



# Anaerobic digestion of fruit and vegetable waste: a critical review of associated challenges

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## Abstract

The depletion of fossil fuels coupled with stringent environmental laws has encouraged us to develop sustainable renewable energy. Due to its numerous benefits, anaerobic digestion (AD) has emerged as an environment-friendly technology. Biogas generated during AD is primarily a mixture of CH<sub>4</sub> (65–70%) and CO<sub>2</sub> (20–25%) and a potent energy source that can combat the energy crisis in today's world. Here, an attempt has been made to provide a broad understanding of AD and delineate the effect of various operational parameters influencing AD. The characteristics of fruit and vegetable waste (FVW) and its feasibility as a potent substrate for AD have been studied. This review also covers traditional challenges in managing FVW via AD, the implementation of various bioreactor systems to manage large amounts of organic waste and their operational boundaries, microbial consortia involved in each phase of digestion, and various strategies to increase biogas production.

**Keywords** Acidogenesis · Acetogenesis · Methanogenesis · Hydraulic retention time (HRT) · Biomethane potential (BMP) · Organic loading rate · Co-digestion · Biogas

## Introduction

Population explosion coupled with rapid industrialization and urbanization has amplified municipal solid waste (MSW) generation across the globe. MSW primarily comprises refuse, garbage, food waste, and garden waste. According to a report published by the World Bank in 2012, annual MSW generation was 1.3 billion tonnes, with a projected increase to 3 billion tonnes by 2025. Furthermore, by 2100, the production rate is predicted to reach 11 million tonnes per day (World Bank 2013). As much as 15% of fruit and 25% of vegetables are lost at the bottom of the supply chain (FAO 2014), contributing to approximately 1748 million tonnes of FVW annually across the globe (Edwiges et al. 2018). Indian markets hold more than 30% share in the production of vegetables and fruit (Ravi et al. 2018; Ji et al. 2017), contributing about 50% of all the putrescible organic waste. Currently, in major cities of India, organic waste is

largely dumped into low-lying areas in the city's outskirts. This contaminates groundwater through leachate percolation and adds greenhouse gases to the atmosphere, thus causing a hazard to the environment and human beings (El-Fadel et al. 2002; Cheng and HU 2010). Environmental legislation across the globe emphasizes reducing, reusing, and recycling municipal solid waste's inorganic residues such as glass, metal, paper, and plastics and recommends resource and material recovery from organic waste. Anaerobic digestion (AD) has emerged as an environment-friendly solution to treat this massive quantum of waste while generating biogas as a commercially important by-product. The generated biogas is rich in methane content (about 50–75% CH<sub>4</sub>) and CO<sub>2</sub> (25–50%), along with other gases like N<sub>2</sub> (0–5%), NH<sub>3</sub> (0–0.5%), H<sub>2</sub>S (0–0.05%), water vapor (1–5%), and H<sub>2</sub> (0–2%) (Chen et al. 2015; Surendra et al. 2014). Biogas can be utilized as biofuel as it has become imperative to look for an alternate source of gaseous fuel that can meet current and future energy requirements.

Bioenergy will provide more than half of the renewable energy required to meet the fixed goals in the upcoming years. Biofuels have a strong potential for long-term sustainability because they may be created from locally available renewable resources and have a positive carbon balance when compared to fossil fuels. FVW, an essential

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class of municipal solid waste (MSW) residue, is mainly generated during harvest, transport, storage, and marketing of FV and also from household garbage. FVW produced in India has not been widely used for energy production owing to lack of efficient segregation technique, inadequate technical acquaintance of processing procedures, and insufficient funding resulting in the build-up of immense amounts of trash (Joshi and Ahmed 2016). Conventionally, FVW is used for animal feeding, composting, landfilling, and incineration. However, each of the practices mentioned above has pros and cons. Animal feeding under controlled conditions can be the safest (Salemdeeb et al. 2017). It also renders the partial substitution of conventional feed with substantial environmental and health consequences. In developed countries, the percentage of FVW recycled as feed is recorded as high (35–45%). Nevertheless, this substrate holds strong potential to produce up to 4000 mm<sup>3</sup> of biogas annually and an energy potential of roughly 86,000 TJ per year, which will help in mitigating energy crisis as well as provide an eco-friendly solution to waste management (Vijay et al. 2015).

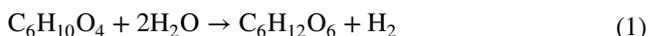
FVW is characterized by high moisture content, low total solids (TS), and high volatile solids (VS), making it a potent feedstock for AD, for AD is best suited for organic materials with high moisture content or semi-solid waste. AD is a series of complex metabolic reactions carried out by different microbial consortia that can survive in an anaerobic environment and form biogas and sludge. Digestate obtained as the by-product of AD holds strong potential to replace inorganic fertilizers owing to its rich N, P, and K content (Tambone et al. 2010; Peng and Pivato 2019). However, the amount of biogas generated substantially depends on various factors like the nature of substrate, moisture content, low TS, high VS, C/N ratio, nutrient availability, temperature, pH, and type of digester.

### Anaerobic digestion: a promising technology for FVW management

AD is a microbe-assisted biochemical process, which transforms complex organic waste into biogas and a highly concentrated sludge through the processes of hydrolysis, acidogenesis, acetogenesis, homeoacetogenesis, and methanogenesis (Gerardi 2003). Biogas is primarily a mixture of methane and carbon dioxide along with other gases like N<sub>2</sub> (0–5%), H<sub>2</sub> (0–2%), NH<sub>3</sub> (0–0.5%), H<sub>2</sub>S (0–0.05%), and water vapor (1–5%) (Chen et al. 2015). Digestate generated as the by-product of AD contains a high concentration of nutrients while being low in pollutants (Elango et al. 2007; Krugel et al. 1998; Kübler et al. 2000). Various biochemical routes of anaerobic digestion are illustrated in Fig. 1.

### Hydrolysis

During this phase, hydrolytic and fermentative bacteria break down complex polymeric matters such as polysaccharides, carbohydrates, and proteins into soluble monosaccharides, sugars, amino acids, purines, pyrimidines, glycerol, and long-chain fatty acids (LCFAs) by releasing extracellular enzymes (Barlaz et al. 1990). These hydrolyzed products pass through cell membranes and undergo further degradation. Cellulases, proteases, and lipases play a vital role in decomposition, released by a group of hydrolytic bacteria such as *Clostridia*, *Micrococci*, *Bacteroides*, *Butyrivibrio*, *Fusobacterium*, *Selenomonas*, and *Streptococcus*.

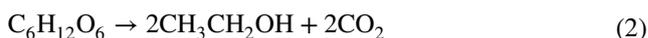


Generally, complex organic matter is represented by the chemical formula C<sub>6</sub>H<sub>10</sub>O<sub>4</sub>. Hydrolysis of complex organic matter is indicated by Eq. (1), in which complex substances are decomposed to glucose. Noike et al. (1985) studied the degradation of cellulose, soluble starch, and glucose. They reported that the specific rate of substrate utilization decreases from higher fatty acids to low-chain fatty acids. However, the rate of hydrolysis for cellulose was prolonged, and hence, this was the rate-limiting step in AD.

### Acidogenesis

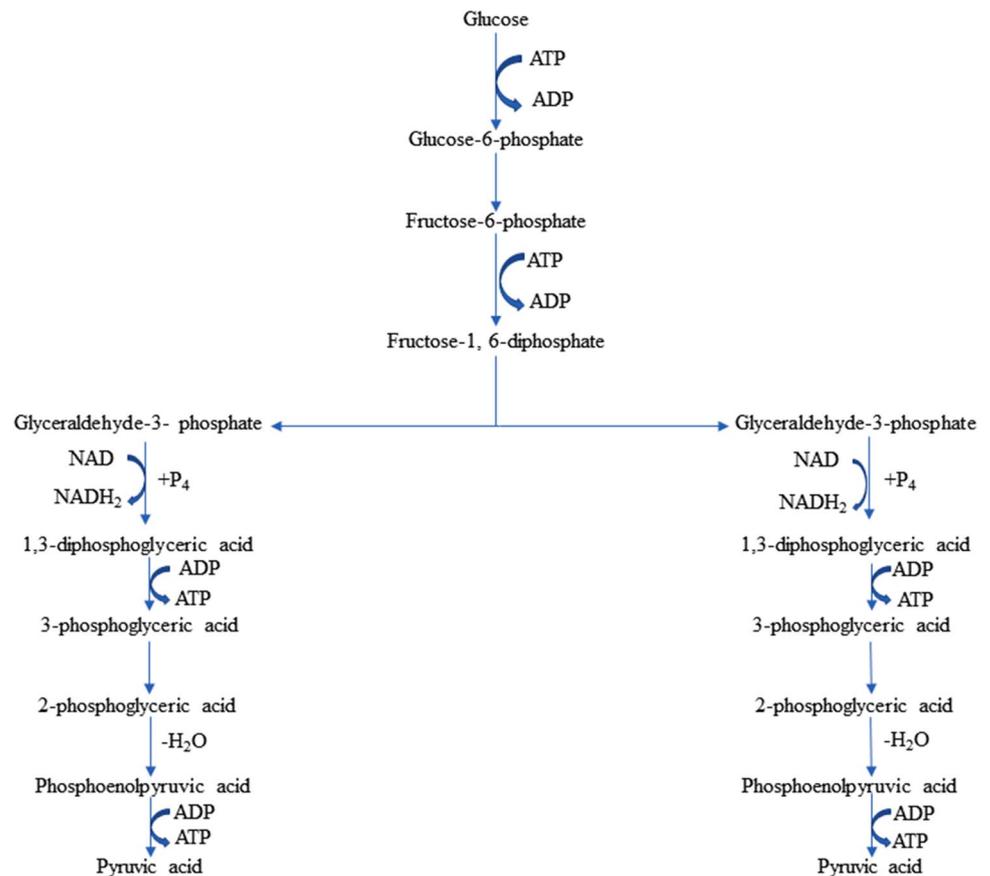
Usually, acidogenesis is the quickest reaction in the overall AD process (Mosey and Fernandes 1989). During this phase, long-chain fatty acids (LCFAs) and amino acids, i.e., by-products of hydrolysis, are utilized as substrates by acidomers (*Lactobacillus*, *Bacillus*, *Escherichia coli*, *Salmonella*). These are further degraded to organic acids along with short-chain fatty acids (SCFAs), hydrogen, and carbon dioxide (Gujer and Zehnder 1983; Kalyuzhnyi et al. 2000).

Typical acidogenesis is indicated by the following biochemical reactions.



The conversion of organic acid from complex substrates results in a pH drop in the system, which is ideal for acidomers and acetogenic bacteria because they are highly efficient in the pH range of 4.5 to 5.5 (Christy et al. 2014). During conversion of glucose to organic acid, pyruvic

**Fig. 1** Glycolytic Embden-Meyerhof-Parnas (EMP) pathway (Todar 2020)



acid is also formed as an intermediate product via the glycolytic Embden-Meyerhof Parnas (EMP) pathway, which results in volatile fatty acids (Demirel and Yenigün 2002). According to Chang et al. (2004), a rise in the operating temperature of the digester boosts the hydrolysis and acidogenesis phases. In contrast, it slows down the metabolic activity of the acetogenic and methanogenic bacteria by raising the system's pH, thus resulting in the inhibition of biogas production.

## Acetogenesis

In this process, acetogenic bacteria convert the organic acids and alcohol into acetate, an intermediate product, which is utilized by methanogens. These are strict anaerobes. They use the acetyl coenzyme-A route that contains enzymes that are particularly sensitive to  $O_2$  (Wood 1991). They grow slowly and are sensitive to organic loadings and alterations in the environment. Furthermore, they require long lag times to acclimate to new environmental conditions (Xing et al. 1997). During acetogenesis, hydrogen ion concentration in the liquid continues to rise, resulting in the agglomeration of lactate, ethanol, propionate, butyrate, and higher volatile acids, which are difficult to degrade by methanogens (Fig. 2). Hence, these must be first degraded by acetogenic

bacteria such as *Syntrophomonas wolfeii* and *Syntrophobacter wolinii* to produce acetate, hydrogen, and carbon dioxide (Björnsson et al. 2000; Chatterjee and Mazumder 2016).

