, wherein anaerobes of the genera *Bacillus*, *Pseudomonas*, *Micrococcus*, *Clostridium*, *Flavobacterium,* and *Proteobacteria* like *Enterobacteriaceae* break down the simpler molecules derived from hydrolysis into short-chain organic acids (formic, acetic, butyric acids, etc.), alcohols (methanol and ethanol), hydrogen and carbon dioxide [50, 51]. In one study, it was reported that *Proteobacteria* make up approximately 53.2% of the total microbial community present in an up-flow anaerobic sludge blanket reactor, making them a crucial phylogenetic group in this process owing to their involvement as glucose, butyrate, propionate, and acetate-consuming microbes [52, 53]. The organic compounds produced during acidogenesis can serve as both electron donors (dehydrogenation) and acceptors (hydrogenation). The accumulation of electrons in the form of organic acids is a bacterial response to the increasing hydrogen concentration in the solution, which may not always be directly used by methanogenic bacteria for biogas production, thus necessitating an intermediate step called acetogenesis. Both hydrolysis and acidogenesis are carried out in acidic pH within the range of 5.2–6.3 [54]. During acetogenesis, bacteria of the genera *Syntrophomonas*, *Syntrophobacter*, *Methanobacterium*, etc. metabolize the organic acids to produce acetic acid along with ammonium, hydrogen gas, carbon dioxide. Acetogenesis determines the efficiency of the AD process because approximately 70% of total methane produced in the AD process is derived from the acetate produced during acetogenesis. In addition, this step accounts for approximately 25% of the total acetate as well as 11% of the hydrogen gas formed during AD [55]. The final stage of AD is methanogenesis and is assisted by the activities of both acetotrophic (*Methanosaeta*) and hydrogenotrophic methanogens (*Methanosarcina*). In methanogenesis, the acetotrophic methanogenic bacteria decompose acetate produced during acetogenesis to methane [56], while the hydrogenotrophic methanogens convert the hydrogen and carbon dioxide gas into methane [57]. Generally, filamentous *Methanosaeta* dominates the microbial population at low concentrations of acetate. But higher concentrations of toxic byproducts of digestions, like volatile fatty acids, hydrogen sulphide, etc. inhibit *Methanosaeta* and allow the growth of hydrogenotrophic methanogens like *Methanosarcina*.

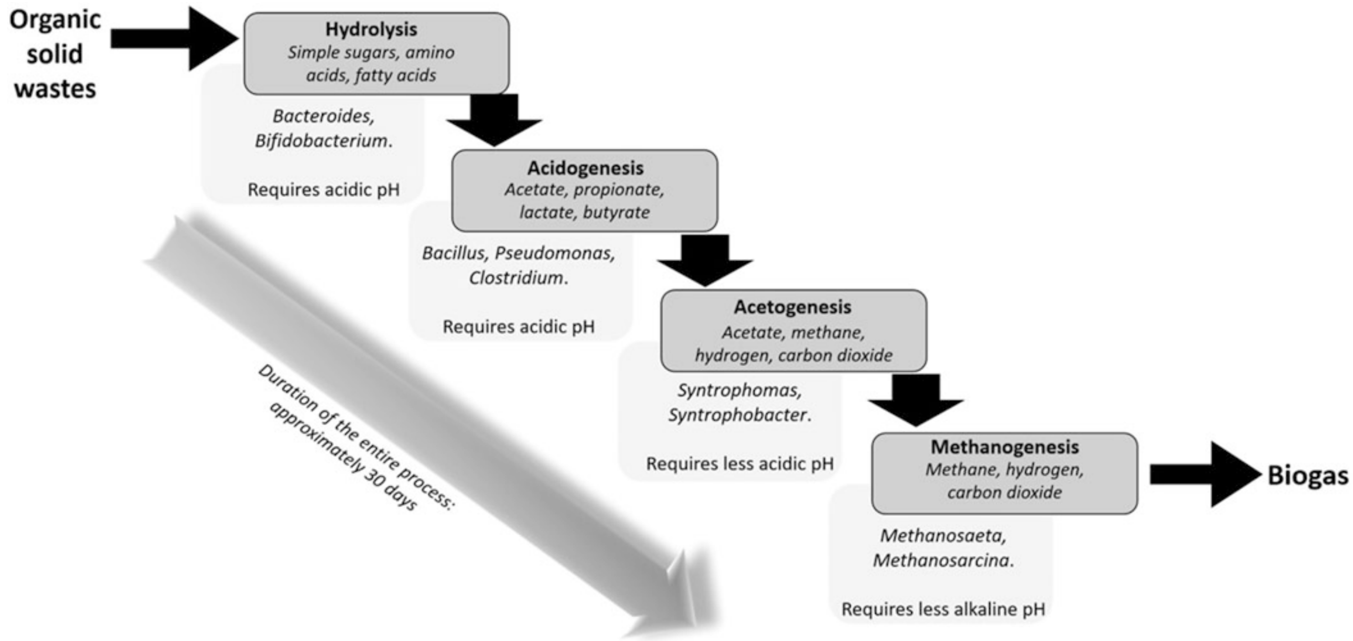

Fig. 6.2 Stages in anaerobic digestion. The organic wastes are digested via microbial action in four stages, i.e., hydrolysis, acidogenesis, acetogenesis, and methanogenesis to produce biogases. The entire process can take up to 30 days to complete.

