Chapter 9
Effect of Short-Chain Fatty Acid Production on Biogas Generation

Marina Celant De Prá, Andréia Anschau, Cleverson Busso, Naiana Gabiatti and Marcelo Bortoli

Abstract The short-chain fatty acids (SCFAs) are generated in the acidogenesis step of the anaerobic digestion, and their production is very important for the global process and efficient biogas production. SCFA production takes place inside the cells of fermentative microorganisms, which are the first ones to start the complex soluble carbon degradation. The SCFA reactions are the most energetic among the anaerobic digestion steps, which means that this step will hardly be limiting for biogas production in normal conditions—except if the previous hydrolysis is rough or impaired. The SCFAs produced are subsequently converted to acetic acid, which is effectively converted to methane by methanogenic acetoclastic archeas. Nevertheless, acetic acid production from SCFA releases a large amount of hydrogen in the medium, and in some situations, it will reduce the process pH to inhibitory levels for methanogenic archeas and consequently suppress biogas production. This chapter will focus on these events, approaching SCFA formation, the functional microorganisms involved, and their importance for the global process.

Keywords Biogas production · Short chain fatty acids (SCFAs) · Acidogenesis

9.1 Introduction

As discussed in previous chapters, anaerobic digestion involves four processes: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. The individual degradation steps are carried out by different consortia of microorganisms. Through

M. C. De Prá (✉) · A. Anschau · N. Gabiatti
Federal University of Technology—Parana, Campus of Dois Vizinhos,
Dois Vizinhos, PR, Brazil
e-mail: marinapra@utfpr.edu.br

C. Busso
Federal University of Technology—Parana, Campus of Toledo, Toledo, PR, Brazil

M. Bortoli
Federal University of Technology—Parana, Campus of Francisco Beltrão,
Francisco Beltrão, PR, Brazil

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these steps, and depending on the balance among them, complex polymers are bioconverted to biogas. To study and detail the effects of short-chain fatty acids (SCFAs) on biogas production, this chapter will focus mostly on the first two steps, hydrolysis and acidogenesis processes, since they are the most important and responsible for SCFA formation.

The biogas quality is directly related to the final methane concentration formed by the anaerobic digestion process. On the other hand, methane formation is dependent on the presence of SCFAs, because they are the methanogenesis substrates. Several studies have already observed that in addition to dependence, methane production is also improved with higher concentrations of SCFA. This is mainly because methanogenic microorganisms become more active when there are increases in the SCFA concentration (Buyukkamaci and Filibeli 2004).

The straight SCFA amount influence on methane production has been widely studied, and most of these studies correlate methane production with the total concentration of SCFAs, which is also called as total volatile acidity (Anderson and Yang 1992).

In general, the influence of SCFAs on biogas production is not limited only to the amount that is present in the same medium during anaerobic digestion. Several studies show that there is a correlation among the quantity of SCFAs and other process variables associated with methane production, such as organic loading rate (OLR) (Rincón et al. 2008; Nagao et al. 2012; Jiang et al. 2013; Ferguson et al. 2016), pH (Zhang et al. 2009; Endres and Barberg 2007; Dai et al. 2013; Dong et al. 2016), and hydraulic retention time (HRT) (Dong et al. 2016; Koch et al. 2015, 2016). The temperature associated with SCFA concentration also influences methane production, in an indirect way, though. The decrease in temperature causes a decrease in microbiological activity in general, leading to an inhibition in all activities of anaerobic digestion steps and consequently in the methane production and biogas generation (Bowen et al. 2014).

Despite its relevance, the amount of SCFA may be inhibitory for anaerobic digestion, especially in imbalanced or low alkalinity systems. When there is an abrupt decrease in the methanogenic microorganism activity or SCFA production is somewhat uncontrolled, SCFA accumulation may occur in the medium, causing a reduction in the systems' pH. This pH reduction can reach critical acidity values and cause inhibition of the anaerobic digestion process. Irreversible inhibition levels could be reached, causing a system collapse (Chen et al. 2008; Intanoo et al. 2016).

Therefore, it is important to understand that the operational conditions, the key control factors, and its direct relationship with the SCFA concentration are determinant for the efficient biogas production and success of anaerobic digestion. SCFAs are substrate for biogas generation and concomitantly can cause imbalances in the buffer system medium until process inhibition, which justifies a better understanding of the SCFA mechanism formation, interferences, and functional microorganisms responsible for biochemical reactions, as we will see in the following topics.

