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REVIEW

Recent advances on anaerobic digestion of swine wastewater

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Abstract
Anaerobic digestion is a valuable technology for the elimination of biodegradable pollutants and stabilization of agro-industrial wastes. This review focuses on the AD of wastewaters derived from swine production within the scope of wastewater treatment and bioenergy production. Fundamental principles of AD as a biochemical and microbiological process are described. The effect of essential parameters is also examined in the context of swine wastewater, especially the relation between chemical and operational factors, process stability and performance. The review continues with a discussion of current trends and future achievements in AD research of swine wastes, namely co-digestion with carbon-rich substrates, integration with pretreatment methods, process enhancement via the use of additives, and digestate management. Finally, a brief outlook on the economy of biogas utilization is provided to better understanding the future of AD as biotechnology for on-farm treatment of swine wastewater.

Keywords Anaerobic digestion · Swine wastewater · Microbiology · Inhibition · Biogas · Digestate

Introduction

Livestock production industries are one of the most traditional agricultural subsectors in Europe, with primary importance in the economy of several European countries. Agro-industries such as pig production farms have recently been in the spotlight regarding waste management due to intensive methods of production and high polluting loads of the wastes generated (Bayo et al. 2012; Lahav et al. 2013; Shen et al. 2015). As industrial facilities, pig production farms generate significant amounts of animal waste with high concentrations of organic and inorganic compounds, which are particularly harmful to the environment (Girard et al. 2009; Xu et al. 2016). With environmental regulations becoming tighter in recent decades, combining the historical focus on wastewater treatment with waste to energy has become a matter of growing interest in the industry. Another driving force has been the need to increase profit margins and competitiveness. As such, using swine wastewater (SW) for bioenergy production offers a promising alternative to deal with both economic and environmental issues as a way of reducing production costs and ecological impacts in large farms (Jimenez et al. 2015; Araújo et al. 2016).

Biological processes are by far the most popular traditional methods for wastewater treatment in agro-industries. Among these technologies, anaerobic digestion (AD) has presented high effectiveness in the elimination of biodegradable pollutants from complex agro-industrial effluents, as well as bioenergy production through biogas. As the biotechnological application of methanogenesis, AD is a valuable pathway to stabilize wastewaters with high polluting loads, such as the liquid fraction of pig production effluents. The process is mediated by complex microbial communities which degrade the organic matter in the absence of oxygen, thus yielding biofertilizers and a mixture of gaseous end products comprising mainly CH_4 and CO_2 (O'Flaherty et al. 2010). In addition, AD provides several benefits outside the potential for energy recovery such as cost-effectiveness, and less sludge production when compared to other biological treatments. Low energy requirements, higher odor control, and enhanced organic matter removal as measured by biological oxygen demand (BOD_5) are other advantages mentioned in the literature (Dupla et al. 2004; Ward et al. 2008). Despite the importance of the process, full-scale digesters often face stability issues due to the complex biochemical

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and microbiological phenomena occurring inside (Dupla et al. 2004). In fact, poor practical and operational stability are two of the main bottlenecks preventing AD from being fully used in agro-industries (Amani et al. 2010). An overall assessment of relevant research progress is, therefore, essential for further development. Research activities have recently taken a step forward by paying particular attention to process performance and stability, including modeling and optimization (Wu et al. 2013; Jurado et al. 2016; Yang et al. 2016). Another focus has been the study of process inhibition and toxicity (Cerrillo et al. 2016; Riya et al. 2018). The aim of the present paper is thus to overview the most relevant applications of AD in the treatment of SW to understand the advantages and limitations of the process for on-farm application. The first sections outline fundamental biochemical principles, process microbiology, and recent achievements in terms of chemical and operational parameters. The review ends with a discussion of current trends and future developments in the context of SW anaerobic degradation, including pretreatment, process enhancement through co-digestion and additives, digestate management, and economic aspects of biogas utilization.

SW composition and environmental impacts

Chemical composition is fundamental to determine whether wastes can be used as a raw material to produce energy or valuable products (e.g., biofertilizer, energy carrier). Generally, SW contains suspended solids, organic matter (biodegradable, but also refractory), and micronutrients from animal food digestion, i.e., animal excreta in the form of urine and feces. In addition, wastes derived from pig houses usually contain the remains of food, bedding (straw, sand, sawdust), and washing waters (Villamar et al. 2012; Marszałek et al. 2014; Makara and Kowalski 2015). Based on this, wastewater composition greatly varies depending on farm characteristics, such as animal feed, housing system, manure management practices, and environmental regulations. Wide variations intra-farms and inter regions are prevalent, and a broad range of values have been reported in previous studies (Villamar et al. 2012; Hai et al. 2015; Córdoba et al. 2016).

Nevertheless, a complete picture of SW composition can be given by reviewing the literature. Important parameters in SW characterization include pH and concentration of organic and inorganic components. The physical and chemical properties of the particles are also important to optimize different treatments. Generally, SW has low total solids content (TS< 12%), with about 70–75% comprising organic materials in the form of volatile solids (VS) (Christensen et al. 2009). Carbohydrates compose the most significant fraction of the organics present, followed by proteins, lipids, lignin, and volatile fatty acids (VFAs) (Jensen and Sommer 2013). Indirect measures of the presence of organic compounds include BOD_5 and chemical oxygen demand (COD). SW has high values of both parameters depending on farm circumstances (6500–7200 mg L^{-1} and 4684–63,724 mg L^{-1}, respectively) (Villamar et al. 2012; Hai et al. 2015; Córdoba et al. 2016). Regarding the pH, SW is usually neutral (6.8–7.3) due to the presence of short-chain VFAs. Particle charge and ionic strength are generally high due to the presence of salts and affect the electrical potential around the particles. Conductivity often exceeds 10 mS cm^{-1} as a consequence (Hjorth et al. 2011). These wastewaters also contain high amounts of total nitrogen (TN, 1062–2222 mg L^{-1}) and total phosphorus (TP, 32–181 mg L^{-1}) as pigs usually excrete a relatively high proportion of the N and P intake (Villamar et al. 2012; Hai et al. 2015; Córdoba et al. 2016). Nitrogen occurs in organic combinations (proteins, amino acids, urea, uric acid) and mineral combinations (ammonia, nitrates). Dissolved ammonia is the most common nitrogen form present (948–1558 mg L^{-1}) (Villamar et al. 2012; Hai et al. 2015; Córdoba et al. 2016). P compounds are generally present in the particulate fraction of the waste. Less than 30% is dissolved in the liquid phase, with more than 80% in the form of orthophosphate (PO_4^{3-} (aq)) (Christensen et al. 2009). SW also contains high amounts of Cu and Zn. Other trace elements such as Fe, Mn, Cd, and B are also present.