6.3.2 Synthetic Biology and Genetic Engineering in AD

The efficiency of biogas production, particularly methane, from organic wastes depends on the composition of the microbial consortium used, as well as the behavior or action of the consortium. As the optimal conditions (e.g., temperature, pH, etc.) of each stage are different, engineering the microbial consortium of each stage can help scientists design and optimize the process to improve the biogas production [58]. Till date, wild-type strains of anaerobic microbes are widely used for facilitating the process of AD, however, the advent of genetic and metabolic engineering can assist in improving the performances of these strains. Previously the only option for genetic engineering was to create changes in the DNA sequences, but the development of synthetic biology and metabolic engineering can provide means to radically manipulate bacterial and fungal genes to change their characteristics in order to produce enzymes that can improve the AD of wastes [59]. In recent years, toolsets documenting the different bacterial consortia present in anaerobic digestion cultures, their genomic information, and their physiology have become available, which act as valuable resource for conducting further research. So far, genomic sequences of 21 *Archaebacteria* and 205 *Eubacteria* have been sequenced, out of which approximately 80% of the *Archaebacteria* comprises of methanogenic bacteria typically found in sludge or other anaerobic environments [59]. The availability of the genomic and physiological properties can allow the discovery of non-cultivable bacteria in the consortia [60] as well as enable genetic engineering of either the hosts or particular enzyme activities for the enhancement of biogas production from AD [61]. Other tools such as q-PCR, RT-PCR, Sanger sequencing, T-RFLP, next-generation sequencing (NGS) etc., can benefit the researchers to make libraries and identifying markers and genes from microbial consortia involved in AD to assist in targeted redesigning of the entire metabolic pathway using synthetic biology. Additionally, since most methanogenic enzymes function optimally at high K^+ ion concentrations, genes from other organisms encoding K^+ transporters and channels can be cloned into the microbes present in AD to increase their electrochemical activity, thereby increasing the efficiency of AD process to produce a higher amount of biogas [62, 63].

Also, for easier analysis of microbial genome and their characteristics, analyzing the 16S rRNA gene in the microbial community of AD has been proposed [58]. In order to assess the microbial community in each stage of AD, the 16S rRNA gene can act as a marker to help scientists specify the identity of organisms in the anaerobic digester [64]. Rivière et al. [65], analyzed the microbial community present in seven anaerobic digesters by creating a total of 9890 16S rRNA clones. The analysis revealed that the Archaea community is represented by the following operational taxonomic units (OTUs): *Methanosarcinale*, *Methanomicrobiales*, and Arc I. Further phylogenetic affiliation and statistical analysis of the library revealed that the bacterial community present in the anaerobic digesters can be grouped under three categories: (1) a core group of phylotypes, which is common to most digesters; (2) another group of phylotypes shared among a few digesters; and (3) a third group phylotype specific to each digester [65]. Finally, it is imperative that for improving biogas yield through synthetic biology techniques, key points such as developing efficient genome-editing tools, mapping and cloning of key genes from important phylotypes associated with biogas production, creating metagenomics-based data mining method, as well as further experiments from lab and pilot-scale to full-scale application needs to be conducted for furthering AD research.

6.3.3 Insights into Microbial Community Dynamics in AD

To further understand and investigate the factors and mechanisms governing AD process, studies on the microbial community dynamics are indispensable. They can also be useful in investigating the transformation of compounds during the whole AD process. Analyzing microbial community dynamics can also provide an idea on the interactions and relative abundance of the microbes under different conditions and thereby, help us in creating an appropriate and robust microbial consortium for efficient substrate degradation and biogas production. For example, reactor performance, as well as microbial community dynamics studies on solid-state AD of corn stover conducted at mesophilic and thermophilic conditions revealed that thermophilic AD resulted in faster reduction of cellulose and hemicellulose in the first 12-days, compared to mesophilic conditions. It was found that there was a shift in population of microbes over the 38 days of culture, compared to the initial inoculum. When mesophilic cultures were used as inoculum for thermophilic conditions, it was observed that the populations of thermophilic cellulolytic and xylanolytic microbes were about 10–50 times greater than those in mesophilic ones [6]. The same group investigated the effect of inoculation ratio on microbial community dynamics in solid-state AD and highlighted that non-microbial factor of the inoculum, such as alkalinity, were found to be more decisive on the final methane yield of corn stover. Instead, the microbial population of methanogens affected the kinetics of volatile fatty acids (VFA) consumption and methane production [5, 66]. Determination of microbiome composition and their temporal succession in thermophilic and mesophilic solid-state AD, as well as acidified solid-state AD reactors using Illumina sequencing of 16S rRNA gene amplicons showed that the genus *Methanothermobacter* dominated in the thermophilic solid-state AD reactors, while *Methanoculleus* dominated in the mesophilic reactors [5, 67, 68]. Also, acetate oxidation coupled with hydrogenotrophic methanogenesis was found as an important pathway for biogas production during thermophilic solid-state AD, and the abundance of *Methanomassiliicoccus* was positively correlated to daily biogas yield in the mesophilic solid-state AD process [67]. Additionally, studies were also conducted to study the effect of inhibitors on the microbial community present in solid-state AD. It was found that increasing acetate concentration impacted the population dynamics of dominant hydrogenotrophic methanogenic microbial species including *Methanobacterium*, *Methanosarcina*, and *Methanocorpusculum*