Biochemical reactions during the acetogenesis process progress in the following sequence:



## Homoacetogenesis

Homoacetogens are responsible for the reduction of  $H_2$  and  $CO_2$  (a by-product of acetogenesis) to acetate (Nie et al. 2007; Diekert and Wohlfarth 1994), which is the precursor for methanogenesis. They boost acetate production by assimilating and converting  $H_2$  and  $CO_2$  following the Wood-Ljungdahl route to generate acetyl CoA by reducing CO or  $CO_2$  along with  $H_2$  and preserving energy. Furthermore, they also assimilate  $CO_2$  for cell carbon synthesis (Drake et al. 2008). In the absence of hydrogenotrophic methanogens, the concentration of  $H_2$  rises as they utilize

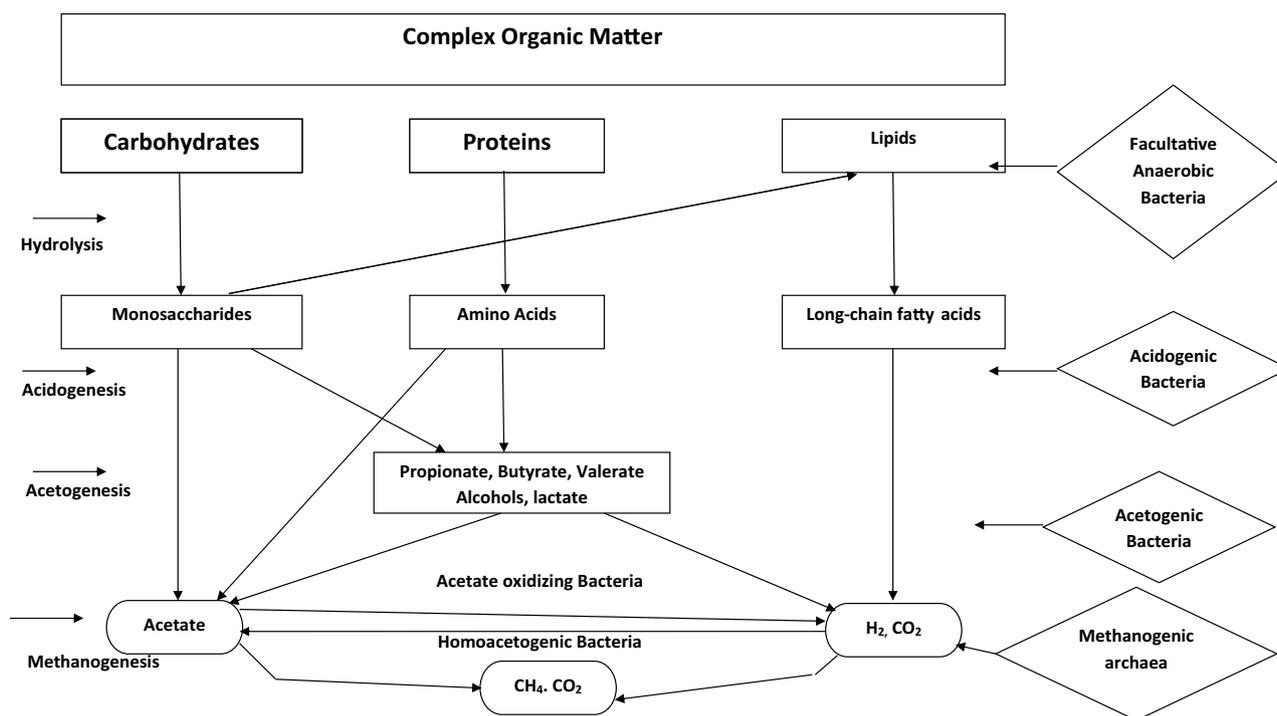


Fig. 2 Schematic representation of anaerobic decomposition (Christy et al. 2014)

$H_2$  for methane formation, resulting in higher hydrogen partial pressure. Higher HPP offsets the activity of acetate-forming bacteria, which results in lower acetate formation (Luo et al. 2012) or vice versa. Therefore, the role of hydrogenotrophic methanogens is very crucial for maintaining low HPP (Wegener Kofoed et al. 2021). Acetogenic bacteria of the genus *Syntrophomonas* also produce acetate by oxidizing VFA. However, the conversion of VFA to acetate depends on HPP, which has to be maintained below  $10^{-4}$  atm. (Anderson et al. 2003). The syntrophic relationship between homoacetogens and hydrogenotrophic methanogens is very crucial for efficient AD.

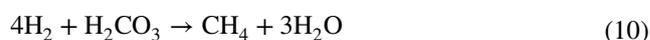
## Methanogenesis

Methane is the biofuel generated by methanogenic bacteria in an anaerobic environment. Methanogens utilize  $H_2/CO_2$ , formate, or acetate as their carbon sources for growth and survival. Methanomers utilize two pathways for methane production. The first is by reducing carbon dioxide and hydrogen, and the second is by dissociating acetic acid to carbon dioxide and methane (Ostrem 2004). The most prevalent metabolic process for converting  $CO_2$  and  $H_2$  to methane is hydrogenotrophic methanogenesis (Eq. 8).

The following equations show the biochemical reaction during methanogenesis.



Carbonic acid and methane are formed as by-products of  $CO_2$  hydrolysis.



Methanomers are broadly classified into two categories, namely, hydrogenotrophic methanogens and acetoclastic methanogens. *Methanospirillum hungatei* and *Methanococcus receptaculi* are hydrogenotrophic methanogens.  $H_2$  is used as an electron acceptor to form methane by hydrogenotrophs (Garrity et al. 2004) and  $CO_2$  is reduced to methane in a stepwise manner by special coenzymes like coenzyme M, methanofuran, and tetrahydromethanoptein. Some hydrogenotrophic methanogens require an additional source of carbon for their growth (Vogels et al. 1988).  $CO_2/H_2$  reduction normally requires a 4:1 molar ratio of  $H_2$  to  $CO_2$ , yet  $H_2$  is typically at nM concentration while  $CO_2$  is at mM concentration in natural systems. Thus, substrate limitation of hydrogenotrophic methanogenesis leads to a lack of electron donor. Hence, 45% of the hydrogenotrophic methanogens can substitute formate for  $H_2$  in the reaction (Garcia et al. 2000).

Hydrogenotrophic methanogens use chemoautotrophic processes in which  $H_2$  is the source of both energy and electrons, and  $CO_2$  is often both an electron sink and the source of cellular carbon. Therefore, hydrogenotrophic methanogens have a longer doubling time. On the other hand, acetotrophs are only 10% of the population of methanomers. *Methanosarcina* and *Methanosaeta* are acetoclastic methanomers. *Methanosarcina* can utilize a wide variety of substrates and therefore, show higher potential growth rates. Acetotrophic/acetoclastic methanogens do not require external electron acceptors and can utilize multiple substrates; hence, they exhibit higher growth rates. Furthermore, hydrogenotrophic methanogens are vulnerable to pH change and require a slightly alkaline environment for their performance. The optimal pH range for methanogenesis is 6.8 to 7.8 (Chatterjee and Mazumder 2018a, b). However, if the pH of the digester drops below 6, methanogenesis becomes the rate-limiting step by offsetting the biogas production.

## Operating parameters influencing anaerobic digestion

Optimal environmental conditions are necessary for breakthrough performance of the microbial community during anaerobic digestion as they are susceptible to environmental changes. Biogas production via anaerobic digestion depends on several factors such as seeding, pH, temperature, mixing speed, C/N ratio, organic loading rate (OLR), volatile fatty acids, and hydraulic retention time (HRT).

### Seeding

Seeding is considered the most crucial parameter when initiating the anaerobic digester. It should be a perfect blend of necessary microbial consortia to degrade organic matter, including hydrolytic bacteria, acidomers, and methanogens. Seeding plays a vital role for smooth functioning and attaining the stability of the bioreactors. Therefore, depending on the operating parameters of the reactor, a proper choice for the seed source should be made. Commonly adopted seeds are cattle manure, waste activated sludge, soil manure, compost, and sludge from primary and secondary clarifiers of the activated sludge process (ASP). Seeding helps stabilize organic waste by providing the necessary environment through the incubation of microbes (Chatterjee and Mazumder 2016).

### Operating temperature

The operating temperature of the digester plays a vital role in stabilizing organic waste as the conversion of acetic acid into

methane is mainly temperature-dependent. Various bioreactors associated with AD work in two temperature regimes: mesophilic (30–38 °C) and thermophilic (49–57 °C). The ideal mesophilic temperature is around 35 °C, while the ideal thermophilic temperature is around 55 °C (Hartmann and Ahring 2006; Kim et al. 2002). According to Speece et al. (2006), the environmental temperature significantly impacts anaerobic microbial systems, affecting metabolic rate, ionization equilibrium, substrate solubility, lipids, and iron bioavailability. From the past literature, it has been concluded that anaerobic digesters operating under a thermophilic regime yield more biogas than those operating under a mesophilic one. This may be attributed to the increased rate of hydrolysis and acidogenesis, which enhances the biodegradability of the waste, thus resulting in higher production of biogas. Moreover, thermophilic digesters were found to be superior in many ways, such as being suitable for higher organic loading rates, higher process efficiency in terms of COD and volatile solid reduction, and higher removal of pathogens (Mao et al. 2015). On a laboratory scale, Boualagui et al. (2004) compared the effects of temperature in the thermophilic (55 °C) regime with those in the psychrophilic (20 °C) and mesophilic (35 °C) regimes in tubular anaerobic digesters for the stabilization of fruit and vegetable waste. Biogas production from the thermophilic digester was found to be 144% more than that of the psychrophilic and 41% more than mesophilic digesters, respectively. At 4% solid concentration, methane yield was recorded as 58%, 65%, and 62% of total biogas produced at temperatures of 20 °C, 35 °C, and 55 °C, respectively. However, methane yields of 57% and 59% were found for solid concentrations of 8% and 9%, respectively. Castillo et al. (2006) claim that a rise in temperature by 15 °C above room temperature leads to a threefold higher methane yield. According to them, the optimal temperature in the mesophilic range is 35 °C. A slight deviation in the temperature range from 35 to 30 °C may decrease the microbial activity, subsequently causing a reduction in biogas yield. However, in single-stage digesters, an operating temperature beyond 38 °C results in rapid hydrolysis of the substrate and may result in accumulation of VFA, thereby inhibiting the methane production. This problem can be mitigated by deploying multistage reactors, which provide a suitable environment for hydrolytic, acidogenic, and methanogenic microbes. A relatively new technology, namely TPAD (temperature-phased anaerobic digestion), has been invented by the Iowa State University (Schmit and Ellis 2001), which combines the benefits of thermophilic as well as mesophilic anaerobic digestion, nullifying the drawbacks of either process. A short thermophilic pre-treatment stage (SRT of 1–3 days) is followed by a second mesophilic step with a longer retention time. The thermophilic stage accelerates hydrolysis and acidogenesis, which are regarded to be the rate-limiting activities in the

AD process, while the mesophilic stage maintains continuous syntrophic acetogenesis and methanogenesis. In TPAD, the thermophilic stage promotes hydrolysis, while the mesophilic step provides system stability by reducing the danger of inhibition owing to ammonia and VFA accumulation.