Based on this chemical composition, inadequate SW management poses severe environmental impacts. As an example, SW is considered a significant cause of point source pollution from uncontrolled disposal as high amounts of N, P, and organic matter can contaminate water bodies and cause regional eutrophication and soil overfertilization (Chen et al. 2009). Waste streams derived from pig production are also a source of odors, gas emissions (methane, ammonia), steroids, antibiotics, microbiological, and heavy metal contamination, which can contribute to climate change and lead to human health hazards (Aarnink and Verstegen 2007; Chen et al. 2009; Sagastume Gutiérrez et al. 2016). Farmers are trying to adopt efficient technologies for effluent processing and management to mitigate these problems. As already mentioned, the most common approach to improve the characteristics of SW is anaerobic digestion.

Anaerobic degradation of SW

Microbiological fundamentals

Metabolism of SW by anaerobic microorganisms

AD is a well-established technology which involves a complex multistage biochemical process promoted by anaerobic microbial communities. Several descriptions relating the microbiology to the biochemical reactions occurring inside the reactor can be found by reviewing the literature. Generally, the process is systematized in three main stages performed by different metabolic groups of microorganisms: hydrolysis/acidogenesis (hydrolytic fermentative bacteria), acetogenesis (proton-reducing acetogenic bacteria), and methanogenesis (hydrogenotrophic methanogens and acetoclastic methanogens) (O'Flaherty et al. 2010). This approach helps to describe the generalized pathway of AD, but fails to provide a fundamental understanding of the critical metabolic activities occurring in a digester. In this sense, two previous papers (Abram et al. 2011; Lin et al. 2016b) have brought great clarity to the study of the metabolic functions of the microbial species involved in AD. Both papers use a metaproteomic approach to analyze the proteins expressed in the studied digesting system, thus relating some of the functions of the identified proteins with the main stages of AD. Lin et al. (2016b) investigated the functional insights of a digester treating swine manure and their results particularly stand out. Figure 1 shows a description of the fundamental metabolic pathways of AD based on the microbial involvement at the enzyme level, as described by Lin et al. (2016b), and a brief discussion follows.

Bacteria are unable to directly metabolize particulate organic matter due to the existence of the cell barrier. The consequence is that the solubilization of biopolymers such as carbohydrates, proteins, and lipids is the first stage of the degradation of complex substrates inside an anaerobic reactor (Kim et al. 2010). Coincidentally, Lin et al. (2016b) verified that about half (46%) of the proteins identified in the digester under study were involved in metabolism, including proteins associated with energy production and conversion (28%), amino acid transport and metabolism (9%), carbohydrate transport and metabolism (5%), and lipid metabolism (1%).