[69, 70]. It was also discovered that increasing OLR impacted acetotrophic methanogens more than hydrogenotrophic methanogens. This imbalance between the two phylotypes (and the associated metabolic pathways) could lower methane production.

6.3.4 Modeling of AD Systems to Study Molecular Mechanisms

To gain insights into the molecular mechanisms of the reactions involved in the AD process in the reactors, modeling has been used as an effective approach, which can also help in facilitating process design as well as predicting system performance [71]. Theoretical models developed for solid-state AD are diverse and utilize different parameters like reactor designs, reaction kinetics, and mass transfer along with the rate-limiting steps to provide better insights into the complex system mechanisms. However, many of these models cannot be applied for robust simulation of varying process conditions and input substrates as they are structurally and numerical complex [72]. In parallel, liquid-state AD models have also been developed and the most popular among them is the Anaerobic Digestion Model No.1 (ADM1) [73]. In the development of ADM1 researchers have utilized both biochemical reactions and physico-chemical reactions that takes place within an AD process. More importantly the reactor design in ADM1 is based on the assumption that the digester is a completely stirred tank reactor with a constant liquid volume and a single input and output stream [73]. Using this most comprehensive liquid-state AD model, the ADM1 as a template, several kinetic models have been further developed that simulate the process of disintegration, acetogenesis and methanogenesis steps of various complex organic substrates. [74–76]. Most of the recent models focused on the effect of total solid content on methane yield and production rate. These models assume that the total solid content is a key parameter that affects the mass transfer of VFAs, H_2, CO_2, etc., between the gas-liquid-solid phases. It was also assumed that the mass transfer effect in turn affected the hydrolysis rate constant, the rate of accumulation of inhibitors [74, 76], maximum microbial growth rate or half-saturation coefficient [74], and the maximum microbial growth rate [77].

Additionally, linear regression models have also been created that calculate how total solid content affects methane production in solid-state AD using artificial neural network [69, 78]. Kinetic models, on the other hand, have also been developed empirically and these models mainly captured the heterogeneous distribution of inoculum in the substrate, which in turn caused heterogeneous accumulation of VFA in the reactor. Kinetic model simulations suggest that vigorous mixing, highly dispersed inoculum, and leachate recirculation can affect methane production during solid-state AD, as these conditions result in the acidification of the inoculated organic particles by the VFAs [79]. As the experimental designs for the AD process are becoming more advanced, so are the amount of data being generated as well and utilizing these data for the development of mathematical models will play a major role in revealing further details about the molecular mechanisms in AD process.

6.4 Process Variables that Influence AD

AD, like any other process, can operate under various operational conditions and therefore, factors affecting the efficiency of the AD process can be optimized based on the type of waste used. These factors are as follows:

6.4.1 Temperature

Temperature is one of the most important parameters in AD as microbial metabolism and enzyme kinetics vary with temperature. Therefore, the optimal temperature to be employed to obtain higher biogas production is based on the type of organism employed in the AD process. This is due to the fact that psychrophilic ($T < 20$), mesophilic ($35 < T < 40$), and thermophilic ($50 < T < 65$) organisms prefer to grow better at their optimal temperature [59]. Comparing the preferred temperature of various organism types shows that higher energy is required for thermophilic organisms, compared to mesophilic bacteria while using thermophilic bacteria provides a higher volume of biogas generation and guarantees a faster production rate. On the other hand, in processing waste streams that generate ammonium, mesophilic digestion is more stable compared to thermophilic digestion owing to ammonium toxicity [14]. Therefore, for the wastes containing a high amount of nitrogen, using mesophilic digestion would make the process more efficient.

6.4.2 pH

pH is another critical parameter that can regulate the activity of organisms. For example, methanogenic and acidogenic bacteria prefer different pH for their growth. Various researches have reported different optimum pH for the biogas production in the AD process in which generally, the optimal range for pH is reported to be between 6 and 8 [59]. However, different stages of AD require different values of pH as hydrolysis and acidogenesis bacteria prefer the pH of 5.2–6.3, whereas the methanogenic bacteria desire 6.8–7.5 [80]. Therefore, choosing the AD process pH based upon the AD process stage in operation would make the process more efficient.