9.2 Mechanism and Principle of SCFA Formation

The SCFA production process is performed by different microorganisms and goes through several transformations until its formation. The SCFA generation reaction can be described as below (Zygmunt and Banel 2009).

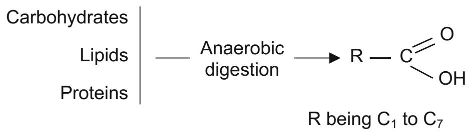

Figure 9.1 shows a graphical representation of the different steps of anaerobic digestion and its metabolic pathways in a simplified way. In the case of SCFA production process, the limiting step is represented by the hydrolysis, in which the majority of the complex and polymerized organic compounds must be degraded

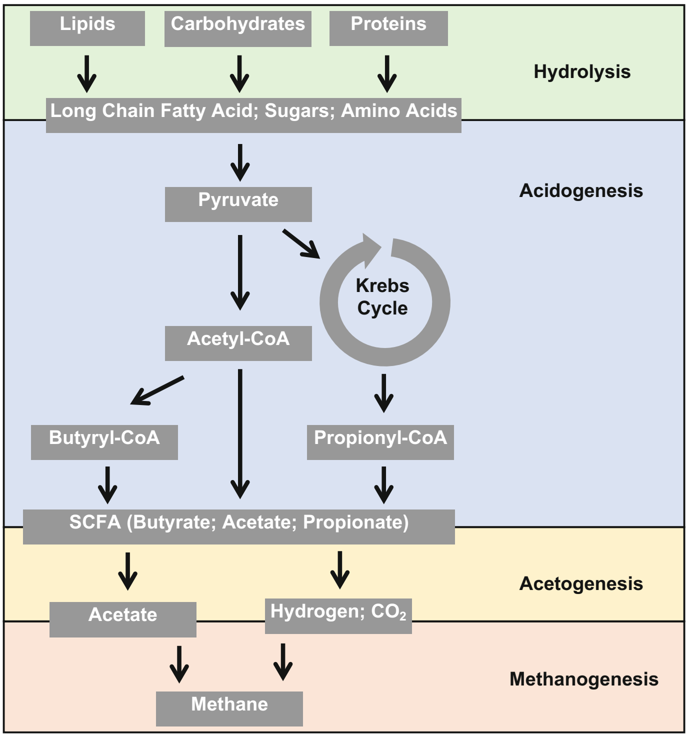

Fig. 9.1 Schematic pathway of anaerobic fermentation for SCFA production from metabolic pathway intermediates. In the diagram are indicated the four stages for the biogas formation (hydrolysis, acidogenesis, acetogenesis, and methanogenesis)

into simpler products. During hydrolysis, the hydrolytic bacteria, using extracellular enzymes, cleave organic compounds, such as cellulose, hemicellulose, starch, pectin, proteins, and lipids, to smaller compounds such as amino acids and volatile fatty acids (VFAs) also known as long-chain fatty acids, LCFA (Evans and Furlong 2011).

Subsequently, in the acidogenesis step (Fig. 9.1), these simplest organic compounds are transformed again. During acidogenesis process, sugars and the other compounds generated in the previous step, including long-chain fatty acids, and other low-molecular weight molecules are bioconverted to SCFA, CO_2, H_2, NH_3, SO_4^{-2}, and alcohols by the acidogenic bacteria activity. SCFAs are the major nutrients produced by acidogenic microorganisms in charge of solubilized hydrolysis product metabolization. The bacteria involved in this step proliferate very quickly, about 30–40 times faster than methanogens, and can survive in extreme conditions such as low pH, high temperatures, and high organic loads (Amani et al. 2010). SCFAs produced during acidogenesis include formic acid (CH_2O_2), acetic acid ($C_2H_4O_2$), propionic acid ($C_3H_6O_2$), butyric and iso-butyric acid ($C_4H_8O_2$), iso-valeric and valeric acid ($C_5H_{10}O_2$), caproic and iso-caproic acid ($C_6H_{12}O_2$), and heptanoic acid ($C_7H_{14}O_2$) (Tchobanoglous et al. 2013). Figure 9.1 shows the steps of biochemical conversion of the acidogenesis products into other compounds for the biogas production (acetogenesis and methanogenesis).