In TPAD, the thermophilic step can be run at either an acidic or neutral pH. The hydrolysis and acidogenesis stages are favored at acidic pH, impeding the methanogenesis step. However, a dynamic equilibrium between hydrolysis/acidogenesis and methanogenesis is established at neutral pH. As a result, the subsequent mesophilic stage is used as a polishing stage, removing the drawbacks of thermophilic digestion and boosting methane generation at both acidic and neutral pH levels (Lv et al. 2010, 2013). The combination of both mesophilic and thermophilic regimes results in faster degradation of the feedstock, thereby resulting in higher production of biogas. This process is mostly used to stabilize anaerobic sewage sludge. In some cases, it is also applied for food waste degradation (Kim et al. 2011). Borowski (2015) deployed TPAD for the AD of municipal sewage sludge and organic fraction of municipal solid waste (OFMSW) with a 1-day thermophilic phase and 14-day mesophilic phase. In comparison to the single-stage mesophilic continuous stirred tank reactor (CSTR), the TPAD system exhibited improved process performance in terms of methane yield and VS reduction, according to their findings. For the TPAD system, methane yield was 333 L CH<sub>4</sub>/kg VS and 52.1% VS reduction, whereas for the single-stage CSTR, methane yield was 230 L CH<sub>4</sub>/kg VS and 37.23% VS reduction.

### Effect of pH

AD is a microbe-assisted technology in which complex organic substances are converted into biogas. pH of the bioreactor is crucial to ensure the stability and performance of the reactor, as the associated microbial community is sensitive to pH change. Different microbes operate at different pH ranges, and even minor changes in pH can inhibit biogas production. The pH range for hydrolytic bacteria under a mesophilic regime is between 5 and 6 (Menzel et al. 2020), while for acidomers, it is between 5.5 and 6.5 (Kim et al. 2003a, b; Yu and Fang 2002). However, the methanogens thrive best in the pH range between 6.8 and 7.3 (Ten Brummeler and Koster 1989). Mosey and Fernandes (1989) reported that pH below 6.8 adversely affects the growth rate of the methanogenic bacteria, as acidomers prosper best in the pH range of 5 to 6. However, in this pH range, microbial activity of the methanogens is hindered due to the formation of undissociated volatile acids, which are generally present at pH less than 6.8 because they can permeate bacterial cells without resistance. At an alkaline pH greater than 7.3, the formation of ammonium nitrogen takes place, which

ceases the microbial activity of the methanogens, thereby offsetting the production of biogas (El-Fadel et al. 2013). Therefore, in this view, multistage systems are more robust in operation and stability and offer pH flexibility during the different digestion phases.

Tsigkou et al. (2020) studied the effect of pH 4.5 to 7.5 on biohydrogen production for the co-digestion of FVW and hydrolyzate from disposable nappies. According to their findings, at acidic pH (4.5 and 5), biohydrogen production ceased due to inhibition of the microbial activity of methanogens. However, at pH around 6, the highest biohydrogen production was recorded at 1008.1 mL and 1.345 LH<sub>2</sub>/L reactor.

Dwivedi et al. (2020) deployed dark fermentation for the production of H<sub>2</sub>, using FVW as a potent substrate using heat pre-treated mixed anaerobic culture. They emphasized that pH of the system plays a vital role in H<sub>2</sub> production. An increase in pH increased H<sub>2</sub> generation, and an alkaline pH range of 7–7.5 was discovered to be the ideal.

Alkalinity is essential for maintaining the pH of an AD system. An AD system's total alkalinity should be between 7400 and 27,000 mg/L as CaCO<sub>3</sub> equivalent (Chatterjee and Mazumder 2016). An AD system's bicarbonate alkalinity, on the other hand, should be in the range of 2400–5400 mg/L (Ferrer et al. 2010; Chatterjee and Mazumder 2016). The major carbon source for autotrophic methanogens is bicarbonate alkalinity, which represents the system's real buffering capacity. As accumulation of VFA affects buffering capacity much before the drop in pH (Ward et al. 2008), pH measurement is a less reliable predictor of digester imbalance than buffering capacity (Ward et al. 2008). The VFA to alkalinity ratio ( $\alpha$ ) indicates the balance between alkalinity and VFA accumulation (Poggi-Varaldo and Oleszkiewicz, 1992). It is represented by the following equation:

$$\alpha = \frac{\text{Aceticacidequivalents}}{\text{Calciumcarbonateequivalents}}$$

The value should be maintained below 0.3. However, in some exceptional cases, the value between 0.3 and 0.4 is acceptable. If the value is higher than 0.4, the digester is at risk of VFA accumulation (Schoen et al. 2009; Chatterjee and Mazumder 2020, 2016). This can be mitigated by withholding feeding until the additional VFA is digested, or by bio-augmentation using pure hydrogenotrophic culture/hydrogenotrophic-rich seeds (Angenent et al. 2004).

### C/N ratio

The C/N ratio determines the reactor's stability and performance. Optimal C/N ratio is essential to ensure proper nutrient balance required by the microbial consortia for their growth as well as to maintain stable environment. Microbial consortia responsible for the degradation of

organic matter require nitrogen for their growth, but the amount of nitrogen required will vary according to their species (Hills 1979; Kivaisi and Mtila 1997; Rao and Singh 2004; Bouallagui et al. 2005; Yen and Brune 2007). The ideal C/N ratio for food waste is 25:1–30:1 (biodegradable carbon). This implies that microbes require 25–30 times the amount of carbon than that of nitrogen (Kayhanian and Tchobanoglous 1993). OFMSW is often found deficient in nitrogen content and hence, it is supplemented by external sources such as manure, clean sewage sludge (biosolids), and urea (Kondusamy and Kalamdhad 2014). If low nitrogen content persists in the anaerobic digester, it results in lower density of the microbial consortia and may take a longer time to digest the available carbon (Kondusamy and Kalamdhad 2014; Chatterjee and Mazumder 2016). On the contrary, excess nitrogen results in ammonia inhibition (Mao et al. 2015), which can be countered by using a well-balanced blend of the C/N substrate. Lin et al. (2011) carried out biomethanation of the FVW. Decreased biogas production of 0.30 m<sup>3</sup> kg VS<sup>-1</sup>, was observed in the study, which was mainly attributed to the lower C/N ratio of the substrate (15.6).

### Organic loading rate

The organic loading rate is the amount of substrate processed per unit volume of the reactor per day; it is represented by the following equation:

$$OLR = \frac{S_o}{HRT} = \frac{S_o Q}{V}$$

where *OLR* = organic loading rate, on the basis of VS or COD (g/L day).

*S<sub>o</sub>* = influent substrate concentration VS or COD (g/L).

*Q* = flow rate (L/day).

*HRT* = hydraulic retention time (day).

OLR is affected by various parameters such as substrate type, digester configuration, and digestion type. Higher OLR may lead to severe acidification because of the formation of VFA and the other intermediate compounds over a short period of digestion, which has the potential of inhibiting the methanogenesis process and ceasing biogas production (Franke-Whittle et al. 2014). Moreover, the formation and accumulation of propionate lead to the instability of the reactor. In order to achieve process stability, there should be a gradual increment in the OLR after the other process parameters are stabilized. The type of digestion also influences OLR, with wet digestion requiring a higher OLR and vice versa (Chatterjee and Mazumder 2016). Furthermore, single-stage reactors have lower OLR as higher OLR may result in rapid and spontaneous acidification. This may hamper the

biogas yield of the digester as acidogenesis occurs at a faster rate as compared to methanogenesis, which is responsible for the slower degradation of the VFA by methane-forming bacteria (Bouallagui et al. 2005; Ward et al. 2008). Therefore, in this view, multistage reactors can be deployed for higher OLR as it will not result in VFA accumulation and thus, higher stability of the process can be achieved.

E. A. Scano et al. (2014) evaluated biogas yield from the AD of FVW at pilot-scale and full-scale power plants. The entire experimental study was conducted in four different phases—start-up phase (during the winter with average ambient temperature 15 °C), phase I (during the spring with average ambient temperature 19 °C), phase II and phase III (during the summer with average ambient temperature 26 °C). The result of pilot-scale study revealed that VFA/alkalinity ratio was mainly affected by the chemical composition of the feeding substrate mostly because of the simple sugar present in the fruit waste. They reported that VFA/alkalinity ratio often reached above 0.4, which could be countered by utilizing a well-balanced blend of the feedstock to facilitate the smooth functioning of the digester. Moreover, the VFA/alkalinity ratio reached 0.65 due to increase in OLR—the reduction in OLR reduces the VFA/alkalinity ratio. Their finding shows that optimum OLR ranges from 2.5 to 3.0 kg VS/m<sup>3</sup>/day with an average biogas output of 0.78 Nm<sup>3</sup>/kg VS and a specific methane yield of 0.43 Nm<sup>3</sup>/kg VS.

Nagao et al. (2012) investigated AD of household food waste (FW) consisting of vegetables (53.6%) and fruits (24.8%) in a mesophilic regime for a period of 225 days. They emphasized that the digester can achieve best performance VS reduction (91.8%) and methane yield (455 mL/g VS) by ensuring wet (TS below 5%) to semi-dry (5–10% TS) conditions, even at a higher OLR of 9.2 kg VS/(m<sup>3</sup>/day). This ensured a high mass transfer rate of VFAs through the liquid phase and a sufficiently high methanogenesis rate to avoid acidification.

### Micronutrient

Trace elements are essential for the cell growth of microbes. However, their deficiency is often ignored due to the diverse sources of trace elements present in the substrate. AD systems work efficiently when they are complemented with macro and micronutrients in addition to the required microbial consortia. A lack of these nutrients in the system hampers the biomethane potential of the substrate. Moreover, Kayhanian and Rich (1995) reported that the inclusion of several macronutrients, such as Co, Cu, Fe, Mo, Ni, Se, W, and Zn, not only enhances the biogas yield by up to 30% but also improves the digester stability, which is attributed to the right balance of macronutrients (N, K, P, and S) and micronutrients (trace metals). Jiang et al. (2011) reported

that vegetable waste is deficient in cobalt content and hence, it must be supplemented from an outside source in an easily accessible form for the microbes. However, the quantity of the trace metals to be supplemented is determined by the digestion technique (mono or co-digestion), the nature of the substrate to be digested, and the digester type (Demirel and Scherer 2011; Chatterjee and Mazumder 2016, 2020). Co-digestion of different waste streams enhances the synergistic relationship between different waste streams and microbial consortia. It also compensates for the essential nutrients required for the operational stability of the process. Selection of the co-substrate plays a major role in the overall process of AD. Therefore, it should be chosen precisely to promote macro and micronutrient synergy (Mata-Alvarez et al. 2011; Astals et al. 2011). The composition of the substrate ensures the type and amount of trace elements to be included. For instance, vegetable waste is deficient in cobalt concentration (Jiang et al. 2011; Chatterjee and Mazumder 2016), which is essential for microbial growth and must be supplemented externally. Furthermore, sometimes micronutrients are available in the digester but they remain inaccessible to the microbial species due to numerous interferences—for example, sulfide precipitation reduces the availability of iron, cobalt, and nickel (Barber and Stuckey 2000; Chatterjee and Mazumder 2016). This can be alleviated by raising the system's pH ( $\text{H}_2\text{S}$  converted to the less toxic  $\text{HS}^-$  form). Hence, it can be concluded that supplementation of macro and micronutrients (trace elements) is necessary for ensuring stability and smooth functioning of the digester.

The influence of important micronutrient in trace quantity on the continued AD of food waste was examined by Zhang et al. (2012). After augmenting with a model trace element solution, they noticed an improvement in process performance. However, inclusion of the trace element could not benefit the prolonged and continuous AD of food waste. Furthermore, a long-term analysis of the association between process performance and trace element profile discovered that decreased trace element concentrations, particularly Co, Mo, Ni, and Fe, were most likely to blame for the declining performance.

Effect of inclusion of both macronutrients and micronutrients for the degradation of organic waste was investigated by Kayhanian and Rich (1995). A pilot plant operating in the thermophilic regime was supplemented with Co, Cu, Fe, Mo, Ni, Se, W, and Zn, with an increment of nearly 30% in the biogas yield and enhanced digester stability.

In another study, Feng et al. (2010) investigated the effects of trace metals such as Co, Ni, Mo, B, Se, and W in the AD of food waste. According to them, maximum generation of methane was observed at high concentrations of Se (0.08–0.8 mg/L) and W (0.018–1.80 mg/L), coupled with low levels of Co (0.06–6 mg/L).