6.4.3 Hydraulic Retention Time (HRT)

Hydraulic retention time, also hydraulic residence time (HRT) is the average time that liquids remain in the anaerobic digester [81]. It is an important operational parameter in AD as its duration depends on the type of feedstock and can affect the conversion of volatile solids into biogas. Each AD requires a minimum HRT period for completion. A low HRT can result in the accumulation of volatile fatty acids, while a high HRT increases the process operation cost due to longer run-time. Hence, researchers often attempt to lower the HRT to a certain period, where the biogas production is optimum and the volatile fatty acids production is lowered [82, 83]. Another form of retention time is called solid retention time (SRT) which is the average time that microbes are in the digester. SRT and HRT will be the same when the microbial culture and waste are present in the same phase which happens while the waste is in the liquid form; however, when solid wastes are used, HRT and SRT will have a different value. Obviously, the HRT changes with the nature of the feedstock. The average reported HRT is 15–30 days for treating solid organic wastes under mesophilic condition for the biogas production in AD [59]. Recalcitrant wastes containing a high content of fiber or fat require a high HRT, while other easily digestible wastes such as animal manure need a lower HRT. Besides, digestion by mesophilic organisms needs a longer HRT as they are efficient at lower temperatures, whereas thermophilic digestion can be accomplished at a higher rate leading to a shorter HRT. It should also be noted that the size of waste particles can also influence HRT. Due to their high surface area, smaller waste particles lower the HRT and therefore faster digestion will happen.

6.4.4 Organic Loading Rate (OLR)

The amount of SOW necessary to be fed to the anaerobic digester per day per unit working volume is called the organic loading rate [84]. As all the process variables affecting the efficiency of the AD process are interconnected, various OLR have been reported based upon the operating condition. Therefore, the temperature, pH, feedstock characteristics, and hydraulic retention time can influence the organic loading rate. A high OLR means a higher workload on the anaerobic bacteria to convert wastes into biogas, which would result in the availability of a high amount of VFAs in the anaerobic digester that leads to bacteria inhibition, whereas a low OLR may reduce the nutrient availability and therefore, disrupt the performance of the microbial community [85].

6.4.5 C/N ratio

One other factor that affects the AD operation is the C/N ratio. Bacteria need the right supply of carbon and nitrogen for their optimal growth and metabolism and therefore, the C/N ratio of the feedstock is critical. The C/N ratio from 20:1 to 30:1 has been reported as optimal for AD [86]. A high C/N ratio results in a less efficient AD as nitrogen is a vital element for microbial protein synthesis. On the other hand, a low C/N ratio leads to build-up of ammonia and therefore, causes ammonia toxicity [87]. It is noteworthy to mention that the C/N ratio is feedstock specific and it cannot be changed unless wastes with different C/N values are mixed as feed to obtain an optimal C/N ratio. Providing a feed with an optimal C/N ratio for the microbes will maximize biogas production.

6.4.6 Feedstock-to-Inoculum (F/I) Ratio

Feedstock-to-inoculum ratio (F/I) is another important factor to be considered in the AD of solid organic wastes and it can affect the pH as well as inhibitor production. A very high F/I could result in the overproduction of VFAs due to excess organic loads that can significantly lower the pH and inhibit the action of the methanogens [88]. It was found that AD of palm oil mill residues achieved the highest methane production rates at the lowest F/I ratio within the range of 2:1–5:1, while rapid hydrolysis at F/I ratio of 4:1–5:1 resulted in a VFAs accumulation and low methane yield [89].

6.5 Pretreatment Techniques

Various techniques have been suggested to improve the biogas production of solid wastes such as the addition of additives, co-digestion and using pretreatment [59]. Using pretreatment techniques is helpful specifically for the wastes containing a high percentage of lignocellulosic materials to increase the rate of hydrolysis and thereby, achieve high biogas yield through maximum digestion of solid wastes. Pretreatment of agricultural waste is generally divided into chemical, biological, physical, and thermal or their combination. It is noteworthy to mention that a pretreatment technique must not only be economical and environmental-friendly but also should not repress or have a negative effect on the biomass or process [90, 91]. Also, the pretreatment technique required for each waste type might be different and factors such as the availability of lignocellulosic materials, crystallinity, the surface area of the particles, availability of acetyl groups, and the degree of polymerization should be considered [8].