Despite the acidogens' adaptation ability to such harsh circumstances, it is extremely important to control and monitor the operational conditions to ensure the proper process progress. Acidogenesis is influenced by several factors such as substrate characteristics, environmental conditions, and other parameters such as temperature, pH, medium agitation, retention reactor time, and the organic volumetric load applied (Gavala et al. 2003; Jiang et al. 2013). Furthermore, an unbalanced environment is not favorable to methanogenesis, thus avoiding the consumption of the produced SCFA for methane formation (Zhang et al. 2009).

The phenomenon that interrelates the biochemical and microbial activities of acidogenic and methanogenic community, controlling the global function of the reaction, is known as syntrophy (McInerney et al. 2009). Considering a stable reactor operated under optimal conditions for microbial growth, in the absence of stress factors, acidogenic, acetogenic, and methanogenic steps should be synchronized, so that there will be an equilibrium in the intermediate compounds' production and consumption rates.

On the other hand, the accumulation of acetate and hydrogen in the dissolved phase could lead to thermodynamic inhibition of important metabolic conversions. The medium acidification resulted from SCFA accumulation contributes to alkalinity consumption, and pH decreases intensification (Yuan et al. 2015). This explains why volatile fatty acids, pH, and alkalinity are important indicators of anaerobic reactors stability.

Knowing the mechanisms involved in SCFA production and consumption as well as operation control strategies is the key for maintaining the system stability and process efficiency. These are topics that will be covered in the following topics.

9.3 Key Control Factors

Particular features, such as substrate and product composition, as well as bioreactor types generally drive the operation conditions for SCFA production. Regarding the bioreactors and the rate of biomass decomposition, Lee et al. (2014) recommended that the batch or semi-continuous operation mode is better than the continuous system for anaerobic sludge bed reactor (UASB), packed bed biofilm reactor (PBBR), and anaerobic fluidized bed reactor (AFBR).

Besides the operation mode, the ideal operating conditions such as pH, temperature, organic loading rate (OLR), and retention time vary largely according to substrate conditions and different types of bioreactor systems. Some specific actions, such as hydraulic flushing and sludge pre-treatment, can help in the bioreactor acidification process and hence maximizing the SCFA production through anaerobic digestion.

9.3.1 Substrate Influence on SCFA Generation

The rapid-to-moderate biodegradable matter content of matter, such as carbohydrates, proteins, and lipids, is related to the microorganisms' development and, therefore, affects qualitatively and quantitatively the intermediates and biogas production (Maciel and Jucá 2011). According to Zhang et al. (2012), the substrate volatile solids concentration (SV) also refers to its biodegradable organic matter. Therefore, solids characterization can be a reliable low-cost alternative parameter to evaluate a substrate SCFA and biogas potential generation.

Substrates rich in organic matter like waste-activated sludge (WAS) and primary sludge (PS) obtained from municipal wastewater treatment plant have been considered as potential sources for SCFA production due to the great volumes generated from biological wastewater treatment (Jiang et al. 2007a, b). Regardless the substrate organic matter concentration, an important reference to ensure the system balance is the ratio of SCFA $\times$ alkalinity in the medium which, according to Sánchez et al. (2005), should be around 0.1 and 0.5.

Table 9.1 shows the different SCFA compositions after the acidogenesis step according to the substrate type and characteristic. Wastes rich in protein, also meaning high nitrogen content, present a high organic matter, high biological oxygen demand (BOD), and low C/N ratio (Esposito et al. 2012). In general, acetic acid is the dominant SCFA. Authors show that the SCFA composition varies from 20 to 75% acetic acid, followed by propionic acid with concentrations varying between 3 and 35%, butyric acid between 8 and 35%, and other SCFAs to a lesser extent (Q. Yuan et al. 2011; Ucisik and Henze 2008).