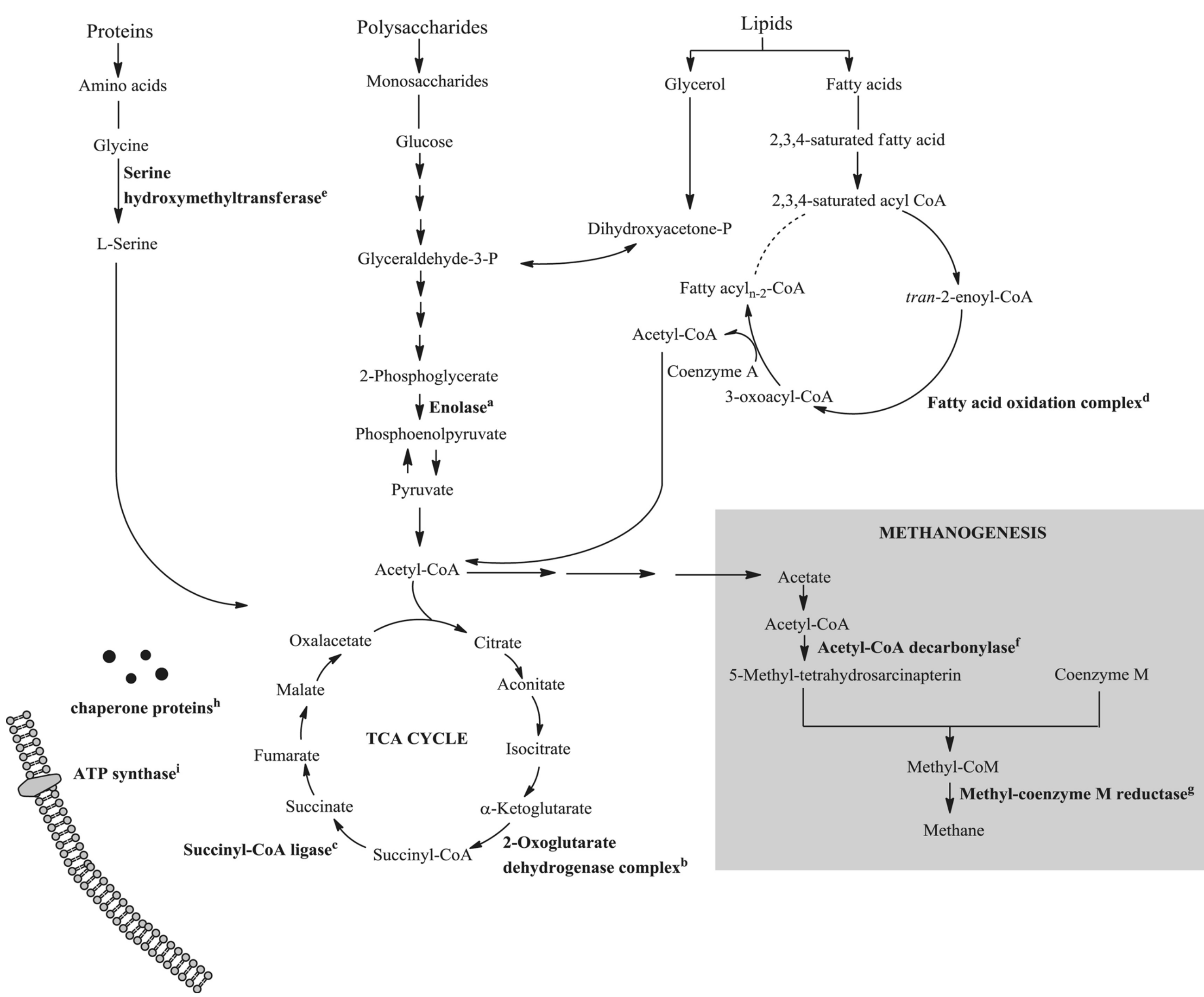

Fig. 1 Example from the literature: Fundamental metabolic pathways of AD based on the microbial involvement at the enzyme level. Reproduced with permission from (Lin et al. 2016b). Copyright 2016 Elsevier

For protein degradation, extracellular proteases help cleave off amino acids into smaller peptides which can then be broken down into individual amino acids in the cell's cytoplasm. For instance, Lin et al. (2016b) found glutaminase–asparaginase from *Pseudomonas fluorescens bv. A* active in their study, thus concurring with the fact that the bioreactor was treating swine manure, which is very rich in protein. Glycine and other individual amino acids are eventually enzymatically deaminated to remove the amino group with the remaining organic acids then ready to be transformed into pyruvate. Pyruvate can be fermented to produce CO_2 and Acetyl-CoA or enter the TCA cycle (Lin et al. 2016b).

Regarding carbohydrates, AD research has identified the presence of enzymes which use sugars as a carbon source, thus generating pyruvate via the Embden–Meyerhof–Parnas pathway. Lin et al. (2016b) isolated a group of proteins associated with carbohydrate metabolism called enolases, which matched several bacterial species (*Pseudomonas putida GB-1*, *Clostridium novyi NT,* and others.)

Lipids, in turn, are also hydrolyzed in an anaerobic digester by the action of lipases. The products from this process are glycerol and fatty acids. On the one hand, glycerol usually undergoes phosphorylation and can be oxidized to dihydroxyacetone phosphate, which then enters the glycolysis pathway; on the other hand, the released fatty acids are oxidized via beta-oxidation, thus generating Acetyl-CoA. In the same study, Lin et al. (2016b) found an essential protein associated with this pathway, namely fatty acid oxidation complex subunit alpha from *Pseudomonas fluorescens* Pf-5. This process degrades fatty acids by sequentially removing two-carbon acetyl groups from the ends of the chain (Lin et al. 2016b).

In summary, proteins, carbohydrates, and lipids are the primary components of organic substrates suitable for AD. The pyruvate and Acetyl-CoA produced after hydrolysis and acidogenesis of these biomolecules are degraded through different pathways to organic acids (e.g., lactic acid, propionic acid, formate, butyric acid, and others) and alcohol (ethanol). The organic acids are then beta-oxidized to acetate by hydrogen-producing acetogens. Hydrogen-utilizing acetogens also generate Acetyl-CoA by the reduction of CO_2 via the Wood–Ljungdahl pathway. In anaerobic conditions and in the presence of certain species of bacteria and archaea, methanogenesis is the preferred pathway for Acetyl-CoA utilization. Lin et al. (2016b) verified that a key enzyme associated with methanogenesis was present in the digesting system studied, which indicated that methanogenesis occurred from both the acetoclastic and hydrogenotrophic pathways (Acetyl-CoA decarbonylase from *Methanosarcina* spp). In fact, this enzyme is known to produce methane from both acetate decarboxylation and CO_2 reduction. Another enzyme identified in the same study (Lin et al. 2016b) can use methyl groups (e.g., methylamines, methanol, formate (Ferry 1999)) for methane production via the reduction of methyl coenzyme M (methyl coenzyme M reductase from *Methanothermobacter thermautotrophicus*, *Methanosarcina barkeri*, and *Methanothermus fervidus)* (Lin et al. 2016b).

Microbial dynamics under different process stages and conditions

AD is a complex process due to the involvement of a diverse microbial group supporting different biochemical reactions. Regarding the digestion of swine-derived wastes, many studies have been conducted on the dynamics and abundance of this microbial community (both bacteria and archaea) in changing conditions (Cho et al. 2013; Lin et al. 2016a, b; Zhou et al. 2016; Shaw et al. 2017).

Bacteria

Large numbers of bacterial populations with specific properties take part in the degradation of swine wastes in anaerobic conditions. At the phylum level, *Firmicutes* and *Bacteroidetes* are common and abundant throughout the process. *Firmicutes* are a well-known acetogenic and syntrophic group of bacteria which can degrade VFAs. *Bacteroidetes* are proteolytic bacteria usually involved in the degradation of various proteins relevant in AD. Lin et al. (2013) identified several anaerobes involved in the digestion of swine manure and grouped them into *Bacteroidetes* and *Firmicutes*, as determined by 16S rRNA gene clone analysis.