Kumar et al. (2006) studied the influence of trace elements, namely Ni (II), Zn (II), and Cd (II) when treating potato waste along with cattle manure at the doses of 2.5 ppm and 5 ppm. They reported a boost in the methane yield with the inclusion of trace elements at a concentration of 2.5 mg/L. The % increase in biogas sample over the controlled sample was highest for Cd (II), followed by Ni (II) and Zn (II).

### Role of the microbial community in different phases of AD

Different microbes deployed during the AD process are hydrolytic, fermentative, acetogenic, and methanogenic bacteria.

#### Hydrolytic bacteria

Hydrolytic bacteria can break complex organic substances such as carbohydrates, lipids, and protein into soluble monomers. Hydrolytic genera include *Clostridium*, *Peptococcus*, *Vibrio*, *Micrococcus*, and *Bacillus*. These extracellular hydrolytic enzymes can access large substrate molecules that are incapable of crossing the bacterial cell wall due to their size. Anaerobic digesters contain  $10^8$ – $10^9$  hydrolytic bacteria per mL, comprising both facultative and obligate anaerobes (Anderson et al. 2003).

#### Acidogenic bacteria

Acidogenic microbes convert the product of hydrolysis into VFAs, organic acids, ammonia ( $\text{NH}_3$ ), hydrogen gas ( $\text{H}_2$ ), carbon dioxide ( $\text{CO}_2$ ), hydrogen sulfide ( $\text{H}_2\text{S}$ ), and low alcohols. Acidogenic cell counts in anaerobic digesters typically range from  $10^6$  to  $10^8$  per mL (Archer and Kirsop 1990). These bacteria prefer a slightly acidic environment for their growth and reproduction. Hydrolytic acidogens require large surface areas to colonize and hydrolyze feedstock rich in cellulose and agricultural wastes, which are insoluble and recalcitrant in nature but undergo enzymatic hydrolysis to form volatile acids. They operate actively in the acidic range (Chyi and Dague 1994). Various cellulolytic microorganisms producing cellulases to hydrolyze cellulolytic biomass are mentioned in Table 1.

#### Acetogenic bacteria

Acetogenic bacteria are broadly classified into two groups depending on their metabolism, namely, obligate hydrogen-producing acetogens and homoacetogens. Obligate hydrogen-producing acetogens (OHPA), commonly known as proton-reducing acetogens, produce acetic acid, hydrogen, and carbon dioxide from propionate and butyrate and

**Table 1** Role of various microbial communities involved during different AD phases (Paritosh et al. 2017)

Reaction type	Microorganism	Active genera
Hydrolysis	Hydrolytic bacteria	<i>Bacteroides</i> , <i>Lactobacillus</i> , <i>Propionibacterium</i> , <i>Sphingomonas</i> , <i>Sporobacterium</i> , <i>Megasphaera</i> , <i>Bifidobacterium</i>
Acidogenesis	Syntrophic bacteria	<i>Ruminococcus</i> , <i>Paenibacillus</i> , <i>Clostridium</i> <i>Bacillus</i> , <i>Thermomonospora</i> , <i>Bacteriodes</i> , <i>Erwinia</i> , <i>Acetovibrio</i> , <i>Microbispora</i> , and <i>Streptomyces</i>
Acetogenesis	Acetogenic bacteria	<i>Desulfovibrio</i> , <i>Aminobacterium</i> , <i>Acidaminococcus</i>
Methanogenesis	Methanogens (Archaea)	<i>Methanosaeta</i> , <i>Methanolobus</i> , <i>Methanococcoides</i> , <i>Methanohalophilus</i> , <i>Methanosalsus</i> , <i>Methanohalobium</i> , <i>Halomethanococcus</i> , <i>Methanolacinia</i> , <i>Methanogenium</i> , <i>Methanoculleus</i>

other higher fatty acids (valerate, isovalerate, stearate, etc.) via  $\beta$  oxidation. These species are mainly responsible for  $\beta$  oxidation of LCFA arising from lipid hydrolysis. Obligate hydrogen-producing acetogens will prosper in the environments that maintain low concentration of hydrogen partial pressure (Anderson et al. 2003).

*Syntrophomonas wolfei* and *Syntrophobacter wolini* are examples of OHPA. Homoacetogenic species (strictly anaerobes) belong to the genera *Acetobacterium*, *Acetonaerobium*, *Acetogenium*, *Butyribacterium*, *Clostridium*, *Eubacterium*, and *Pelobacter*. Population of homoacetogens in anaerobic digesters is approximately  $10^5$  per mL (Anderson et al. 2003). Homoacetogens catalyze the formation of acetate from  $H_2 + CO_2$  (Borja et al. 2003). The first isolated acetogen was *Clostridium aceticum*, which works best in the mesophilic range (Chatterjee and Mazumder 2016). However, because the bacterial strain was thought to be lost during the 1940s, further research aimed at the discovery of a second acetogen, *Clostridium thermoaceticum* (Fontaine et al. 1942). Thereafter, it was renamed as *Moorella thermoacetica* (Collins et al. 1994) and served as the model organism for the Wood–Ljungdahl delineation of the acetyl CoA pathway (Ljungdahl and Wood 1969).

### Methanogenic bacteria

In methanogenesis, methane is generated by acetotrophic, hydrogenotrophic, and methylotrophic methanogens (Gerardi 2003). However, most of the methane is produced by acetotrophic methanogens, converting acetate into  $CH_4$  and  $CO_2$  (André et al. 2016). The optimum pH range for methanogens is between 6.8 and 7.3 (Chatterjee and Mazumder 2016). Methanomers are broadly classified into two distinct groups, namely hydrogenotrophic and acetotrophic bacteria depending on the utilization of the substrate for methane production. Hydrogenotrophic methanogens convert  $H_2$  and  $CO_2$  to methane. They are responsible for maintaining hydrogen partial pressure (HPP) less than  $10^{-4}$  atm. It may be noted that acidogenic bacteria only function well when HPP is below  $10^{-4}$  atm. (Batstone et al. 2004). As a result, hydrogenotrophic methanogen activity is critical to

the digesting process's stability and efficiency. A rise in the level of  $H_2$  leads to a pH drop in the digester, thus causing inhibition of the methanogenic bacteria. More precisely, a build-up of hydrogen can result in inhibition of the growth of hydrogen-producing organisms (Oremland 1988), and alter electron flow, which further leads to the formation of more reduced products such as lactate, butyrate, or ethanol instead of acetate. Inhibition of hydrogen-producing bacteria occurs due to the prevention of hydrogenase generation in these bacteria (Oremland 1988). Higher concentration of hydrogen leads to inhibition of syntrophic bacteria responsible for  $C_3$  and  $C_4$  acid oxidation.

The AD of simple, soluble substrates relies on the efficiency and activity of the hydrogenotrophic methanogens. Acetotrophic methanogens are obligatory anaerobes belonging to the genus *Methanosarcina* that converts acetate to methane and carbon dioxide. Acetotrophic methanogenesis is more common than hydrogenotrophic methanogenesis, as nearly 70% of the biomethane is produced by processing acetic acid. Methanogenic bacteria that utilize hydrogen belong to the family of Methanobacteriaceae. Earlier studies have shown that the *Methanosarcina* species have reproduction rates of 1.0–1.2 days (Anderson et al. 2003).

### Fruit and vegetable waste as a substrate for AD

FVW are extremely putrescible due to high moisture content and are often classified with minimal pH and cellulose content. They are characterized by a very high content of volatile solids, making them a suitable substrate for anaerobic digestion (Raynal et al. 1998; Viturtia et al. 1989). FVW is characterized as waste with a higher amount of organic matter, sugar, and hemicellulose with a minimal amount of lignin (5%) and cellulose (9%) along with other nutrients (Verrier et al. 1987). However, the presence of simple cellulose in high concentrations in FVW might cause acidification and eventually impede the production of methane, particularly in single-stage anaerobic digesters. Aside from that, seasonal and geographical variations in characteristics

of FVW lead to a range of biodegradability potential. As a result, the unique characteristics of FVW can offer significant obstacles to its successful treatment by AD (Chatterjee and Mazumder 2020). However, the high percentage of VS in FVW necessitates its control with wet AD (TS concentration of 10%). Under mesophilic temperature regimes, semi-dry (10–20% TS concentration) and dry AD of FVW can be chosen. This is due to the fact that under thermophilic temperatures (49–57 °C), quick degradation of the carbohydrates takes place to generate a high concentration of organic acids, which would result in a pH drop of the system and inhibition of methanogenic activity (Chatterjee and Mazumder 2020). This is more detrimental when stabilizing FVW using a single-stage digester, where the optimal pH should remain between 6.5 and 7.5 for efficient digestion. In the case of excess VFA production, acidification of the digester would occur, ultimately lowering the pH below 6. VFA/alkalinity ratios between 0.3 and 0.4 are ideal for methanogenic activities.

Due to the earlier-mentioned properties of FVW, aerobic processes are not recommended for their treatment due to the high organic loading rates (OLR) in this process, which

requires an additional pre-treatment step to minimize the same (Bouallagui et al. 2005). FVW are often found to be deficient in the required C/N ratio (Bouallagui et al. 2005), which is crucial for determining the extent of biodegradability. According to Bouallagui et al. (2005), an ideal range for the (C/N) ratio for FVW lies between 25 and 32. Bouallagui et al. (2009a, b) studied common vegetables such as tomatoes, lettuce, courgettes, apples, carrots, pear, oranges, and potatoes. Their proximate analysis yielded total solids of 8.3% and volatile solids of 93%.

The biomethane potential of some fruits and vegetables studied by past researchers is depicted in Table 2. Proximate analysis of the fruit and vegetable waste studied by a few past researchers is shown in Table 3.

### Common problems associated with the AD of FVW

The fermentation potential of popular fruits and vegetables has been investigated in the past to underline the benefits of their utilization as potential substrates for anaerobic

**Table 2** Biomethane potential of some fruits and vegetables

S. no	Waste type	Biomethane yield (m <sup>3</sup> kg VS <sup>-1</sup> )	References
1	Apple	0.317	Raynal et al. (1998)
2	Banana peel	0.289	Raynal et al. (1998)
3	Banana waste stem	0.081–0.196	Kalia et al. (2000)
4	Banana waste	0.4	Chanakya et al. (2009a, b)
5	Mango peel	0.370–0.523	Gunaseelan (2004)
6	Pineapple peel	0.357	
7	Pineapple leafy shoot	0.355	
8	Pomegranate (rotten fruit)	0.43	
9	Pomegranate (peel)	0.312	
10	Orange peel	0.455	
11	Orange pressing	0.502	
12	Sugar beet pulp	0.43	
13	Lemon residue	0.473	
14	Potato peel	0.267	
15	Onion peel	0.4	
16	Cauliflower leaves	0.19	
17	Cauliflower stems	0.331	
18	Cabbage leaves	0.309	
19	Cabbage stems	0.291	
20	Brinjal stalks	0.374	
21	Lettuce (residue)	0.473	
22	Carrot leaves	0.241 ± 0.008	
23	Potato pulp	0.332	Kryvoruchko et al. (2009)
24	FVW	0.47	Scaglione et al. (2008)
25	Green pea shells	0.194–0.220	Scaglione et al. (2008)
26	Potato waste	0.32	Parawira et al. (2004)

**Table 3** Proximate analysis of the FVW

Parameters	FVW	FVW	FVW	Raw shredded FVW
Total solids (TS)	90.4	95	83	100
Volatile solids (VS)	82.9	87.4	77.19	88
Total COD	104.5	-	-	120
Total suspended solids (TSS)		-	46.3	74.4
Total Kjeldahl nitrogen (TKN)	2	-	-	3.8
Cellulose	9.2	14.95	-	
Sugars, hemicellulose	62	77.8	-	
Lignin	4.5	5.6	-	
References	Bouallagui et al. (2005)	Edwiges et al. (2017)	Bouallagui et al. (2009a, b)	Bouallagui et al. (2004)

digestion to recover energy in the form of biogas. However, some unique qualities of this waste can make practical applications a challenge. FVW is characterized by high moisture content, high total solids, and acidic pH (Garcia-Peña et al. 2011; Chatterjee and Mazumder 2020). Chemical composite analysis of FVW yielded a cellulose content of 5–75% of hemicellulose and 1% of lignin (Ferrer et al. 2014). These properties pose the following roadblocks in processing FVW via AD.