Table 9.1 SCFA proportions in function of different substrate characteristics

	Acetic (%)	Propionic (%)	Butyric (%)	Other (%)	
Food waste	66.9	3.7	29.4	–	Jiang et al. (2013)
Food waste	37.6	20	32.1	10.3	Yin et al. (2014)
Waste-activated sludge	26–31	43–49	14–18	–	Ucisik and Henze, (2008)
Waste-activated sludge	24.7	14.3	24.6	36.4	Xiong et al. (2012)
Waste-activated sludge	41–69	8–29	9–21	11–15	Yuan et al. (2011)
Swine manure	56.7	19.7	11.8	11.8	Bortoli et al. (2013)
Dairy cattle manure	72.8	16.8	10.4	–	Patni and Jui (1985)
Sewage sludge	47.0	47.6	3.7	1.8	Buyukkamaci and Filibeli (2004)

9.3.2 Temperature

Temperature plays an important role on SCFA accumulation by anaerobic digestion. Q. Yuan et al. (2011) reported SCFA accumulation by waste-activated sludge (WAS) at distinct temperatures (4, 14, and 24.6 °C). The highest SCFA production (2154 mg/L) was achieved at 24.6 °C. Lower temperatures resulted in 782 mg/L (4 °C) and 2149 mg/L (14 °C) of SCFA. The yield of SCFA was also improved at psychrophilic and mesophilic temperature ranges (Q. Yuan et al. 2011; Zhuo et al. 2012).

The results obtained by these authors could be explained by the higher carbohydrates and protein solubility at high temperatures. The hydrolysis rate also increased at higher temperatures (Liu et al. 2012).

Some authors observed that temperatures variation during production process had no straight influence on the produced SCFA type. Q. Yuan et al. (2011) also verified that the composition of the SCFA produced at 4, 14, and 24.6 °C presented no significant modifications. However, the authors reported that mild temperature increases leaded to a reduction in the acetate production, while butyrate and propionate had its production improved in the same condition.

Zhuo et al. (2012) investigated the influence of temperature on ultrasonic pre-treated WAS cultivation at alkaline pH. The results indicated no significant variation in the SCFA is produced. In another study, the temperature increase up to 70 °C did not result in positive effect on SCFA production (Yu et al. 2013). On the other hand, Zhuo et al. (2012) reported a reduction of 40 °C in total SCFA production when the temperature decreased from 40 to 37 °C.

It should be highlighted that microorganisms in different waste materials will present significant differences in growth rate at diverse temperatures. In addition, the changes identification in distinct microorganisms' growth rates could be a promising investigation study in the temperature influence in SCFA production.

9.3.3 pH

The primary factor that directly affects the content of SCFA produced is the amount of organic matter being hydrolyzed. Just like substrate composition, pH has a significant function in the yield and production rate of SCFA in anaerobic digestion.

When different pH values were compared to test SCFAs accumulation in excess sludge, alkaline conditions (pH = 10) were able to maximize the SCFA content (Jie et al. 2014). This result was supported by another study (Wu et al. 2010) in which primary sludge was used in alkaline fermentation for SCFAs formation.

Acidogenic microorganisms are inhibited in low pH (below 3) or high pH (above 12) (Liu et al. 2012). Although it was mentioned above that the optimal pH for sludge hydrolysis was around 10, the waste source used may require a diverse pH value, which can vary from 5.25 to 11 (Lee et al. 2014). Anaerobic digestion of kitchen waste resulted in optimum pH of 7.0 (Tang et al. 2016) whereas for wastewater treatment the optimum pH was around 6.0 (Bengtsson et al. 2008).

Acidic or alkaline conditions can affect the acidogenic (Zhang et al. 2012) and methanogenic (Ghosh et al. 2000) microorganisms activity in SCFA production from WAS anaerobic fermentation. Yuan et al. (2006) showed that the accumulation of SCFA from WAS was greatly enhanced in alkaline systems (such as pH 10). Nevertheless, in the full-scale sludge treatment plant, the pH control during the whole process is still challenging. Kang et al. (2011) studied the effect of initial pH adjustment on sludge hydrolysis and acidification by ultrasonic pre-treatment.

The highest SCFA concentration in anaerobic digestion by the excess of sludge can also be determined by fermentation with inoculum and the reactor hydraulic retention time (HRT), resulting in changes at the optimum pH of the process. For example, Wang et al. (2014) investigated the influence of pH on different inoculum types, in eight different batch bioreactors, over 20 days of fermentation. The results indicated the maximum yield (918 mg/g VSS removal) and concentration (51.3 g-COD/L) for SCFA at pH of 6.0.