Similarly, Wang et al. (2018) reported large populations of *Bacteroidetes* (36.8%–41.2%), *Firmicutes* (26.1%–30.2%), and *Proteobacteria* (15.8%–21.1%), while investigating the anaerobic degradation of swine wastes in the presence of chlortetracycline (a broad-spectrum antibiotic) and Cu. Both studies reported marked differences in microbial dynamics as AD progressed. Specific populations of the mentioned phyla were dominant in the beginning while decreasing at the last stages of the process, thus suggesting different metabolic functions. For instance, certain classes of bacteria which belong to *Firmicutes* and *Bacteroidetes* such as those of *Clostridia* and *Bacteroidia* are known to hydrolyze macromolecules, thus generating organic acids. Accordingly, Wang et al. (2018) identified *Ruminococcaceae* and *Lactobacillaceae*, two families of the class Clostridia, in the early stages of their digesting system, and noted that the dominance of this microorganisms decreased as the process advanced. The identification confirmed the importance of these bacteria to perform hydrolysis and acidogenesis. In fact, both families are associated with the degradation of carbohydrates and proteins by different mechanisms. *Ruminococcaceae* are obligate anaerobes known to degrade cellulose and produce hydrogen and other by-products (Sträuber et al. 2012; Tian et al. 2015; Shaw et al. 2017). The members of *Lactobacillaceae*, in turn, are facultative anaerobic bacteria which metabolize simple and complex sugars to lactate and also acetate, ethanol, CO_2, formate, or succinate (Felis and Pot 2014).

On the other hand, *Bacteirodetes* have been found in previous studies (Lin et al. 2013; Wang et al. 2018) mainly represented by *Porphyromonadaceae* (a family of bacteria which generates acetic and propionic acids via the degradation of proteins and carbonaceous compounds (Hahnke et al. 2015)), *Prevotellaceae* (strict anaerobes related to the utilization of starch, non-cellulosic polysaccharides, and simple carbohydrates (Chen 2005), and *Bacteroidaceae* (another carbohydrate-utilizing family of bacteria (Klocke et al. 2007)). While *Bacteroidetes* play a vital role in the initial steps of digestion (hydrolysis and acidogenesis), at late stages of the process (acetogenesis) the population of these microorganisms generally decrease due to the lack of growth substrate. This phenomenon is coupled with an increase in VFAs, which have to be further degraded by syntrophic bacteria. Syntropic bacteria degrade mid- and short-chain fatty acids, such as propionic and butyric acid to produce hydrogen and acetic acid. These compounds are further used by methanogens to produce methane (Wang et al. 2018). Zhang et al. (2017) and Wang et al. (2018) identified acetogenic bacteria such as those belonging to *Syntrophaceae* and *Syntrophomonadaceae* at the late stages of digestion in a system with swine wastes. These syntrophic bacteria play an essential role in efficient biogas formation since acetate serves as a substrate for methane-forming bacteria. Other potential shares of acetogenesis as a general metabolic pathway have been recently suggested at the phylum level; for example, Shaw et al. (2017) indicated that phylum *Spirochaetes* might play a role in syntrophic acetate oxidation. Wang et al. (2018) found the same *Spirochaetes* to become more abundant as AD progressed.

In addition to the different stages of the process, environmental and biochemical aspects also regulate bacterial populations. For example, AD research has indicated a strong *Firmicutes* dominance under stressful conditions. Regarding temperature, Shaw et al. (2017) pointed out that *Clostridia* from the phylum *Firmicutes* accounted for 79% of bacteria in a thermophilic digester running on swine manure. On the other hand, Lin et al. (2016b) found that the bacterial community present in another thermophilic digester degrading swine wastes was mainly composed of *Proteobacteria* (87%). However, this difference may depend on the approach used (Tuan et al. 2014; Lin et al. 2016b). Indeed, Shaw et al. (2017) used a 16S rRNA analysis rather than a metaproteomic approach. At milder temperatures, a co-dominance of *Bacteroidetes* and *Firmicutes* seems to occur. Pampillón-González et al. (2017) reported similar abundances of *Bacteroidetes* ($23.6 \pm 3.0\%$) and *Firmicutes* ($20.1 \pm 4.1\%$) in a lagoon-type anaerobic digester operated in a pig farm, each dominated by *Bacteirodia* and *Clostridia*, respectively. Liu et al. (2009) also found most bacterial operational taxonomic units (OTUs) at mesophilic temperatures as belonging to *Firmicutes* (47.2% of total clones) and *Bacteroides* (35.4%). They also identified *Spirochaetes* (13.2%), a phylum of gram-negative bacteria that are generally assumed to be glucose fermenters (Lee et al. 2013).

Other process parameters such as total ammonia nitrogen (TAN) also significantly affect the bacterial community involved in AD. Studies focused on the digestion of ammonia-rich wastes such as SW have suggested a shift from acetoclastic methanogenesis to hydrogenotrophic methanogenesis and syntrophic acetate oxidation (Cho et al. 2017; Nordgård et al. 2017). Cho et al. (2017) showed that *Thermoanaerobacterales* dominated in low ammonia concentrations, while *Clostridiales* and *Bacteroidales* were the key functional taxa at high ammonia levels. Consistently, De Vrieze et al. (2015) identified a significantly higher relative abundance of *Clostridiales* in high ammonia conditions. These bacteria are involved in syntrophic acetate oxidation, which is the main pathway for acetate removal at elevated ammonia concentrations (Karakashev et al. 2006; Lü et al. 2013). As for *Bacteroidales*, Ruiz-Sánchez et al. (2018) recently suggested that some species might harbor novel syntrophic partners of hydrogenotrophic methanogens during methane formation.

Finally, bacterial communities have also been observed to differ at distinct pH during the digestion of swine wastes. Zhou et al. (2016) reported higher genetic diversity of bacterial community at neutral pH as compared to pH 6.0 and 8.0. Their results suggested that some *Clostridiales* and *Bacteroidales* tolerated slightly acidic environmental pHs. On the other hand, *Solibacillus*, *Porphyromonas,* and *Clostridium* abounded in alkaline conditions. These genera participate in the hydrolysis and acidogenesis of various organic compounds.

Archaea

Regarding archaea communities, the phylum *Euryarchaeota* has been found to dominate the functional pathways that culminate in methane formation. Due to the inherent low diversity of methanogens, the same orders are frequent in anaerobic digesters digesting different wastes. These orders include *Methanomicrobiales*, *Methanobacteriales*, *Methanococcales* (which are hydrogenotrophic), and *Methanosarcinales* (which are mixotrophic methanogens). These communities have been found to shift when growing in different media and are thus susceptible to different process conditions (Zhou et al. 2016; Cho et al. 2017).

Temperature, for example, is an important selective factor in terms of archaea community structure. Lin et al.