1. The low pH value of the substrate hampers hydrolysis and methanogenesis, which subsequently results in low biogas output. Therefore, an optimum pH has to be maintained during the reactor's operation to enable an appropriate environment for the reproduction and growth of the microbial community. Khanal (2008) reported that an optimum pH for hydrolytic and acidomeric bacteria is 5.5 to 6.5, whereas for methanogens, it ranges from 7.8 to 8.2. However, single-phase digesters are often prone to inhibiting biogas formation as methanogens are extremely sensitive to pH change. A slight deviation in pH results in inhibition of their metabolic activity. However, this can be mitigated by adjusting the pH in the digester using a buffering agent. Hence, it is recommended that the pH modification is worked out well in advance to enable the appropriate environmental conditions for acidomers and methane-forming bacteria. Moreover, using a multistage reactor facilitates the hydrolysis of the waste in different reactors, which allows dispersion of the long C-chain SCFA into acetate and CO<sub>2</sub> that contributes to the addition of alkalinity in the dissolved state. Furthermore, the breakdown of TKN would result in the formation of NH<sub>3</sub>-N and total ammonia nitrogen (TAN), raising the alkalinity of the system (Chatterjee and Mazumder 2019, 2020), thus ensuring the smooth functioning of the digester.
2. The biodegradability potential of FVW is assessed by the C/N ratio, which determines the stability of an

anaerobic digester. This is attributed to the formation of ammonia nitrogen and volatile fatty acids in the anaerobic digester (Shanmugam and Horan 2009). The C/N ratio in the range of 25–30 indicates high-performance efficiency and stability of the AD process (Ros et al. 2013; Yen and Brune 2007). Although the C/N ratio can vary significantly with the composition of different fruits and vegetables present in the waste, the average value is around 20. Due to the relative scarcity of nitrogen, it is necessary to incorporate an additional nitrogen source during fermentation. As per Garcia-Peña et al. (2011), the addition of an external nitrogen source may yield higher biogas output. Furthermore, it may also raise the pH of the fermentation broth to some extent, thus helping to tackle the waste's low pH.

3. Another problem associated with FVW is the presence of minimal cellulose content. Hydrolysis cannot be considered a rate-limiting step for processing FVW via anaerobic digestion (Bouallagui et al. 2009a, b). The unique characteristics of FVW, i.e., low total solid and high VS (Table 2), result in quick hydrolysis during anaerobic digestion, causing acidity and accumulation of VFA, which inhibits methane formation.

The problems mentioned earlier can be resolved by codigestion of FVW with another substrate, pre-treatment of the raw substrate prior to AD, and stage separation in different AD phases, i.e., by deploying multistage or three-stage anaerobic digesters to ensure acidogenesis and methanogenesis in separate chambers.

## Co-digestion

AD has emerged as a techno-economically viable solution for effectively managing a massive quantum of organic waste (Ge et al. 2010). It is environment-friendly and provides an opportunity for resource recovery in terms

of methane. This process has the potential to help mitigate the energy crisis (Lv et al. 2010). In the recent era of co-digestion, the organic waste with other substrates has become an attractive techno-economic option. It not only serves the inoculum's nutritional requirements by the introduction of a variety of different substrates but also helps neutralize the pH and improves AD kinetics (Garcia-Peña et al. 2011; Astals et al. 2014). Furthermore, co-digestion of different waste streams eliminates the inhibitory effects of ammonia and long-chain fatty acids (LCFAs) (Astals et al. 2014). Selection of the co-substrate plays a major role in the overall process of AD and it should be chosen precisely to promote macronutrient and micronutrient synergy (Mata-Alvarez et al. 2011; Astals et al. 2011). However, the effect of an individual substrate on AD and the relationship among different substrates are factors yet to be established. Various metabolic processes breakdown complex organic substances at different rates, resulting in different yields of methane (Angelidaki and Sanders 2004). Therefore, there is a pressing need to study the effect of substrate composition to determine the exact proportion of co-substrate that will prevent the haphazard mixing of two or more waste streams. Various benefits of co-digestion are illustrated in Fig. 3. The addition of low nitrogen content co-substrates such as waste activated sludge and wheat straw balances the C/N ratio by reducing the ammonia concentrations and increasing biogas generation (Lin et al. 2011).

In general, co-digestion of FVW is carried out to achieve an optimal C/N ratio of 25–30 (Wang et al. 2012; Mata-Alvarez et al. 2014). Lin et al. (2011) deployed mesophilic CSTR for the co-digestion of FVW and FW with C/N ratios of 15.6 and 17.2, respectively, at an OLR of  $3 \text{ kg/m}^3 \text{ day}^{-1}$ . The removal of VS was discovered to be optimal. This indicates the synergy between FVW and FW, enhancing the process stability and ensuring higher biogas production and VS reduction. In another study by Wang et al. (2014), they investigated the feasibility of co-digestion of FVW with wheat straw (WS). FVW and WS were mechanically pulverized prior to main AD, thus reducing the size of the substrate and thereby increasing the surface area. These wastes were then subjected to hybrid two-stage anaerobic digesters with an OLR of  $1.37 \text{ kg VS m}^3 \text{ day}^{-1}$ . Investigation revealed a biogas yield of  $0.53 \text{ m}^3 \text{ kg VS}^{-1}$ , with a methane concentration of 64.9–76.7% and a VS removal efficiency of more than 85.0% (Table 4). This confirms the suitability of agricultural waste as a co-substrate along with FVW. Alvarez and Lidén (2008) conducted a lab-scale study in four digesters with a total volume of 2 L and an OLR ranging between 0.3 and  $1.3 \text{ kg VS m}^3 \text{ day}^{-1}$  to investigate the co-digestion feasibility of slaughterhouse

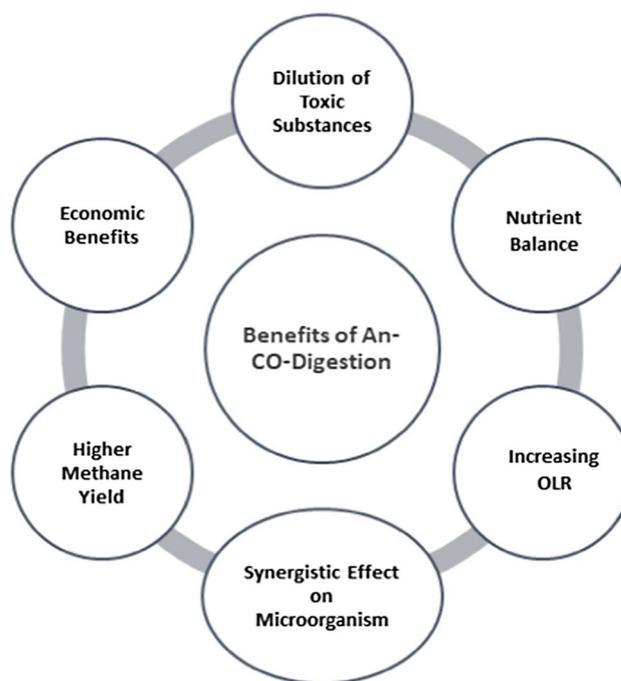


Fig. 3 Benefits of anaerobic co-digestion

wastes and FVW in conjunction with organic manure. The results showed a methane output of  $0.3 \text{ m}^3 \text{ kg VS}^{-1}$ , whereas VS reduction was only 54–56% of the total. This could be due to an excess of OLR. Low pH was noticed due to VFA accumulation, which results in digester failure due to the substrate's low nitrogen concentration. Overall, the C/N ratio is a significant factor in determining the process performance.

Higher biogas yield was reported by Ros et al. (2013) and Di Maria et al. (2014) during the co-digestion of sludge with FVW. However, the efficiency and stability of AD was found to completely depend on the syntrophic activity of the hydrolytic bacteria, acidogens, acetogens, and methanogens (Werner et al. 2011) as well as their interactions with other environmental factors and process performance (Di Maria and Barratta 2015).

Tsigkou et al. (2020) investigated the effect of pH on biohydrogen production for co-digestion of FVW and the hydrolyzate from disposable nappies. They concluded that co-digestion of the mixed waste stream led to a threefold increment in  $\text{H}_2$  volume compared to monodigestion of FVW. This may be due to microbial consortium enhancement from the disposable nappy hydrolyzate. Also, pH 7.5 is favorable for methanogens and hence, a higher yield of biohydrogen, equivalent to  $3021.91 \text{ mL}$  and a yield of  $4.02 \text{ L H}_2/\text{L reactor}$ , was reported.

**Table 4** Various co-substrates used for AD of FVW

Reactor and volume	Feedstock + Co-substrate	Operating temperature (°C)	OLR (kg VS m <sup>-3</sup> day <sup>-1</sup> )	HRT (days)	Methane production (m <sup>-3</sup> kg <sup>-1</sup> VS)	Optimal C/N ratio	References
CSTR (4 L)	FVW + food waste (FW)	35	3	30	0.49	15.6 (FVW) + 17.2 (FW)	Lin et al. (2011)
CSTR single phase (8 L)	FVW + FW (5:8 ratio)	35	0.5–3.5	30	0.31–0.405	18.9 (FVW) + 11.5 (FW)	Shen et al. (2013)
CSTR two-phase (5 L + 8 L)	FVW + FW (5:8 ratio)	35	0.2	10 + 10	0.351–0.455	18.9 (FVW) + 11.5 (FW)	Shen et al. (2013)
ITPAR	FVW + wheat straw (WS)	35	1.37	27	0.53	15.6 (FVW) + 49.67 (WS)	Wang et al. (2014)
ASBR (2 L)	FVW + abattoir waste water (AW)	55	0.3–1.3	20	0.3	9.45 (FVW) + 3.418 (AW)	Alvarez and Liden (2008)

## Pre-treatment techniques to enhance FVW bio-digestion

Pre-treatment of the substrate helps in accelerating the hydrolysis and acidogenesis phases by breaking macromolecular structures, due to which the digestion proficiency improves. Various factors like particle size, pH, and enzymes generated by the microorganisms influence the degree of hydrolysis. The stabilization of the organic substances is aided by enzymes secreted by various microbial consortia associated with the process. Pre-treatment alters the surface area of particulate wastes by loosening the molecular bonds of complex substrates, allowing polymers to break down and enhance the surface area (Zia et al. 2020).

Traditional pre-treatment procedures include physical (mechanical pulverization), chemical (acid–alkali, ozonation), biological (micro-aeration), and thermal pre-treatment methods, which can be adopted for various types of organic wastes. However, the choice of the particular treatment relies upon the composition of the substrate (Pham et al. 2014). Various mechanical pre-treatment procedures that are used to reduce the size of waste in order to enhance the surface area have an impact on the degree of waste solubilization and anaerobic digestion efficiency. Modern pre-treatment approaches such as pulsed electric field (PEF) treatment and ultrasonic pre-treatment have been combined with traditional pre-treatment approaches to improve FVW hydrolysis and anaerobic digestion (Safavi and Unnthorsson 2017; Shanthi et al. 2018; Karouach et al. 2020).

### Physical pre-treatment

Mechanical pulverization effectively decreases the particle size of waste, thereby leading to increased surface area;

this is beneficial to both chemical and biological processes (Kumar and Sharma 2017). As a result, all materials must be pre-treated in some way to improve efficacy of the hydrolysis process and microbial decomposition because substrate utilization rate is inversely proportional to particle size (Esposito et al. 2011). However, though the size of organic wastes must be kept to a minimum, there is a limit to the extent of reduction. Particle size less than 0.7 mm can cause VFA accumulation, which in turn results in lower process stability and performance as acidic intermediates may be toxic for the methanogens (Izumi et al. 2010).