6.5.1 Physical Pretreatment

Organic wastes come in different particle sizes, and knowing the fact that smaller particle size gains a higher surface area, physical pretreatment can be the first solution to enhance the efficiency of any type of organic solid wastes. In physical pretreatment, neither microorganisms nor chemicals are involved. Examples of physical pretreatments are high-pressure homogenizer, electrohydrolysis, microwave, milling, crushing, steam explosion, and ultrasound. Milling not only provides a higher surface area of particles but also decreases the degree of crystallinity and polymerization. Other physical pretreatments such as high-pressure homogenization make an abrupt expansion to rupture the lignocellulosic biomass structure and therefore increase the AD performance. Steam explosion of wheat straw increased the methane yield by 30% [92]. Microwave pretreatment can be applied to the substances that contain water inside their cell in which the sudden increase in water volume, the cell will be destroyed, yielding a higher AD efficiency [93].

In order to reduce the particle size, proper equipment should be used regarding the substrate type and the type of anaerobic digester to be used to not damage the equipment and causing process failure. It should also be noted that the size of the particle has to be within an optimum range as smaller particles might cause media acidification in dry digestion as the result of acid production during fermentation, while they might lead to the formation of foams in the wet digestion [33].

6.5.2 Chemical Pretreatment

Chemical pretreatment includes using acids, alkalis, ionic liquids, oxidants, etc., to enhance the hydrolysis rate. The selection of suitable chemical pretreatment depends on the type of substrate and its characteristics. Generally, the use of chemical pretreatment has received more attention compared to physical pretreatment due to its higher effectiveness on biogas production. It has been suggested not to use acid pretreatments for readily degradable materials as it might cause the accumulation of VFA, along with the degradation of soluble sugars to inhibitory compounds like furfural [94]. However, this type of pretreatment (acid) is mostly used for lignocellulosic substrates as the strong acid disrupts lignin and thereby, releases the cellulose and hemicellulose rendering them more susceptible to enzymatic hydrolysis [95]. On the other hand, dilute acid pretreatment is better to be applied on food wastes, along with thermal pretreatment [96].

As mentioned, the generation of toxic or inhibitory chemicals in the chemical pretreatment is likely to happen, and therefore, actions such as neutralizing the pH of the biomass are recommended. Due to this fact, chemical pretreatment cost is mostly higher than that of physical pretreatments, and therefore economical assessment of chemical pretreatment in the industrial scale should be investigated for the process design. Currently, alkali hydrolysis is majorly used on solid organic wastes with low lignin content in the industrial scale [97].

6.5.3 Biological Pretreatment

Biological pretreatment is being done by using biological agents such as enzymes that can improve the degradation of biomass by breaking the covalent cross-linkages and non-covalent bonds between hemicellulose and lignin without the generation of any inhibitory chemicals [98], therefore, it can be very useful for AD of agricultural and yard wastes.

Biological pretreatment includes enzymatic, bacterial, and fungal pretreatment. The merits of using biological pretreatment are its low operation cost, less energy requirement for operation, and environmental-friendly. However, the need for a long process time is the main disadvantage of using biological pretreatment, which precludes its application at industrial scale. Also, some bacteria have the ability to degrade cellulose along with hemicellulose, resulting in the reduction of final biogas production [99].

***Enzymatic*:** Laccase and versatile peroxidase are examples of enzymes used for enzymatic pretreatment of lignocellulosic biomass as they can degrade lignin [100]. Schroyen et al. [101], have investigated the effect of various enzymatic pretreatment on corn stover and found 25% and 17% increase in biomethane production after 24 h and 6 h incubation using laccase and peroxidase enzyme, respectively. In another study, enzymatic pretreatment of sugar beet pulp and spent hops yielded 19% and 13% increase in biogas production, respectively, compared to control [102].

***Bacterial*:** Studies on the microbial pretreatment of SOWs have also shown a positive effect on biogas production. In the study of Zhang et al. [103], a microbial consortium pretreatment was applied on cassava residues and 97% increase in methane production from 131.95 mL/g-VS to 259.46 mL/g-VS was observed. Findings of another research study also showed 35% decrease in the digestion time of corn straw AD using a complex microbial agent pretreatment compared to untreated feed [104]. In their study, pretreatment with microbial agents yielded 33% and 76% increase in total biogas and biomethane production, respectively.

***Fungal*:** Shi et al. [105] performed fungal pretreatment of cotton stalks by using *Phanerochaete chrysosporium* and observed 19–36% of lignin being degraded under various pretreatment conditions compared to the control (untreated). Another study by Ge et al. [106] showed 24% lignin degradation using fungal pretreatment on *Albizia* biomass that improved the methane yield by 3.7-fold. A study on biological pretreatment by the fungus *Ceriporiopsis subvermispora* [107] that produces ligninolytic enzymes [108] showed 106% increase in methane production from 21.6 L/kg volatile solid (control) to 44.6 L/kg volatile solid after pretreatment. In general, studies on fungal pretreatments showed that lignin degradation of biomass often improves the methane production.