(2016a) related a lower diversity of functional pathways to an improved conversion to methane at thermophilic conditions. This enhancement of central functional pathways works by centering most cellular activities and resources in methanogenesis (Lin et al. 2016a). At the family level, Shaw et al. (2017) reported that *Methanobacteriaceae* and *Methanomicrobiaceae* predominated under thermophilic SW digestion. These two methanogenic groups belong to the order *Methanomicrobiales* and *Methanobacteriales*, respectively. On the other hand, *Methanocorpusculaceae* and *Methanoregulaceae* (order *Methanomicrobiales*) dominated at mesophilic temperatures (Shaw et al. 2017). At the genus level, Tuan et al. (2014) observed an archaeal community composed of 77% *Methanobacterium*, 13% *Methanosarcina*, 9% *Methanothermobacter,* and 1% *Methanobrevibacter* in a thermophilic anaerobic digester. This microbial structure is consistent with the previous report of *Methanobacteriaceae* predominating at high temperatures (Shaw et al. 2017). In fact, the genera *Methanobacterium*, *Methanothermobacter,* and *Methanobrevibacter* belong to the family *Methanobacteriaceae*. These archaea are strict anaerobes, which obtain energy for growth from formate or CO_2 reduction with H_2 (Oren 2014).

The abundance of methanogenic species has also been found to vary according to the ammonia levels in anaerobic reactors. Cho et al. (2017) observed a 40% decrease in acetoclastic methanogenesis at total ammonia nitrogen (TAN) concentrations of 4000 mg L^{-1} when compared to 1200 mg L^{-1} (Fig. 2). They found that *Methanosarcinales* co-dominated in low-ammonia environments, but suffered inhibition for free ammonia nitrogen (FAN) concentrations as high as 0.64 g L^{-1} (Cho et al. 2017). Interestingly, Calli et al. (2005) previously observed that some *Methanosarcinales* formed large multi-cellular structures to avoid ammonia inhibition, but the clusters disintegrated at FAN levels above 0.7 g L^{-1}. Other methanogens such as those belonging to the genus *Methanosaeta* can also adapt to FAN concentrations as high as 1.0 g L^{-1} using the same strategy, i.e., growing in aggregates with other microbes (Nordgård et al. 2017).

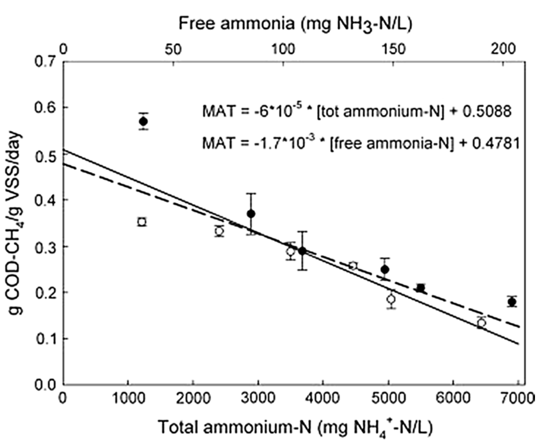

Fig. 2 Example from the literature: Acetoclastic methanogenic activity (in g COD-CH_4 g VSS^{-1} day^{-1} at different TAN (bottom axis) and FAN (top axis) at 25 °C and pH 7.7. A ~ 40% inhibition of the methanogenic activity was found at TAN concentration of 4000 mg L^{-1} when compared to 1200 mg L^{-1}. Reproduced with permission from (Garcia and Angenent 2009). Copyright 2009 Elsevier

Also, pH levels have been proved important for the structure of methanogenic bacteria. Zhou et al. (2016) reported the dominance of *Methanocorpusculum* at neutral pH, a mesophilic genus of microbes able to produce methane from formate and H_2/CO_2. In deviating conditions, the dominant archaea belonged to *Methanosarcina*, a group of neutrophilic or alkaliphilic microorganisms which use several substrates (other than formate) for energy generation (Oren 2014).

Despite the above discussion, clear relations between specific microbial taxonomy groups and process parameters relevant in AD are difficult to establish due to the complexity of biological systems. Nevertheless, further research should be able to improve the understanding of microbial communities and provide more functional insights into the metabolic and physiological activities occurring in a digester. If this is the case, metaproteomics should be considered a tool with great potential to elucidate specific functional aspects of AD via the identified proteins that catabolize the digestion process. On the other hand, note that the interaction between the different environmental and chemical parameters determines not only the microbial structure inside the digester but also the performance and stability of the overall process. Based on this, there is considerable interest in understanding how the parameters associated with AD relate to process performance in terms of biogas production. The next section discusses these chemical and operational aspects in the context of swine-derived substrates. Table 1 presents a general summary of the parameters reviewed.

Chemical factors and process stability

pH and buffering capacity

The activity and growth of methanogens can be strongly affected by culture pH. For anaerobic treatment, performance is good within the pH range of 6.8–7.2 (Gerardi 2003). However, pH levels inside an anaerobic reactor can shift during the process as a result of several chemical interactions within a dynamic environment. During start-up, pH typically decreases due to the generation of VFAs resulting from the hydrolysis and acidogenesis of biomolecules. After the consumption of these organic acids and the production of alkalinity, the system recovers and pH increases (Gerardi 2003). Note that fermentative bacteria grow faster than the methanogens (Amani et al. 2010). This ability of a digesting system to minimize changes in pH is called buffer

Table 1 Influence of different parameters on anaerobic digestion efficiency and stability