### Biological pre-treatment

Biological pre-treatment enables the breakdown of complex molecules into simple monomers, resulting in higher organic material solubilization and enhances the AD process performance (Fdez-Güelfo et al. 2011). Biological pre-treatment is divided into three main categories, namely enzymatic, anaerobic, and aerobic. In enzymatic pre-treatment, exogenous enzyme addition is done during anaerobic digestion to improve the hydrolytic step of complex organic substrates (Brémond et al. 2018); however, in the case of aerobic pre-treatment, this is accomplished by simple aeration, in which the presence of oxygen enhances the degradation capacity of facultative microbes. They utilize oxygen as a final electron acceptor and produce CO<sub>2</sub>, water, sulfate, and nitrate as end products. Furthermore, addition of the pure culture of the microbes possessing the required degradation properties quickens the hydrolysis process. In aerobic consortium pre-treatment, aerobic consortia (mixed culture) under liquid or solid forms are inoculated instead of pure culture to enhance the degradation of complex organic waste. Biological pre-treatment is more cost-effective, although the by-products can cause marginal inhibition of digestion. However, in moderate conditions, biological pre-treatment

is favored because it does not require addition of expensive chemicals (Sindhu et al. 2016). Aerobic pre-treatments such as composting are preferred over AD as they lead to higher production of hydrolytic enzymes, which results in better hydrolysis of complex substrates (Lim and Wang 2013; Zhang and Zhang 1999). However, biological treatment method is mostly adopted for agricultural wastes as they have a high percentage of lignocelluloses. Also, given the recalcitrant nature of this waste, it becomes mandatory to carry out its pre-treatment. Addition of enzymes via the biological route breaks down the lignocellulosic component thereby enhancing the hydrolysis process.

Zhou et al. (2013) conducted a pilot-scale study to investigate the feasibility of co-digestion of sewage sludge along with FVW and kitchen waste. They also assessed the effect of thermal hydrolysis on the physical and chemical properties of the substrate. Thermal hydrolysis boosted digestibility up to 115%. The disadvantages of biological pre-treatments include the need for more space, a longer HRT, optimal environmental conditions, and the loss of cellulose, which hampers anaerobic digestion of the substrate (Sindhu et al. 2016).

### Chemical pre-treatment

Alkali treatment is one of the most effective techniques used for solubilizing complex compounds in this sequence: sodium hydroxide > potassium hydroxide > magnesium hydroxide and calcium hydroxide (Kim et al. 2003a, b; Kondusamy and Kalamdhad 2014). Chemical pre-treatments with alkalis, oxidants, or strong acids are generally deployed for lignocellulosic biomass owing to their recalcitrant nature. However, alkalis are often added to the anaerobic digester to treat food waste or fruit and vegetable waste to ensure optimal pH condition during different phases. These treatments might be harmful to microorganisms and may result in reduced biogas yields. Chemical pre-treatment of agricultural residues and FVW was conducted and it was unsuitable for fast decomposable materials. Also, excessive levels of Na<sup>+</sup> or K<sup>+</sup> have been linked to the suppression of AD (Neves et al. 2006). In addition to alkali pre-treatment, a combination of thermal and ultrasonic pre-treatment has also been used. Yiyang et al. (2009) studied alkali and ultrasonic pre-treatment for activated sludge waste and reported an increase in COD solubility as compared to mere ultrasonic or alkali pre-treatment.

### Thermal pre-treatment

Thermal pre-treatment ruptures the outer skin of the substrate, thereby increasing solubility, which, in turn,

maximizes the rate of COD solubilization. Thermal pre-treatment also removes pathogens, improves dewatering performance, and minimizes the viscosity of the digestate. Liu et al. (2012) investigated the viability of thermal pre-treatments for organic waste and they reported that steam explosion at 175 °C for 60 min improved both physical and chemical characteristics, decreased viscosity, improved dewaterability, and increased solubility of soluble organic content such as protein and organics (molecular weight > 10 kDa). They claimed that 58.5% of organics in the liquid phase separated after the wastes were thermally pre-processed.

Thermal pre-treatment promotes total efficiency of anaerobic digestion by increasing biogas output. It can also be deployed for the pre-treatment of agricultural biomass as they have high amount of lignin present and their recalcitrant nature makes it difficult to undergo hydrolysis, which is an essential step of AD. In such scenario, thermal pre-treatment provides better opportunity for the effective utilization of such waste. Li et al. (2016) used thermal pre-treatment for FVW and found a 24% increase in methane output when compared to untreated biomass. In a separate research work by Zhou et al. (2013), pyro-hydrolysis was utilized as a pre-treatment approach for co-digestion of FVW, kitchen trash, and sludge from a wastewater treatment plant. The yield of biogas and the dissolution of volatile suspended solids increased by 15% and 38.3%, respectively. When B. Ruggeri et al. (2013) looked at the viability of various pre-treatment techniques for FVW wastes and compared the performance of AD, they found that combining thermal and alkali pre-treatment resulted in a tenfold increase in methane output.

### Ultrasonic pre-treatment

When compared with bacterial, thermal, and chemical pre-treatments, ultrasonic pre-treatment emerged as the most effective (Rasapoor et al. 2016). Ultrasonic treatment damages the cell structure and flocs, like mechanical pre-treatment does. It works through two mechanisms: cavitation, which arises at low frequencies, and chemical reactions, which occur at high frequencies due to the release of OH<sup>-</sup>, HO<sub>2</sub>•, and H<sup>+</sup> radicals. During cavitation, bubbles collapse and add free radicals, which improves the chemical structure of the waste (Grönroos et al. 2004). Physical disintegration boosts microbial activity, thereby enhancing biogas yield (Kwiatkowska et al. 2011). When AD of fruit and vegetable market waste (FVMW) was pre-treated in an ultrasonicator for 18 min at 20 kHz frequency and 80 m amplitude, it yielded an 80% increase in methane output (Zeynali et al. 2017). Prior to sonication, the wastes were subjected to mechanical pulverization to the size of 3–5 mm.

Shanthi et al. (2018) used both surfactant-assisted sonic pre-treatment (SSP) and ultrasonic pre-treatment (UPT) for FVW. At a surfactant dosage of  $0.035 \text{ g g}^{-1}$  suspended particles and a specific energy input of  $5400 \text{ kJ kg TS}^{-1}$ , the SSP approach reduced suspended solids by 16% and 26% as compared to ultrasonic pre-treatment. In addition, the SSP method was reported to have a 0.9 energy ratio, indicating development in energy and economics. According to the findings, a surfactant combined with ultrasonic pre-treatment possibly improves the AD process even more.

## Electrical method

Electrical pre-treatment is a method of disrupting the cell structure by passing waste via an electrical field. To pre-treat refractory wastewater, biomass, and biosolids, pulsed electric field (PEF) with high-intensity external electrical fields was used (Safavi and Unnthorsson 2017). The phospholipids and peptidoglycans in a cell are exposed to a high-voltage electric field, which causes the cell membrane pores to open for a prolonged time and destroy the cell's integrity. This causes the cell to release some of its cytoplasm and breaks the biomass down into smaller colloids by a process known as electroporation (Rittmann et al. 2008; Gerlach et al. 2008; Salerno et al. 2009). In comparison to traditional physical pre-treatment methods, PEF treatment not only improves VS and COD removal but also boosts biomethane potential using very little energy (Neumann et al. 2016). To pre-treat lignocellulosic components, electron beams can be used in conjunction with PEF treatment. To change the biological and chemical bonds, the method uses ionization energy (Sindhu et al. 2016).

Electrical pre-treatment was investigated in a study for the stabilization of FVW and landfill leachate at variable electrical intensities of 15, 30, and  $50 \text{ kW h/m}^3$  (Safavi and Unnthorsson 2017). The PEF treatment method was used to pre-treat waste from fruits and vegetables, which resulted in yields even higher than hydrothermal pre-treatment. After achieving a 7% increase in methane yield at  $15 \text{ kW h m}^3$ , production began to fall. In comparison to a blank, there was a 17% increase in COD removal for waste from fruit/vegetables. Electrical intensity plays a vital role in both methane production and COD removal.

Thus, the various approaches of thermal, ultrasonic, and electrical pre-treatments, in addition to physical treatment, are reportedly successful in improving hydrolysis of FVW. However, further research is needed to determine the appropriate optimal operating parameters such as temperature, sonication frequency, and electrical field intensity for individual substrates, as well as the synergy between co-digestion of multiple substrates and pre-treatment procedures.

## Bioreactor performance for the treatment of FVW

Batch reactors are the most common owing to their capacity for fast digestion with simple and low-cost equipment and a simple assessment of the digestion rate. They do, however, have downsides such as large variability in gas output and efficiency, biogas loss during bioreactor emptying, and bioreactor elevation limitations. To date, there are many different types of bioreactors in use, but the three most common are batch reactors, single-stage continuously fed reactors, and bi-phase or multistage continuously fed systems. In single-stage AD systems, all four key AD processes, namely hydrolysis, acidogenesis, acetogenesis, and methanogenesis, take place in a single chamber. On the other hand, hydrolysis, acidogenesis, and acetogenesis take place in a single chamber in two-stage AD systems, which are coupled in series with a secondary chamber where methanogenesis takes place. In three-stage AD systems, the four main steps of AD occur in distinct chambers: hydrolysis in the primary chamber, acidogenesis and acetogenesis in the secondary chamber, and methanogenesis in the tertiary chamber. The large range of waste compositions and digester configurations/designs has been matched by the wide range of waste compositions. Despite the development of multistage AD systems to improve ultimate methane output and overall process performance, industries still favor single-stage AD systems for processing large quantities of FVW.

In contrast to multistage AD systems, single-stage AD systems have straightforward designs, allowing lower investment costs and easier monitoring operations. Industries are still looking for a single, reliable digester/structure that can support all major AD phases. Furthermore, since large operations offset the low biogas yield, single-stage AD systems tend to be economically viable.

Bouallagui et al. (2009a, b a) deployed a thermophilic anaerobic sequencing batch reactor (ASBR) to stabilize FVW with an HRT of 15 days wherein the maximum OLR was fixed as  $1.24 \text{ kg VS m}^{-3} \text{ day}^{-1}$ . They acquired the highest biomethane yield of  $0.48 \text{ m}^3 \text{ kg}^{-1} \text{ VS}$  with 60% methane content and VS reduction of 79%. Also, in another literature, Bouallagui et al. (2009a, b b) investigated the impact of hydraulic retention time and temperature variations on the efficiency of an ASBR treating system under mesophilic and thermophilic conditions using FVW with abattoir wastewater as the co-substrate. Performance assessment revealed VS reduction of 73–86% for the co-digestion, with a biogas yield of  $0.3\text{--}0.73 \text{ m}^3 \text{ kg}^{-1} \text{ TVS}$  added. Significantly, variations in HRT under mesophilic conditions had no significant impact on organic matter removal. However, raising the temperature from 35 to 55 °C substantially increased the biogas yield after

20 days of HRT. The HRT of 10 days at 55 °C resulted in overloading and eventual inhibition of digestion and co-digestion processes. This may be attributed to accumulation of hydrogen ions, which resulted in a pH drop in the system. Also, the alkalinity values were observed to rise in tandem with the rise in OLR and temperature, indicating that biogas production was failing.

Effect of OLR on AD of FVW was assessed by Wang et al. (2014) using single-phase CSTR at mesophilic regime. The initial OLR was maintained at 0.5 g VS L<sup>-1</sup> day<sup>-1</sup> and increased gradually with the increment of 0.5. The maximum OLR for single-stage digester was kept at 3.0 g VS L<sup>-1</sup> day<sup>-1</sup>. Increasing the OLR beyond this resulted in the inhibition of the methanogenic activity due to VFA accumulation and higher concentrations of propionic acid (1576.3 g/L) were noticed in the digester and a total increase of 56.8% in VFA was noticed. Maximum biogas production reached to 1.95 L day<sup>-1</sup> with the methane content of 59.65%, and no significant difference was noticed in methane content due to change in OLR.