6.5.4 Thermal Pretreatment

Another type of pretreatment is thermal pretreatment that is applicable to all types of solid organic wastes in a large scale. Thermal pretreatment can improve the solubility of chemical oxygen demand (COD), increase the process efficiency, and reduce the hydraulic retention time. It can also be used for dewatering and improving the digestibility of some type of organic wastes [109]. The two types of thermal pretreatment are (1) thermal, in which only the temperature is controlled, and (2) hydrothermal in which both pressure and temperature are controlled. Hydrothermal pretreatment is a specialized thermal pretreatment process, in which the biomass to be digested is completely submerged in liquid water at both high temperature and pressure. Hence, hydrothermal pretreatment is generally considered suitable for treating wastes already containing high water content.

6.5.5 Combined Pretreatment Techniques

Each pretreatment method described has its own merits and demerits. Although some pretreatment methods have been suggested to be used for some type of substrates, no general suggestion can be made as each substrate type contains a large variety of wastes. Researches have shown that combining two or three pretreatment methods will also further improve biogas/methane production. Table 6.3 presents a literature review on the different pretreatment methods applied to various substrates and their effect on improving biogas production. Figure 6.3 summarizes the various AD process parameters and parameters that need to be monitored during the AD of SOWs, as well as the pretreatment methods that have been employed to improve the product yield.

6.6 Process Operation Types

AD can be carried out in full scale using the following four different types of process operations, depending on the raw material input method as well as number of stages involved: (a) batch, (b) continuous operations, (c) single-stage and (d) multistage operations. Each of the operation types has its pros and cons, and is discussed below in detail.

Table 6.3 Various pretreatment methods used for AD

Pretreatment type	Pretreatment method	Feed	Pretreatment conditions	Methane/ biogas yield increase	Ref.
Physical	Microwave	Organic fraction of municipal solid waste (OFMSW)	145 °C and 8 days of digestion	26% methane increase	[93]
	Steam explosion	Corn stover	160 °C for 2 min	22% methane yield increase	[110]
	High-pressure homogenizer	Municipal solid waste	40 MPa pressure	33% methane increase	[36]
	Ultrasound	Organic fraction of municipal solid waste (OFMSW)	Sonication time 30 mins; Specific energy 7200 kJ/kg TS Power density 0.6 W/mL	15% increase in biogas	[35]
	Electroporation	Organic fraction of municipal solid waste (OFMSW)	field strength: 24 kV/cm frequency 12.5 Hz	20%–40% biogas increase	[111]
	Pulsed electric field	Landfill leachate	50 kW h/m^3 Frequency 1.7 Hz Electric field strength 20 (kV/cm)	44% methane increase	[34]
Chemical	Acid	Rice straw	160 °C for 10 min	161% to 533%	[24]
	Alkali	Wheat straw	5 min with 5% w/w H_2O_2 solid:liquid ratio of 1:20	64% methane increase	[25]
	Alkali	Sugarcane bagasse	5 min with 5% w/w H_2O_2 solid:liquid ratio of 1:20	68% methane increase	[25]
	Alkali	Napier grass (*Pennisetum purpureum*)	1, 2, and 3% sodium hydroxide (NaOH)	9.3% increase in biogas yield	[22]
Biological	Enzymatic	Corn stover	combination of laccase and versatile peroxidase 30 °C for 6 h	50.4% increase in methane production	[100]
	Fungal	Corn Stover Silage	Using *Phanerochaete chrysosporium* at 28 °C for 30 days	23% in methane production	[10]

(continued)

Table 6.3 (continued)

Pretreatment type	Pretreatment method	Feed	Pretreatment conditions	Methane/ biogas yield increase	Ref.
	Microbial	Corn straw	Combination of yeast, cellulolytic bacteria, and the lactic acid bacteria 20–55 °C for 12 h to 20 days	33.07% increase in biogas yield 75.57% increase in methane yield	[104]
Thermal	Thermal autoclaving	Wheat straw	121 °C for 60 min	62% methane increase	[25]
	Thermal autoclaving	Sugarcane bagasse	121 °C for 60 min	58% methane increase	[25]

6.6.1 Batch Operation

Batch operation is one of the most commonly used modes of operation for AD of organic solid wastes. The batch operation is easier to maintain compared to continuous operation as it requires less capital investment and lower operating costs with fewer process control requirements. However, the amount of biogas produced through batch operation would fluctuate with time and a major portion of the biogas would be produced during the peak performance of the AD process. For example, it was reported that in a 55-day batch solid-state AD of corn stover, more than 80% of biogas was produced on day 36, when the AD was at the methanogenic phase [112]. Moreover, the batch operation also requires a large amount of inoculum (i.e., low F/I ratio); a high F/I ratio is known to produce volatile fatty acids in larger amounts compared to biogas [113].