Parameter	Type	Influence on digestion efficiency and stability	References
Temperature	Operational	Higher temperatures usually mean higher digestion rates (microbial growth rates) and enhanced methane production up to a value (90 °C) for which most microbial population die. However, the relationship is nonlinear since higher temperatures were also found to result in higher concentrations of ammonia. There are three AD operational temperature ranges: psychrophilic digestion, which occurs at below 25 °C; mesophilic digestion which occurs between 25 and 45 °C; and thermophilic digestion which occurs above 45 °C	Amani et al. (2010), Lin et al. (2016a, b)
pH and alkalinity	Chemical	Methane production is inhibited at low pH since the activity and growth of methanogenic bacteria are significantly reduced in acidic conditions. On the other hand, pH values above 8.0 will impede acidogenesis and reduce digestion efficiency. In general, most digesters perform well in the neutral range (6.8–7.4) where enough alkalinity (1000–3000 mg L^{-1} as $CaCO_3$ or 7 mg $CaCO_3$ mg BOD^{-1}) acts as a buffer and prevents rapid changes in pH. pH relates closely to other monitoring parameters such as alkalinity and VFA due to the different biochemical and microbiological steps working synergistically during AD	Amani et al. (2010), Gerardi (2003), Sun et al. (2016)
VFA	Chemical	VFAs are the main intermediates of the digestion process and may accumulate during AD due to process imbalances. These imbalances can lead to a decrease in alkalinity and pH, thus inhibiting methanogenesis	Amani et al. (2010), Appels et al. (2008)
Ammonia	Chemical	Insufficient or excessive ammonia is found to be a major cause of microbial population instability as ammonia is membrane-permeable, thus causing proton imbalance and potassium deficiency inside cells	Rajagopal et al. (2013), Sprott et al. (1984), Switzenbaum et al. (1990)
C/N ratio	Chemical	Imbalances in C/N ratio may result in high VFA and total ammonia formation, two strong inhibitors of the digestion process	Gerardi (2003)
Organic loading rate	Operational	Appropriate organic loading rates are necessary for the stability of AD. If OLR is too low, digesters' productivity will be weak and unattractive; however, in cases where OLR is maintained at higher levels, organic overloading may unbalance the digestion process due to the excessive production of VFA	Amani et al. (2010), Gerardi (2003)
Retention times	Operational	Shorter retention times are often insufficient for a stable process since methanogenesis may not have time to take place due to the washout of methanogenic bacteria. Insufficient time for the complete breakdown of polymeric compounds may also be relevant in the case of complex substrates. On the other hand, very high retention times can result in a nutrient deficiency inside digesters	Amani et al. (2010), Gerardi (2003)

capacity and is usually represented by alkalinity (Sun et al. 2016). Alkalinity results from the equilibrium between carbonic acid, bicarbonate, and carbonate ions (H_2CO_3/HCO_3^-/CO_3^{2-}), as well as ammonia and ammonium ions ($NH_3.H_2O/NH_4^+$) (Mao et al. 2017b). Alkalinity can also relate to the release of amino groups ($-NH_2$) during amino acids degradation (Gerardi 2003).

Typically, swine-derived effluents guarantee enough alkalinity for the digestion to proceed without problems. In these systems, initial values ranging between 2000 and

2500 mg L^{-1} as $CaCO_3$ have been reported with excellent methane production efficiency and process stability (Lin et al. 2013; Wijesinghe et al. 2018; Wang et al. 2018). Also, initial pH adjustment can be used to guarantee that a digester has the adequate buffering capacity (Zhang et al. 2015; Zhou et al. 2016; Mao et al. 2017a, b).

From the reviewed literature, biogas production seems to be favored when the initial pH is around neutrality. Mao et al. (2017a, b) found optimal methane yields around pH 7.0 and 7.5 in two studies assessing the co-digestion of swine wastes and corn straw. As a general trend, they observed that cumulative methane production increased with the initial pH before decreasing at pH 8.0, thus suggesting an inhibition of the process in more alkaline environments probably due to high free ammonia (FA) levels. Maximum methane and biogas productions of 220 and 498 mL g VS_{added}^{-1} were reported at 70% manure content and 7.5 initial pH, respectively. Also, minimum production yields were observed in both studies at the initial pH of 6.0. This minimum production demonstrates that the buffering capacity of the reactors operated in such conditions was not enough to recover from VFA buildup after hydrolysis and acidogenesis. The overall digestion process collapsed as a consequence. Zhou et al. (2016) also reported cumulative biogas productions resulting from the mesophilic digestion of swine wastes (7.8% total solids) as being significantly higher at neutral pH. They observed a biogas production of 453.5 mL g VS_{added}^{-1} when pH was kept at 7.0, while at pH 6.0 and 8.0 the biogas yields decreased to 188.9 mL g VS_{added}^{-1} and 265.9 mL g VS_{added}^{-1}, respectively. Zhang et al. (2015) tested five different initial pH levels in the co-digestion of swine manure and maize stalk at thermophilic temperatures and also observed that pH is a key process factor. For 70% swine wastes, they reported methane production rates of 115.5, 131.9. 146.0, 132.8, and 64.2 mL g VS_{added}^{-1} at pH 6.0, 6.5, 7.0, 7.5, and 8.0, respectively. Figure 3 shows an increasing trend for methane production around neutral pH and increasing manure ratios (Zhang et al. 2015).

Ammonia inhibition

Ammonia is also a selective factor for methane production. Free ammonia nitrogen (NH_3–N) or ionized ammonia nitrogen (NH_4^+–N) are naturally present in anaerobic reactors operated with swine-derived effluents as the end product of the hydrolysis and solubilization of organic nitrogen compounds (e.g., proteins, urea, and nucleic acids).

The fundamental science underlying ammonia inhibition of methanogens has been well covered in some recent reviews (Rajagopal et al. 2013; Jiang et al. 2019). It is well known that free ammonia molecules can diffuse through cell membranes into the cells of microbial populations. Following the diffusion of ammonia into the cell, some possible mechanisms of ammonia toxicity have been proposed such as the change of intracellular pH value, the increase in maintenance energy requirement, or the inhibition of specific enzymatic reactions (Wittmann et al. 1995). In two studies, Sprott and Patel (Sprott et al. 1984; Sprott and Patel 1986) concluded that intracellular accumulation of FAN may change the pH and proton imbalance due to NH_3–N conversion into NH_4^+–N. In response, cells increase the energy requirements to keep homeostasis, i.e., keep intracellular pH stable by activating a K^+/H^+ antiporter. This process results in potassium deficiency and thus cell death (Sprott et al. 1984; Sprott and Patel 1986). Based on this, FAN has been identified as the critical inhibition factor in AD. Nevertheless, ammonia is also an essential nutrient for bacterial growth and is beneficial in low levels as a component of the buffer system in anaerobic reactors. FAN is also highly dependent on pH, temperature, and TAN concentration.