For SCFA generation, the SCFA to soluble chemical oxygen demand (SCOD) ratio is referred to the number of soluble components converted into SCFAs (Jiang et al. 2013). Investigations showed that applying a pH range from 5.0 to 6.0 resulted in the highest ratio value of SCFA to SCOD (75%), regardless of the type of inoculum used while producing SCFA from food waste. Nevertheless, this study did not include the results concerning an extreme alkaline level (pH > 10) (Wang et al. 2014).

Zhao et al. (2015) showed that an initial pH of 11 was the optimum condition for SCFA accumulation. Furthermore, the authors verified that the activity of specific enzymes for SCFA generation at alkaline conditions was higher than in acidic or neutral pH.

Although the composition of SCFA produced primarily depends on the substrate composition, changes in pH levels can also affect the SCFA produced at acidogenic fermentation (Lee et al. 2014). Prior to the selective production of any specific SCFA, the optimum pH value needs to be determined.

9.3.4 Retention Time

In anaerobic digestion using waste materials, the microorganisms and the retention time in the reactor are considered as key control factors of the process. Retention time refers to solid retention time (SRT) and HRT to the allocated time for selected prevalent microorganisms and the reactor volume, respectively. SCFA production is affected more by the HRT than to the temperature during the fermentation (Kim et al. 2013).

High HRT provides enough time for the acidogenic bacteria to convert the waste into a soluble component, and consequently, it favors the SCFA yield (Bengtsson et al. 2008). In terms of system, the HRT depends on the composition and type of substrate. In anaerobic LBR digesting a substrate with a high solid matter, an HRT of 1.5 days was used for SCFA accumulation (Cysneiros et al. 2012). In other studies, an HRT of 1.9 days was the best performance for the acidogenic step of the organic fraction of municipal solid waste (OFMSW) (Romero Aguilar et al. 2013).

Prolonged HRT reached to SCFA accumulation. An investigation produced SCFA by acidogenic food fermentation (Lim et al. 2008). The results showed that the SCFA production was higher as the HRT increased up to 192 h, but no further increment in SCFA content was observed until 288 h.

It has been also verified that the methanogenic microorganism's growth rate is slower when compared to the acidogens' growth rate. Low SRT does not let sufficient time for the methanogenic microorganisms to consume SCFA producing carbon dioxide (CO_2) and methane (CH_4) (Lee et al. 2014). The acidogenic microorganisms also require a minimum SRT to hydrolyze the substrate. A long SRT provides enough time to the methanogenic microorganisms activity and consequently improve the biogas production. Wastewater treatment system using submerged anaerobic membrane bioreactors (SAnMBRs) has an SRT going from one to three months (Huang et al. 2013; Khan et al. 2016).

9.3.5 Organic Loading Rate

The OLR is affected by the substrate type and composition of as long as the bioreactor arrangement. Yet, no direct relation has been reported concerning the production rate or yield of SCFA by changing the OLR. Meanwhile, the bias of SCFA production could be foreseen by changes in the OLR (Khan et al. 2016).

Lactic acid fermentation shows that the amount of lactic acid increased raising the OLR. The lactic acid content reached 37.6 g/L at the same time as the OLR rose up to 18 g-TS/L/day). In contrast, the increment of the OLR until 22 g-TS/L/day resulted in a decrease of acid production to 22 g-TS/L/day. These outcomes could be related to the reduction of the hydrolysis rate if the OLR exceeds the optimum level (Tang et al. 2016).

A fermentation research is tested an olive oil mill solid waste (OMW) and different OLRs. In this study, at OLR of 12.9 g-COD/L/day the production of SCFA was maximized (Rincón et al. 2008). A similar trend was observed using food waste as substrate. An increment in the SCFA generation was obtained at an OLR up to 13 g/L/day; however, above this rate the bioreactor became inconstant. Summarizing, the SCFA production was enhanced by the initial increment in OLR and the production rate decreased when OLR was raised later in the process, regardless of the substrate and its composition (Lim et al. 2008). Nevertheless, additional studies are required to characterize the optimum range in OLR together with substrate used and the type of bioreactor.