Another similar study was carried out by Lin et al. (2011) to investigate the feasibility of co-digestion of FVW with food waste at different mix ratios. Lab-scale CSTR was used to evaluate the performance of the digesters at an OLR of 3 kg VS/(m<sup>3</sup> day). Biogas production was estimated as 2.17 m<sup>3</sup>/(m<sup>3</sup> day) with the methane yield as 0.42 m<sup>3</sup> CH<sub>4</sub>/kg VS. However, at the same OLR, mono digestion of food waste ceased biogas production due to accumulation of VFA. This can be explained by the fact that anaerobic degradation of waste with minimum cellulose content would be limited to methanogenesis rather than hydrolysis (Misi and Forster 2002; Bouallagui et al. 2005). Results show that ratio of FVW:FW (1:1) depicted the optimal performance with methane production of 0.49 m<sup>3</sup> CH<sub>4</sub>/kg VS and COD and VS reduction were obtained as 96.1% and 74.9%, respectively. Performance of co-digestion of FVW and food waste was assessed in a single-stage CSTR operating at 35 °C. Optimal mix ratio was chosen as 5:8 (FVW:FW) by varying OLR from 0.5 to 3.5 g VS L<sup>-1</sup> day<sup>-1</sup>. The OLR was varied with the increment of 0.5 g VS L<sup>-1</sup> day<sup>-1</sup>. Daily biogas production was enhanced with average biogas production as 15.5 L day<sup>-1</sup> at OLR 3.5 g VS L<sup>-1</sup> day<sup>-1</sup>. Highest methane content was found to be 60%. Result indicated that increase in VFA concentration from 120.1 to 2302.3 mg L<sup>-1</sup> offsets the biogas production dramatically. They further claimed that for stable performance of the reactor, the OLR should be kept below 2.0 g VS L<sup>-1</sup> day<sup>-1</sup>. Furthermore, single-stage digester claimed 4.1% higher methane yield as compared to two-stage system for OLR below 2.0 g VS L<sup>-1</sup> day<sup>-1</sup> (Shen et al. 2013).

Lab-scale CSTR was used for AD of FVW and municipal sewage sludge (MSS) under mesophilic regime at HRT of 20 days. The effect of OLR and ratio of the FVW:MSS was examined by Arhoun et al. (2019). Co-digestion of FVW

and MSS resulted in higher methane yield as compared to monodigestion of the individual substrate. Alkalinity and pH of digester were found to be stable regardless of the difference in the FVW:MSS of the feed. Performance of the digester was improved to sixfold with the increase in the FVW:MSS ratio and the methane content was obtained as 62–64%.

Edwiges et al. (2018) used AD to study a high variety FVW blend in a continuously stirred tank reactor (CSTR) operating in the semi-continuous mode. They found a maximum biomethane yield of 360 NL CH<sub>4</sub> kg<sup>-1</sup> VS in batch bottles, with a biodegradability of 79%. The performance of the reactor was steadily monitored while the OLR was increased from 0.5 to 5.0 g VS L<sup>-1</sup> day<sup>-1</sup>. At an OLR of 3.0 g VS L<sup>-1</sup> day<sup>-1</sup>, the highest methane output was obtained equivalent to 285 NL CH<sub>4</sub> kg<sup>-1</sup> VS. At an OLR greater than 3.0 g VS L<sup>-1</sup> day<sup>-1</sup>, accumulation of volatile fatty acids (VFA) was identified, which inhibited the AD process. In another batch study, AD of cabbage waste (CW) and potato waste (PW) was carried out by Mu et al. (2017) to optimize the mix ratio of CW:PW in mesophilic regime using anaerobic granular sludge as inoculum. Highest biogas production was obtained for mono digestion of PW, and methane yield was not higher. This may be attributed to the fact that rich starch in the potato waste quickly hydrolyzed the VFA to CO<sub>2</sub> and most of the CO<sub>2</sub> cannot be converted to methane as methanogenic pathway was dominated by aceticlastic methanosaeta (74.2%). However, co-digestion of PW and CW in the ratio of 1:1 revealed enhanced methane yields from 16.6 to 31.7%. This is because of the balanced C/N ratio (20.5) of feed stock and methane content in the biogas varied from 50 to 62%. Batch AD of various mixtures of potato peel and beet leaves were conducted in a mesophilic environment with a 14-day HRT.

Efficacy of co-digestion of FVW, solid slaughterhouse waste, and manure in a mesophilic regime was investigated by Alvarez and Liden (2008). The effect of OLR on the digestion of the above co-substrate was investigated by varying OLR from 0.3 to 1.3 kg VS m<sup>-3</sup> day<sup>-1</sup> in a mesophilic regime for a period of 30 days and the system yielded 0.3 m<sup>3</sup> kg<sup>-1</sup> VS of methane. Linear relationship between methane yield and loading rate was observed for lower loading rates (0.14–0.49 kg VS m<sup>-3</sup> day<sup>-1</sup>). At intermediate loading rate (0.49–1.31 kg VS m<sup>-3</sup> day<sup>-1</sup>), the methane yield is almost constant. After that, it starts decreasing which points to the beginning of the biological stress and decline in methane production. However, for higher OLR (2.03 kg VS m<sup>-3</sup> day<sup>-1</sup>), biogas and methane yield got decreased and this indicates organic overload or inadequate buffering capability which can be countered by adding buffering agent and reducing OLR.

Therefore, it can be summed up that batch digesters are efficient and by maintaining optimum environmental parameters, they can be used for treating FVW at low cost. However, in case of CSTR, reduced HRT may wash out

the biomass which inevitably results in the process failure (Chen and Hashimoto 1980). Moreover, higher OLR may result in accumulation of VFA coupled with low buffering capacity and leads to process failure. Maximum OLR for single-stage digester are suggested as 3.6 kg VS  $\text{m}^{-3} \text{day}^{-1}$  (Ganesh et al. 2014). Co-digestion of different waste streams explored the possibility of treating waste that could not be effectively handled separately, thereby enabling its efficient management, reducing the cost of handling the waste separately, and enhancing biogas production by maintaining synergistic environment in the digester.

The two-stage system is capable of handling highly biodegradable waste and produces more biogas, which may be attributed to the selection and enrichment of different bacteria in each phase of AD (Demirer and Chen 2005). Acidogenic bacteria degrade the complex organic materials into volatile fatty acids and alcohols, which are quickly metabolized by methanogens into methane and carbon dioxide. Furthermore, the overall process stability can be improved by monitoring the acidification step using optimization of the hydraulic retention period to avoid overloading and the build-up of toxic material.

Shen et al. (2013) investigated co-digestion of FVW and kitchen waste in a ratio of 5:8 using single-stage as well as two-stage digesters. They found that in both processes, ethanol-type fermentation occurred and 4.1% higher biomethane yield was recorded in the single-stage digester for OLR lower than 2.0 g VS  $\text{L}^{-1} \text{day}^{-1}$ . The two-phase system achieved stable performance and was operated at double OLR than single phase. This implies higher treatment capacity for same digester volume, thus enhancing economic benefit. Two-phase system produced methane equivalent to 0.351–0.455 L (g VS) $^{-1} \text{day}^{-1}$  indicating 7.0–15.8% rise as compared to single-stage system. Furthermore, higher bioenergy recovery in terms of  $\text{H}_2$  gas rendered the two-stage system as stable and sustainable with higher organic loads.

Performance of lab-scale two phase (UASB-Anaerobic Filter) was assessed in batch mode under mesophilic regime. Cow manure was used as inoculum for the present study. According to them, phase separation enabled them for regulating optimal pH in the hydrolyzer and methanizer. Maximum degradation (nearly 75%) was obtained within the first 2 weeks of operation. Highest methane yield was obtained at HRT of 25 days with methane yield as 0.383  $\text{m}^3 \text{CH}_4/\text{kg VS}$  and overall VS reduction was obtained as 90%. They also studied the effect of recirculation rate on the overall biodegradation and reported increase in the degradation by 4–7% and methane yield by 7% (Virturia et al. 1989).

Yang et al. (2013) investigated co-digestion of FVW and kitchen waste in two-phase anaerobic digester with different proportion of FVW:KW as 25:75, 50:50, 75:25, and 100:0. Higher degree of acidogenesis was obtained with the KW as 25% which is mainly attributed to the fact that the

presence of carbohydrate in the kitchen waste was higher than the lignin, cellulose, and hemicellulose in the FVW. Carbohydrates can be easily degraded as compared to lignin, cellulose, and hemicellulose, thus enhancing the hydrolysis efficiency. Higher amount of KW in the feedstock results in high salinity and fat concentration which can inhibit the microbial activity. Hence, higher acidification rate was achieved at 25:75. Considering the performance of two-stage system using FVW:KW as 50:50 with moderate salinity and fat content of kitchen waste resulted in better stability of the digester with 74.11% degree of acidification at HRT of 15 days. Optimal HRT for the methanogenic reactor for FVW:KW as 50:50 was 3 days. Daily methane production was higher for the FVW:KW as 50:50 ratios as compared to others because of the balanced nutrient in the digester and less effect of salinity and fat content in the feedstock. Thus, the ratio of FVW:KW as 50:50 is the best ratio for the co-digestion of the waste.

In another study, anaerobic co-digestion of waste activated sludge and FVW was carried out in an inclined tubular digester by Dinsdale et al. (2000). CSTR was used as acidogenic reactor and inclined tubular methanogenic digester. OLR was kept as 5.7 kg VS  $\text{m}^{-3} \text{day}^{-1}$  with optimal HRT of 13 days with 3 days in acidogenic and 10 days in methanogenic reactor. Performance evaluation indicated that 40% of VS reduction was achieved with the biogas yield as 0.37  $\text{m}^3 \text{kg VS}^{-1}$  and methane content as 68% with TVFA as 1300 mg/L. However, at HRT 17 days with OLR 4.3 kg VS  $\text{m}^{-3} \text{day}^{-1}$  with HRT in methanogenic reactor as 13 days resulted in VS destruction of 44%. It also reduced the average TVFA to 300 mg/L. The effect of the OLR and effluent recycling was assessed for two-phase AD of fruit and vegetable waste by Zuo et al. (2013). With the increase in OLR from 1.3 to 1.7 g VS/L/day, biogas production and methane content were reduced to 50% in acidogenic reactor, attributed to the rise in VFA concentration because of quick hydrolysis vegetable waste. This hinders the activity of methanogens in the acidogenic reactor and ultimately offsets the biogas production. Daily biogas yield and methane content were increased from 1.2 to 4.4 L/day and from 27.4 to 60.5%, respectively. However, inhibition of hydrolysis in the acidogenic reactor was demonstrated under the OLR of 2.6 g VS/L/day without recirculation, thus indicating system overloading. Effluent recirculation had shown a considerable positive effect on alleviating VFA inhibition and improving biogas production in the acidogenic reactor because of the effect of dilution and pH adjustment, particularly at high OLRs.

Studies have been also carried out on hybrid reactor for stabilization of organic waste. Gulhane et al. (2016) designed anaerobic baffled reactor (ABR) consisting of four chambers which provided longer residence time for the microbes and enabled selection of the different microbes in different section and phase separation enhances biomethane yield (Arun Khardenavis

et al. 2013). The reactor was operated at OLR 0.5 g VS/L/day and HRT of 30 days. The result indicated that biogas and methane yield of 0.7–0.8 L/g VS added/day and 0.42–52 L/g VS added per day respectively. The recirculation of effluent from chamber four to one enhances biogas production and boosts system stability by neutralization of VFA and thus reduced low pH-related damage to methanogens. It also ensures complete VFA conversion to methane in subsequent step, thus increasing biogas yield without compromising the ABR's phase separation ability (Ahamed et al. 2014).

Hybrid reactor, a combination of leach bed reactor (LBR) and UASB, was used by Chakraborty and Venkata Mohan (2018) to evaluate the effect of mix ration of FW:VW on system performance. The integrated system was operated for a period of 30 days at mesophilic regime. LBR was used to determine the rate of acidogenesis and according to their findings, 2:3 (FW:VW) shows higher rate of acidogenesis. Furthermore, VFA composition pointed reduced propionate and lactate concentration and increased acetate production. This is because of the presence of more vegetable waste in 2:3 (FW:VW) ratio which enhanced the hydrolysis process in turn methanogenesis too. Thus, two-stage (LBR-UASB) integrated AD system was found to be sustainable for valorizing vegetable and food waste.