6.6.2 Continuous Operation

Continuous operation is another popular method of operating AD, with a continuous supply of raw materials and resulting in biogas production at a steady state. Continuous operation is primarily affected by OLR, and SRT, and these are the key parameters in designing and evaluating a continuous AD [114]. Contrary to a batch operation, in continuous operations, the solid-to-gas conversion capacity is proportional to OLR. In general, high OLR is preferred as it can achieve a high waste consumption rate in a relatively smaller digester. On the other hand, high OLR can lead to VFA overproduction that can result in an imbalance between acidogens and

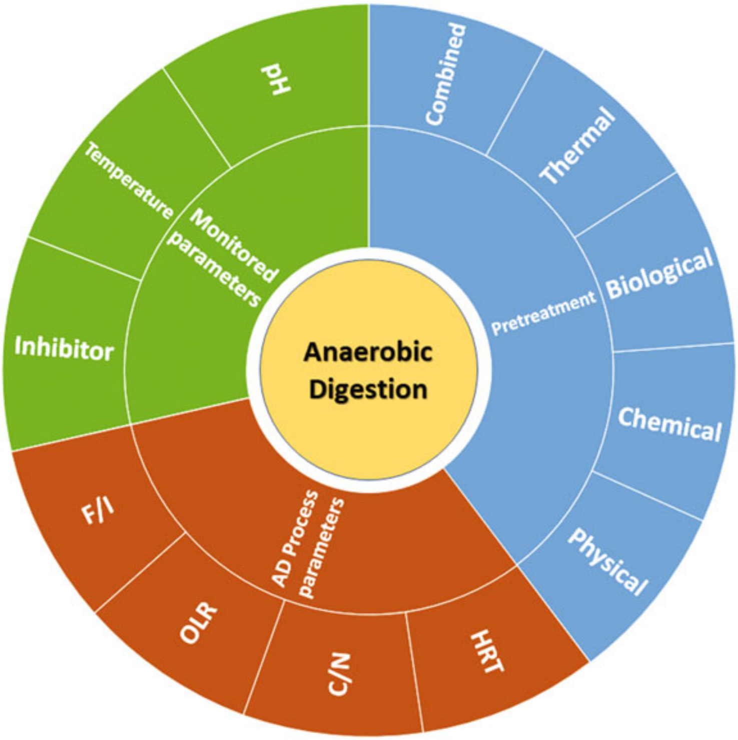

Fig. 6.3 Summary of the various process parameters, parameters to be monitored, and pretreatment techniques. In orange are the AD process parameters: Feed/inoculum ratio (F/I); organic loading rate (OLR), carbon/nitrogen ratio (C/N) in the feed, and hydraulic retention time (HRT). In brown are the parameters needed to be monitored during the AD of SOWs (pH, temperature, and inhibitors). In green are the different pretreatment methods used to improve AD yield

methanogens. A maximum OLR level in solid-state AD depends on various parameters such as reactor design, feedstock characteristics, microbial activity, temperature, pH, and toxicity level [115]. SRT is the second critical factor in continuous operation. In a continuous AD operation with food waste as feedstock, increasing SRT from 15 days to 35 days increased methane yield from 360 mL/kg to 454 mL/kg volatile solids [116].

6.6.3 Single-Stage Operation

In addition to depending on the mode of raw-matter feed, another mode of operation focuses on the stages of operation. In a single-stage AD system, all the four stages of digestion are implemented in a single reactor vessel (Fig. 6.4). Thus, the reactor system is easier to design and can be built with less capital costs. However, a major

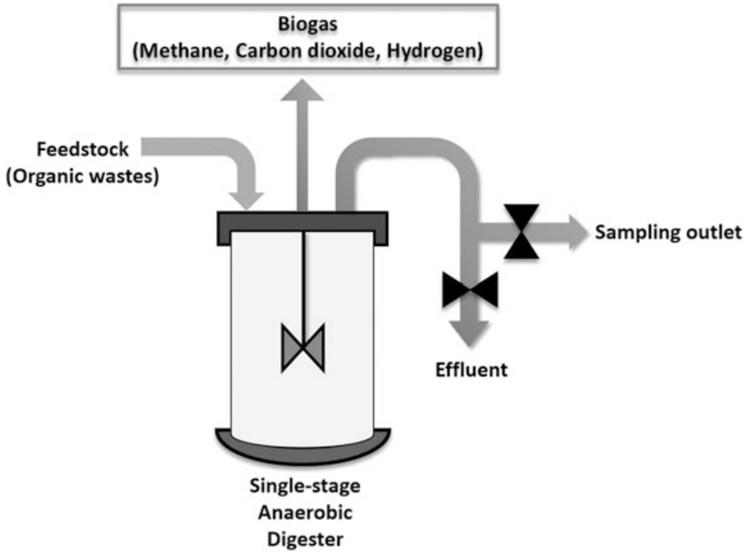

Fig. 6.4 Schematic representation of a single-stage AD operating system. In a single-stage AD all the four stages of decomposition occurs in a single reactor to convert solid wastes into biogas

limitation is the OLR, because excessive OLR can cause rapid pH drop, thus limiting the rate of digestion and overproducing VFAs [4].