9.3.6 Bioreactors for SCFA Production

The mechanisms for SCFA production most used are suspended and attached growth (Tchobanoglous et al. 2013). Both of these types of growth technologies have been used in several bioreactor models. In packed bed bioreactor (PBR), cell mass is attached to packing material but can implicate in clogging. The fluidized bed bioreactor (FBR) is an efficient alternative to prevent the clogging issue, and the cell mass grows linked to a small solid-like sand keeping in suspension by the upstairs fluid movement (Grady et al. 2011). Moreover, the continuous stirred tank reactor (CSTR) is the ideal reactor for completely mixing waste and microorganism cells.

In order to produce SCFAs, reactors can be projected to produce SCFA as primary products (Wang et al. 2014) or as by-products (Peces et al. 2016). Several bioreactors have been designed and afforded promisor SCFA production such as sequencing batch reactor (SBR) (Frison et al. 2013), anaerobic leach bed reactor (LBR) (Cysneiros et al. 2012), packed bed biofilm reactor (PBBR) (Scoma et al. 2016), continuous flow reactor (J. Luo et al. 2014), CSTR (Bengtsson et al. 2008), expanded granular sludge bed (EGSB) (D. Zhang et al. 2011), and two-stage thermophilic anaerobic membrane bioreactor (TS-TAnMBR) (Wijekoon et al. 2011).

Anaerobic fermentation using membrane bioreactor (MBR) for SCFA accumulation has attracted a lot of attention, aiming to produce value-added products and reduce the volume of waste simultaneously (Zhao et al. 2015). In 2017, the global MBR market was around USD 1.54 billion, and by 2026, it is expected to come to USD 5.59 billion (Credence Research Inc 2018).

9.3.7 Other Parameters

Besides the process optimization parameters, other measures can improve the SCFA production rate and yield. Some studies indicated that the hydraulic flush rises the SCFA production by 32% in buffered LBRs where digested substrate was used with high solids matter (Cysneiros et al. 2012).

Feng et al. (2009) reported that the content and composition of SCFA are affected by the carbon to nitrogen ratio (C/N) of the substrate. Moreover, chemical additives can increase significantly the SCFA production. The addition of biosurfactant, for instance, can significantly increase the SCFA content. Huang et al. (2015) reported that the use of surfactin or rhamnolipid can significantly improve the SCFA concentration alkyl polyglycoside (APG seems to be a promissory for SCFA production from WAS). Zhao et al. (2015) related a production of SCFA up to 280 mg of chemical oxygen demand (COD) per gram of volatile suspended solids (VSS) using the biosurfactant APG in a concentration of 0.2 g/g dry sludge (DS) in a MBR sludge anaerobic fermentation. Furthermore, APG can also reduce the fermentation time.

Ji et al. (2010) observed a hydrolysis increase when the surfactant sodium dodecylbenzene sulfonate (SDBS) was added to the mixture of WAS and PS. The increment of the sludge hydrolysis reached higher SCFA accumulation. The addition of SCFA also affects its composition, but as this occurs it is not yet elucidated.

Sodium dodecyl sulfate (SDS) is an anionic surfactant effectively used to speed up the solubilization of WAS and increase the concentrations of proteins and carbohydrates in the aqueous phase. Jiang et al. (2007a, b) related that the SCFA accumulation increased significantly using SDS. Using 0.1 g/g of SDS, a total SCFA concentration of 2243.04 mg COD/L was obtained. Luo et al. (2011) studied the combined effect of enzymes (neuter protease and a-amylase) and SDS on the acidification and hydrolysis of WAS. The authors observed that the composition of the SCFAs produced in SDS plus enzyme systems suggested that acetic acid, propionic acid, and iso-valeric acid were the main SCFAs in WAS hydrolysate.

9.4 Functional Microorganisms Involved

The accumulation of short-chain fatty acids (SCFAs) in sewage sludge systems can be increased when the anaerobic digestion process occurs under alkaline conditions. However, it is observed that temperature variations under different pH conditions result in different levels of SCFA, as well as the constitution of the local microbiota. Analysis with fluorescence in situ hybridization (FISH) and PCR-based 16S rRNA gene clone library have identified that the ratio of bacteria to archaea as well as bacteria producing SCFA change in the following ratio: 20:1 (ambient temperature without pH control); 68:1 (ambient temperature at pH 10); 101:1 (mesophilic at pH 9) and 177:1 (thermophilic at pH 8) (Zhang et al. 2010).