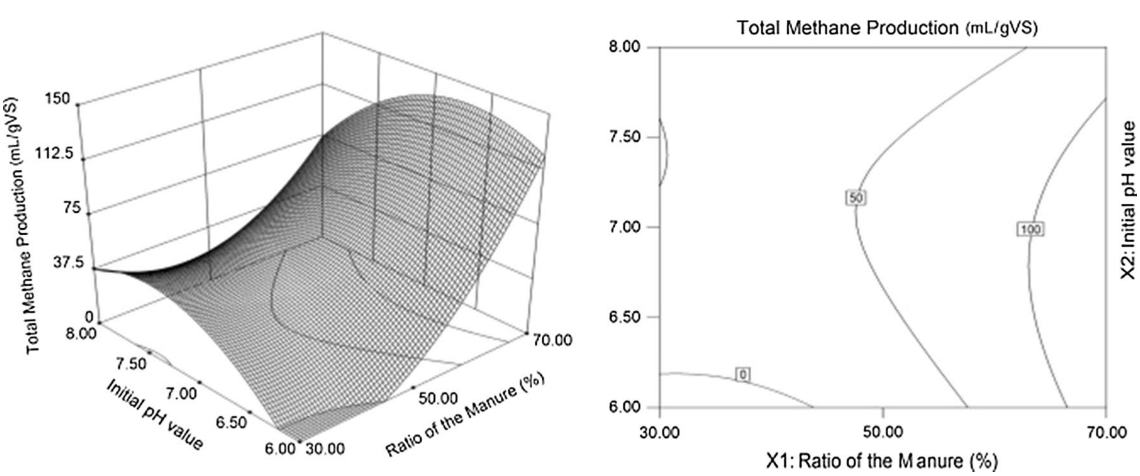

Fig. 3 Example from the literature: Methane production as a function of pH and different manure ratios. Reproduced with permission from (Zhang et al. 2015). Copyright 2015 Elsevier

Research on SW has recently been focused on the evolution of biogas/methane production with changing TAN levels. Generally, TAN concentrations ranging between 1500 and 3000 mg L^{-1} might be inhibitory for methanogenesis especially at high pH values, while concentrations higher than 3000 mg L^{-1} lead to complete inhibition at any pH (Rajagopal et al. 2013). Consistently, Tian et al. (2015) observed no inhibition at TAN concentrations ranging from 1380 mg L^{-1} to 2020 mg L^{-1} in the co-digestion of swine and food wastes. On the other hand, Chen et al. (2015) observed a decline in biogas production when TAN concentrations grew to a maximum value of 3500 mg L^{-1} (FAN higher than 55 mg L^{-1}). They reported a maximal biogas yield of 649 mL g VS_{added}^{-1} at TAN concentrations between 2000 and 3000 mg L^{-1}. Further tests by Nordgård et al. (2017) concurred with these findings. Their results showed that biogas yields were seven times higher at 1900 than at 3700 mg L^{-1}, which indicated the occurrence of process disturbances.

Also, previous studies suggested a more likely inhibitory effect of ammonia under increased temperatures. Experiments by Garcia and Angenent (2009) demonstrated that increasing the temperature from 25 °C to 35 °C decreased methane yield by 13% for high-ammonia reactors (around 4000 mg L^{-1}) compared to low-ammonia reactors (1600 mg L^{-1}). This effect makes sense since temperature affects the chemical equilibrium between ammonia species in an aqueous solution. In fact, higher temperatures and higher pH values increase the ratio of FAN to TAN (Garcia and Angenent 2009).

VFA accumulation

The concentration of VFA is a chemical parameter often monitored in AD. VFAs are immediate precursors of methane production and the accumulation of these organic acids may directly reflect a kinetic uncoupling between acidogenic bacteria and proton-reducing acetogens (Switzenbaum et al. 1990). Therefore, VFA buildup can signal imbalances and disturbances in the process. Generally, inhibition occurs by dropping the pH in the digesting medium. This drop collapses the buffering capacity of the system and creates toxic conditions for microorganisms. In fact, undissociated VFAs can permeate across the cell membrane and drop the pH inside cells, which disrupts homeostasis (Zoetemeyer et al. 1982). During AD, VFAs are dynamically interdependent with other parameters such as pH and alkalinity. These parameters are often monitored together in digesting systems as a consequence (Gerardi 2003).

Several studies have used VFA evolution and VFA/TA ratio as a measure of process stability in digesting systems with swine wastes. At around neutral pH, Kafle et al. (2013) and Zhang et al. (2015) found no significant toxic effects at VFA concentrations up to 10 000 mg L^{-1} and VFA/TA ratios between 0.1 and 0.3, respectively. These VFA levels are typical threshold limits for process inhibition. However, different systems do not respond equally in the same conditions; thus VFA may only indicate the state of an anaerobic process.

For instance, Cuetos et al. (2011) reported VFA levels of almost 14,000 mg L^{-1} in one CSTR reactor fed with swine wastes and crop residues with no inhibition. The final biogas production was 460 mL g VS_{added}^{-1}, with a 72% methane content. On the other hand, Kafle et al. (2013) observed disturbances when an AD reactor operated with swine and apple wastes reached a VFA concentration of 15,000 mg L^{-1} and a VFA/TA ratio of 1.224. During the period of inhibition, the pH dropped from 7.8 to 7.1, and methane content in the biogas dropped from 77 to 44%. This decrease strongly suggested that the buffering capacity of the system was compromised. Zhang et al. (2015) also observed lower biogas yields when the buffering capacity of their co-digestion systems dropped. Increased VFA/TA ratios (0.8–1.6) and reduced pH values (5.0–6.0) indicated that VFA accumulated in the reactors. According to these authors, this accumulation was dependent on the ratio of swine wastes and co-substrate. They found that total biogas yields improved for substrate mixtures with higher SW content due to a more balanced C/N ratio inside the reactor (biogas production was 85, 215, and 308 mL g VS_{added}^{-1} for 30%, 50%, and 70% SW, respectively). More recently, Mao et al. (2017a) also reported a correlation between balanced C\N ratio and lower VFA buildups.