6.6.4 Multi-stage Operation

A multiple-stage operation is another type of AD operation method in which the different conversion stages are carried out in multiple reactor vessels. Generally, the first two stages, i.e., hydrolysis and acidogenesis are carried out in one reactor while acetogenesis and methanogenesis are carried out in a separate reactor (Fig. 6.5) [117]. Thus, all the stages can be operated at their optimal process conditions (pH, temperature, OLR, etc.).

It has been suggested that multistage operation perform better than single-stage operations because the former results in a proper fermentation of the loaded wastes with limited generation of inhibitors or by-products [2]. For example, the solid-state AD of brewery spent grain (BSG) in a single-stage reactor was limited by the inhibitors, such as weak acids, furan derivatives, and phenolic substances, generated in the degradation of lignocellulose in BSG [118]. However, it is noteworthy to mention that although multistage AD system has the advantage of improved AD performance, the need for high capital investments and operating costs hampers its implementation at a commercial scale. As a result, single-stage AD is still predominantly used.

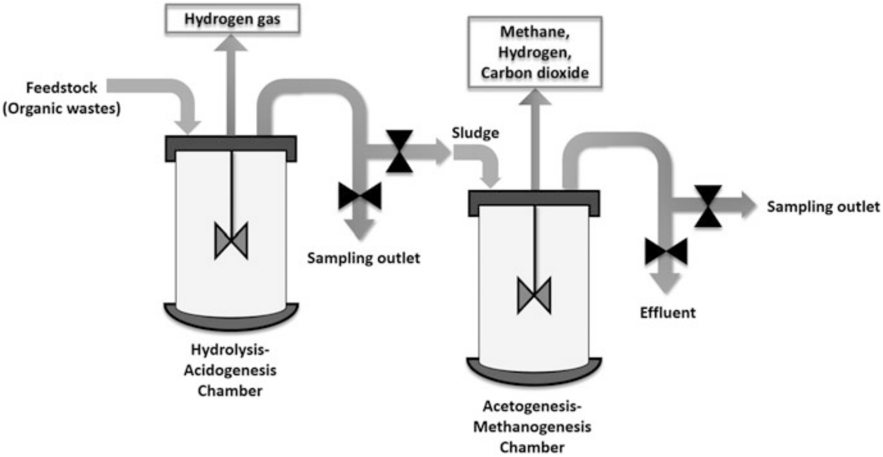

Fig. 6.5 Schematic representation of a multi-stage AD operating system. The reactions occur in separate chambers for conversion of solid wastes to biogas

6.7 Economic Benefits of Biogas Production in AD

As energy production is the main aim for AD operation, a cost-benefit analysis needs to be considered. As stated earlier, apart from biogas production, AD can provide various other benefits such as heat or electricity generation as well as compost and high-quality fertilizer. AD systems can be used in small-scale (approximately 50–500 ft^3) for heat production in rural areas or large-scale up to 300,000 ft^3 [119, 120].

Most of the total cost of AD systems is spent on capital costs. Items such as digester, piping system, liquid and gas pumps, electrical controllers and wiring, power transmission lines, mixing tanks, the land where the whole system is located, etc. are considered as the main contributor of AD. However, the type of feedstock and its shipping costs are other factors that impact the generation costs. Therefore, the use of centralized systems is prevalent in Europe in which co-digestion of animal manure with other agricultural, yard or food wastes of several farms, provides energy and fertilizer for the farmers.

As biogas is composed of methane and CO_2, its heating value (600 btu/ft^3) is less than that of natural gas (1000 btu/ft^3) [121]. Hence, upgrading the biogas to biomethane by removing CO_2 should be evaluated economically, based on the aim and location where the system is located. Biomethane has a similar characteristics as natural gas; thus, it can be used as compressed natural gas (CNG) as transportation fuel or to be transferred to other places.

Overall, a feasibility study is required to determine the payback period of investments for AD or investigating based on the feedstock availability and type, project site, community impact and vicinity, shipping, system size and total energy production estimations, environmental considerations, equipment, and worker costs, etc. based on the location or country where the AD is to be done.

6.8 Conclusions and Future Outlook

Generation of biogas through the AD of organic wastes can not only solve the problems of waste disposal, but also helps energy recovery. The generated biogas can be utilized across different sectors for the production of heat and electricity or be upgraded into biofuels. However, several problems associated with the production of biogas from organic wastes using AD needs to be effectively addressed to implement this method in a largescale globally. One of the primary gases released during the AD process is methane. Methane is a greenhouse gas and therefore, proper design and operation of the AD are required to avoid the release of methane into the atmosphere. Additionally, the process requires a microbial consortium to operate under a given set of operating conditions, lack of which can result in damaging the stability of the system causing inefficient gas production. Moreover, natural gas is readily available, whereas biogas requires the operation of a long lengthy AD process, making the AD-generated biogas costly in comparison to natural gas. In addition to researching the parameters such as temperature, pH, OLR, etc. engineering the microbial consortia could help in maximizing the methane content, which would help inspire future AD developments.

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