Mostly, anaerobic bacteria producing SCFA are members of the Clostridia class, which belong to Firmicutes phylum. In digesters under thermophilic and mesophilic conditions supplied with corn silage, cattle, or pig manure, it was possible to isolate acetic, propionic, and butyric acid forming bacteria. The great majority of isolated bacteria were related to the genus *Clostridium*, e.g., *C. thermoamylovorans, C. sporosphaeroides, C. aminovalericum,* and *C. cochlearium/C. tetani* (Cibis et al. 2016). Acetic acid is produced anaerobically by these microorganisms via the glycolytic pathway and pyruvate intermediates. It is usually a co-product of bacteria such as *Clostridium* and *Propionibacterium* genus that synthesize propionic and butyric acid as major compounds (Baumann and Westermann 2016).

Propionibacterium species utilize the glycolytic pathway and pyruvate to synthesize butyric acid and also succinate intermediates to produce propionic acid (Fig. 9.2) (Wang et al. 2013; Rogers 2006; Dahiya et al. 2018).

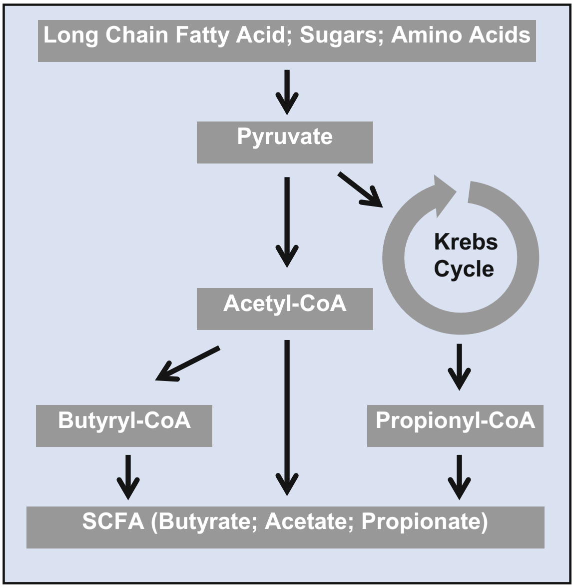

Fig. 9.2 Schematic pathway of short-chain fatty acids production from metabolic pathway intermediates

As previously mentioned, although it is possible to increase the SCFA concentration—especially acetic, propionic, and butyric acid—the increase and accumulation of these substances can result in a reduction of pH, damaging the whole system. An alternative would be blocking the supply of new substrates, which is often not the best option, since optimization for continuous system operation is more advantageous. The separation of acidogenesis and methanogenesis may be an alternative to control the acidification process and consequently prevent the reduction of the population of methanogenic microorganisms during the methane generation stage (Fu et al. 2017).

A study from India demonstrated to be possible to obtain an enriched inoculant from a wastewater bioreactor residue. The use of this inoculant in an anaerobic digestion system allowed an increase in the production of acetic acid and SCFA from food residues adjusted to pH 10. Selective enrichment of the inoculant microbiome was obtained by means of acidic shocks and the operation of the system at alkaline pH. This pre-treatment induced the prevalence of acidic Firmicutes spores and fatty acid-forming Bacteroidetes which together with saccharolytic (*Soehngenia saccharolytica*) and proteolytic bacteria (*Bacillus cellulosilyticus*) induced the effective digestion of complex carbohydrates. Alkaline biodigestion seems to benefit the phosphoroclastic pathway by enhancing the production of acetate and H_2 by the microorganisms, since the pre-treatment of the inoculant agent promotes the acidogenic pathway with parallel inhibition of the methanogenic bacteria without affecting the H_2-producing bacteria (Sarkar et al. 2016).

Proteomic and sequencing analyses of the 16S-rDNA region in a biodigestor containing grass identified a high activity of microorganisms using sugars and producing SCFA in the acidogenic phase. The study was conducted in a two-stage production system under mesophilic conditions (37 °C) and thermophilic conditions (55 °C). In the samples of the mesophilic biodigestor, the prevalence of microorganisms of Bacteroidetes and Firmicutes phyla was observed, producing glycolytic proteins associated with degradation and catabolism of sugars. Among the 1700 proteins quantified at this stage, approximately 120 were expressed at different levels when compared to the 5 thermophilic proteins. However, thermophilic proteins have a high degree of representativity of chaperones, which makes them more stable and suggests their fundamental role in stability on stressful conditions of biodigestion (Abendroth et al. 2017).