Zeshan and Visvanathan (2012) used a pilot-scale thermophilic reactor to investigate the influence of ammonia-N accumulation in dry AD of OFMSW. The OFMSW consists of food waste, fruit and vegetable waste, green trash, and paper waste. These substrates were prepared in two simulations with C/N ratios of 27 and 32. The process performance was evaluated as pH, VFA, alkalinity, ammonia-N, and biogas output. The simulation results revealed that with a C/N ratio of 32 had approximately 30% less ammonia in the digestate than a C/N ratio of 27. The system was found to operate well up to OLR 7–10 kg VS/m<sup>3</sup> day, with a retention time up to 19 days and 50–73% excess energy output.

To summarize, two-stage digester is able to operate at higher OLR as compared to single-stage digester and can achieve better process stability in terms of pH adjustment. These digesters offer higher resistance to organic shock loading and provide more scope for biohydrogen production. Enhanced performance in terms of biogas production and methane generation is noteworthy.

The performances of the various bioreactors studied by the past researchers are described in Table 5.

## Future recommendations

Biogas offers significant advantages over other renewable energy options; however, the economic performance of the AD of FVW should be improved. FVW often lacks in nitrogen content, so co-digestion of the waste with other waste

stream such as slaughterhouse waste, anaerobic sludge, meat residue, and food waste can balance the optimum C/N ratio and this will ensure smooth operation of anaerobic digester. Furthermore, stage separation during the anaerobic digestion of FVW not only prevents the VFA inhibition, but also maximizes the biogas output in commercial scale. Efforts should be made for the scaling up of the anaerobic digester treating FVW and process parameters should be monitored throughout the process. Sorting techniques plays a vital role in the segregation of the waste which should be encouraged. Generation of biogas from anaerobic digestion of fruit and vegetable waste is primary goal. However, it is worth considering for other value added products which can be generated during AD of FVW. For instance, due to high biodegradability of FVW, it can be acidified quickly, so it is possible to maintain acidification during AD for the production of other chemicals such as polyhydroxyalkanoate (PHA) and the raw materials of lactic acid. Furthermore, despite the fact that multiple studies have confirmed the co-digestion of various materials, but the quantitative analysis, the exact assessment of the percentages of chemical indexes such as proteins, lipids, hydrocarbons, and water is still needed. Several studies have been conducted in the past to analyze the effect of trace elements on AD and it indicated that addition of these elements in controlled proportion helps in stabilizing the digester and reduces the risk of VFA inhibition. More research about the impact of these microelements on the anaerobic digestion of fruit and vegetable waste is needed.

Enriching the methane content in the biogas will allow its use with natural gas and can be delivered to the consumers via pipelines in the similar way as LPG. Biogas can be converted into the commercial gaseous fuels such as biomethane, compressed biogas, biohydrogen, and syngas (Budzianowski 2012). This alternative will reduce our dependency on the fossil fuels and also reduce energy crisis. Compressed biogas can be used to power motor vehicle like city buses (Budzianowski 2012). Biomethane production from biogas via cleaning followed by removal of CO<sub>2</sub> technologies such as pressure swing adsorption (PSA), high-pressure water wash (HPWW), and reactive absorption (RA) will increase the calorific value of the methane and can be the future trend of biogas (Budzianowski 2010). Biohydrogen production from biogas via water electrolysis will allow its use in the fuel cell and thus, it holds vast potential of replacing fossil fuels in the transportation sector. Installation of decentralized pilot-scale anaerobic digester can be done within the municipalities and residential communities to avoid costs of long-distance transportation of high moisture content biomass. Centralized large-scale decarbonized biogas-to-electricity power plants can be established by the government to process huge quantum of the organic waste generated (Zhang

**Table 5** Performances of the various bioreactors for FVW treatment

Bioreactor type	Volume	Type of substrate	OLR (kg VS/m <sup>3</sup> /d)	HRT (days)	Efficiency VS red (%)	Biogas yield (m <sup>3</sup> kg <sup>-1</sup> VS)	Methane yield (m <sup>3</sup> kg <sup>-1</sup> VS added) or % CH <sub>4</sub>	References
Tubular reactor	(18 L)	FVW	6% TS	20	75.9	0.707	57% of biogas	Bouallagui et al. (2004)
Two-phase system	(18 L)	FVW	7.5 kg COD m <sup>-3</sup> day <sup>-1</sup>	20	96% of COD	0.705, 0.997 (35 and 55 °C)	64, 61 (35 and 55 °C)	Bouallagui et al. (2004)
ASBR	(2L)	FVW	1.24	20	79	0.48	60% of biogas	Bouallagui et al. (2009a, b)
ASBR	(2L)	Abattoir waste + FVW	2.56	20	86.2	0.73	62% of biogas	Bouallagui et al. (2009a, b)
Semi-continuous	(2 L)	FVW + SW + manure	1.3	30	1.36	0.32	56% of biogas	Alvarez and Liden (2008)
Batch	30 L	FVW	2.4–2.7 kg m <sup>-3</sup> day <sup>-1</sup>	30	80	0.42	0.25 (m <sup>3</sup> /kg TS)	García-Peña et al. (2011)
CSTR single phase	8 L	FVW + FW	0.5	40	NR	NR	0.544 ± 0.006 (CH <sub>4</sub> ) (L g <sup>-1</sup> TS (VS) added)	Shen et al. (2013)
CSTR double phase	5 L, 8 L	FVW + FW	0.5	10	NR	NR	0.478 ± 0.004 (CH <sub>4</sub> ) (L g <sup>-1</sup> TS (VS) added)	Shen et al. (2013)
CSTR (acid phase) + UASB (methane phase); two-phase	Acid—2L Methane—1	FVW	23.2 kg m <sup>-3</sup> day <sup>-1</sup>	3.56	NR	NR	0.348 (CH <sub>4</sub> ) (L g <sup>-1</sup> TS (VS) added)	Wu et al. (2016)
Two-phase LBR + CSTR	LBR – 70 L CSTR – 35 L	FVW	12.47% TS	5	70.90%	51.26 mL/(d g VS)	71% of biogas	Liu and Liao (2019)
Batch	2 L	FVW + OFMSW	19.94%	18	54.60%	493.8 Nml/gm VS	396.6 Nml/gm VS	Pavi et al. (2017)
CSTR	18 L	FVW + cattle slurry + chicken manure	3.19–5:01	21	NR	NR	0.23 to 0.45 m <sup>3</sup> CH <sub>4</sub> kg <sup>-1</sup> VS added	Callaghan et al. (2002)
Batch	15 L	FVW	2	80	81 ± 3% VS removal	NR	0.45 m <sup>3</sup> CH <sub>4</sub> /kg VS	Ganesh et al. (2014)
Batch CSTR	4 L	FVW + FW	3	178	83 ± 2% VS removal	NR	0.47 ± 0.04 m <sup>3</sup> CH <sub>4</sub> /kg VS	Lin et al. (2011)
Single phase	3 L	FVW	1.6	20	96.3% COD removal	NR	0.42 m <sup>3</sup> CH <sub>4</sub> /kg VS	Lin et al. (2011)
Single-phase CSTR	16 L	FVW	3.6	23	88% VS removal	NR	0.47 m <sup>3</sup> CH <sub>4</sub> /kg VS	Mata-Alvarez et al. (1992)
Single-stage batch process	100 L	FVW	2.1	14	83% VS removal	NR	0.37 m <sup>3</sup> CH <sub>4</sub> /kg VS	Verrier et al. (1987)
					-	-	435 NLCH <sub>4</sub> per kg VS feed	Di Maria and Baratta (2015)

Table 5 (continued)

Bioreactor type	Volume	Type of substrate	OLR (kg VS/m <sup>3</sup> /d)	HRT (days)	Efficiency VS red (%)	Biogas yield (m <sup>3</sup> kg <sup>-1</sup> VS)	Methane yield (m <sup>3</sup> kg <sup>-1</sup> VS added) or % CH <sub>4</sub>	References
Two-phase	6 L	FVW	7	-	97.5% VS removal	NR	0.30 m <sup>3</sup> CH <sub>4</sub> /kg COD	Ganesh et al. (2014)
Two-stage system: solid bed hydrolyzer and UASB		FVW	6.8	2.5	94% VS removal		0.35 m <sup>3</sup> CH <sub>4</sub> /kg COD	Rajeshwari et al. (2001)
Two-phase: acidogenic CSTR	Acid: 5 L	WAS/FVW (75:25 VS basis)	5.7	3 acid+10 methanogenic	40% VS removal	0.37 m <sup>3</sup> kg <sup>-1</sup> VS	0.25 m <sup>3</sup> CH <sub>4</sub> /kg VS	Dinsdale et al. (2000)
Methanogenic inclined tubular	Methanogenic 8 L		4.3	4 acid+13 methanogenic	44% VS removal			
Two-stage system: ASBR hydrolyzer and anaerobic filter methanizer	Acid: 2.5 L Methanogenic 10 L	FVW	4.4	7 acid+10 methanogenic	87% VS removal	-	0.34	Raynal et al. (1998)
Two-stage system: CSTR hydrolyzer and anaerobic filter methanizer	Acid: 7 L Methanogenic: 3.8 L	FVW	5.65 (g VS L day <sup>-1</sup> )	2 acid+2.3 methanogenic	96% VS removal	75	0.42 (L g <sup>-1</sup> VS)	Verrier et al. (1987)
Two-stage hybrid reactor	LBR: 1.8 L	FW + VW		30	For FW:VW (2:3) 53.96 FW:VW (2:1) 46.34	-	For FW:VW (2:3) 226.86 mL/g VS For FW:VW (2:1) 218.54 mL/g VS	Chakraborty and Venkata Mohan (2018)
Acidogenic: leach bed reactor (LBR) + methanogenic UASB	UASB: 2.5 L							
Two-stage hybrid reactor	Hydrolytic chamber—4.3 L Anaerobic chamber—16.586 L	FVW		4 days	90% COD removal	12.5 L/day	-	Chatterjee and Mazumder (2020)
Three-stage hybrid reactor	LBR—1.8 L	FW and VW		21	COD removal 78.17%	ALR—324.6 mL/kg of VS	106 mL/kg VS	Chakraborty and Mohan (2019)
LBR + airlift reactor + composter	ALR—2.5 L Composter (33 × 25 × 15 cm)							
UASB	24 L	FVW	10 g VS/(L day)	10	67	-	3.6 LCH <sub>4</sub> /(L day)	Vian et al. (2020)

et al. 2012). To enhance biomethanation, proper process control with monitoring of critical parameter can improve the process and boost the biogas output. Furthermore, the potential generation of biogas systems should be attempted to reduce the capital and management costs. For the AD approach to attain its full potential, policymakers should enforce standardization procedure that will encourage the redirection of “waste to landfill” to “waste to re-use” (Uddin et al. 2021) and the employment of low carbon gas technologies. The government should increase its support for biogas production and consider the possible potential for biogas production. With sustained efforts, biogas will be a remarkable solution for the depletion of GHG emissions, management of waste disposal, and production of renewable energy (Uddin et al. 2021).

## Conclusion

The present review reveals the suitability of FVW as a potent substrate for anaerobic digestion. AD is an environment-friendly technology with numerous advantages such as reduced carbon footprint, biofuel generation, and ability to treat large quantities of waste and low space requirement compared to other disposal methods. The treatment of massive amounts of fruit and vegetable waste by AD will help alleviate the prevailing clean energy crisis. Optimization of the operational parameters is critical for successfully applying AD at the laboratory or industrial scales. Moreover, stage separation during different phases of AD enhances biomethane yield. This may be attributed to the fact that multistage reactors can handle higher OLR and have a shorter retention time, with better synergy between the microbial consortia in each phase. Nevertheless, pre-treatment and co-digestion enhance the accessibility of the substrate by accelerating biodegradation and thus, enhancing the biomethane yield. However, the properties of the individual substrate must be ascertained prior to co-digestion to maintain synergy between the micro and macronutrients.

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## Declarations

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