Although biodigestion for biogas production can be carried out with different types of plant biomasses, a predominance of Firmicutes during the acidogenesis phase can be observed in several studies. This bacterial phylum has a great enzymatic versatility, mainly related to the decomposition of xylans, among them xylanases, xylosidases, and cellulases (Hassa et al. 2018). However, what is observed in a biomass degradation system is a microbial consortium acting together, some secreting free enzymes with different substrate specificities and others, such as the bacterium *Clostridium thermocellum*, constitute a group that secretes subunits of cellulases and xylanases in large multienzymatic complexes known as cellulosomes (Herpoël-Gimbert et al. 2008; Raman et al. 2009).

Metagenomic studies in a microbial consortium called EMSD5 of corn straw identified a population composed mainly of members of the phylum Proteobacteria, Bacteroidetes, and Firmicutes. In the last group, synergistic population activity was attributed to the degradation of polysaccharides in plants, mainly by the expression of xylan-degrading enzymes, including xylanases, β-xylosidases, α-L-arabinofuranosidases, α-glucuronidases, and acetyl xylan esterases. The synthesis of these enzymes is associated with the generation of most SCFA. The study of the microbial composition in a substrate and the association of the enzymes synthesized by these microorganisms are extremely important for the understanding of regulating and optimizing mechanisms in the production of SCFA in acidogenesis.

Although bacteria and archaea are the main constituents of the microbiome in a biodigestor, there are studies suggesting that fungi, although in a smaller amount, have a relevant role in the biodigestion process. Due to its excellent adaptability and versatility in the production of different enzymes, fungi can contribute to the initial hydrolysis process in a biogas reactor. *Saccharomyces cerevisiae* is known to increase the number of viable total cells of cellulolytic bacteria in the cow rumen (Lila et al. 2004). It is believed that this effect can occur in a biodigestor, where the yeast could contribute to increase the number of cellulolytic bacteria optimizing the cellulose degradation process from the plant biomass. This fungus has already been found in a bioreactor containing substrates with more than one year of activity (Bengelsdorf et al. 2012).

Fungi can also be used as pre-treatments in processes to accelerate the decomposition of lignocellulose. Aerobic digestion may be employed in this process, since this constituent of the plant biomass is composed of recalcitrant material, such as cellulose (40–50%), hemicellulose (20–40%), and lignin (5–30%). The use of fungi in pre-treatments of biomass is an alternative to existing physical treatments that present high costs, and can also be a substitute for chemical treatments, which are known to generate toxic products in the digested material inhibiting the microbial activity in the steps of hydrolysis and fermentation.

The use of the fungus *Trichoderma viridae* as aerobic pre-treatment in a municipal waste substrate (plant material, paper, coffee beans, tree debris) resulted in a threefold increase in methane production using a laboratory scale. The availability of nutrients promoted by the cellulolytic enzymatic action of the fungus favors the continuity of the anaerobic digestion process, allowing a greater hydrolytic activity promoted by a bacterial consortium. However, the presence of high moisture content for cellulases is essential in lignocellulosic substrates. The low moisture content inhibits cellulase production due to the lower solubility index of the substrates, preventing swelling and promoting high water stress (Kalogeris et al. 2003; Ghanem et al. 2000).

Acidogenesis is a fundamental step of anaerobic digestion; it is at this stage that the greatest amplitude of microbial diversity occurs, since there is the joint work with the microorganisms involved with the hydrolysis. Factors such as the origin and product resulting from the hydrolysis, pH, temperature, and composition/capacity of the enzymatic activity produced by the microbial consortium end up limiting the subsequent step influencing the quality and quantity of biogas.

After all the information presented in this chapter, it is expected that the effect of SCFA formation and its interference on biogas production has been clarified. This is a small and important part of a larger anaerobic digestion process, which needs more advanced research and still has unresolved challenges. The search for new alternative energy sources and the vision of future development should be a scientific priority in the next decades. About SCFA, controlling their production with the aforementioned alternatives, it is possible to overcome the technological challenges and to improve biogas production.

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