Operational aspects and process performance

Temperature variations

Temperature influences the thermodynamic equilibrium of the biochemical reactions in AD and affects both microbial activity and growth rates (Lin et al. 2016a). An increased process temperature has been positively correlated with methane metabolism and oxidative phosphorylation, which means that AD should be favored at higher temperatures (Lin et al. 2016a) (Fig. 4).

Experiments performed by Shaw et al. (2017) confirmed that thermophilic conditions lead to higher biogas production (0.56 L L^{-1} day^{-1}) as compared with mesophilic conditions (0.36 L L^{-1} day^{-1}). Even at mesophilic conditions, slight variations in temperature have been generally shown to improve biogas production. Chae et al. (2008) observed that temperature variations from 25–30 °C and 30–35 °C resulted in methane yields of 317, 397 and 437 mL g VS_{added}^{-1}, respectively. Similarly, temperature variations from psychrophilic to mesophilic range mean significantly better biogas yields (Chae et al. 2008; King et al. 2011; Deng et al. 2014, 2016;

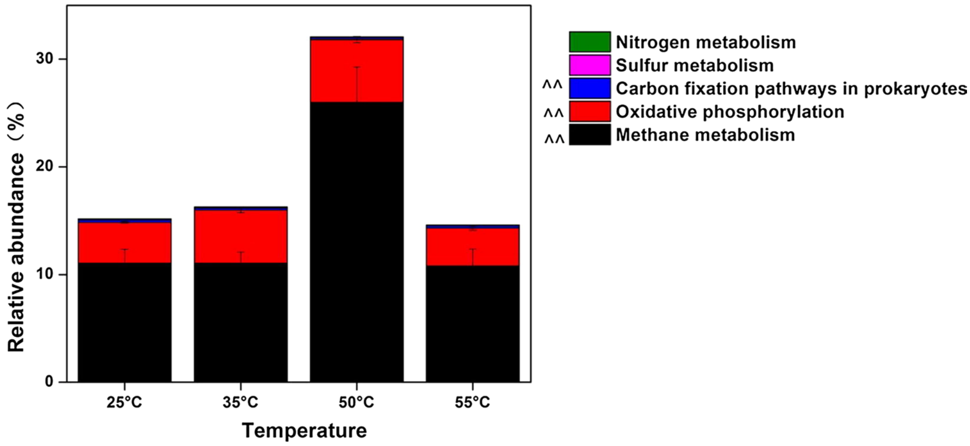

Fig. 4 Example from the literature: Functional pathways at KEGG database (level) 3 affiliated to energy metabolism. Oxidative phosphorylation (ko00190) and methane metabolism (ko00680, mainly referred to methanogenesis in anaerobic environments) were dominant at all temperatures with the latter more pronounced at thermophilic temperatures (50° C). Beyond this temperature, the lower portions of *Euryarchaeota* in the relative abundances of this pathway suggest that 50 °C might be the threshold temperature within which an increased temperature improves methane production in this specific AD system. Reproduced with permission from (Lin et al. 2016a). Copyright 2016 Elsevier

Yang et al. 2016). Deng et al. (2016) obtained low methane productivity at 15 °C with methane yields being 6.5 times higher at 35 °C. The reason for the lower performance is that low temperatures reduce methanogenic activity, which leads to VFA accumulation and acidification of the reactor medium.

Although the process improved by increasing the temperature, economic considerations should be made since overall energy balance may be negative (Deng et al. 2014). As an example, Deng et al. (2016) found that the biogas production rate at 35 °C was 6.77% higher than that at 25 °C. However, energy recovery was only 3.80% in the same conditions. Also, ammonia inhibition can hinder thermophilic digestion for substrates having a high level of nitrogen. Cho et al. (2013) confirmed that process inhibition occurred when operating temperature increased from 35 to 50 °C, which resulted in very high FAN concentrations (1600 mg L^{-1}).

Organic and hydraulic overloading

The variance of swine wastes composition means that digesting systems must be able to operate with a changing substrate. This situation poses some challenges in AD since organic loading rates (OLR) and hydraulic retention times (HRT) must be appropriate to balance process stability and biogas production.

Generally, high OLRs have been associated with higher biogas yields. Molinuevo-Salces et al. (2012) reported that an increase in OLR from 0.4 to 0.6 g VS L^{-1} day^{-1} improved methane yield from 90 to 201 mL g VS_{added}^{-1} in a reactor digesting swine and vegetable wastes. Experiments performed by Yang et al. (2016) further confirmed that daily production rates increase at high OLRs before stabilizing in steady-state conditions. For OLRs ranging between 1.5 and 6.0 g VS L^{-1} day^{-1}, the methane production observed by these authors achieved 0.5–0.9 m^3 m^{-3} day^{-1}, respectively. However, operating at high OLRs may lead to organic and hydraulic overloading, which may disturb the digestion process. When this happens, the immediate response of the system is the excessive accumulation of VFA to inhibitory concentrations, thus leading to a pH drop and acid toxicity. Co-digestion tests by Kafle and Kim (2013) could be interpreted as evidence of this process. These authors observed a rapid increase in VFA concentration when the OLR reached 1.7 g VS L^{-1} day^{-1} coupled with a drop in pH (from 7.8 to 7.1) and a decrease in biogas yields (from 241 to 109 mL g COD_{added}^{-1}). da Mazareli et al. (2016) also observed increasing VFA levels when changing OLR from 5.2 to 11.0 g COD L^{-1} day^{-1}. However, the process remained stable due to the adequate buffering capacity of the system.

OLR is the quantity of organic matter to treat in a given time. Therefore, this process parameter is intrinsically related to HRT. It is well known that HRT changes affect the production of VFA and accumulation of organic acids in digesting systems. Kim et al. (2012) studied the two-stage digestion of swine wastewater with HRTs ranging between 25 and 10 days. They reported good COD removals (average 65.8%) and methane yields (between 450 and 1090 mL g VS_{added}^{-1}). However, further HRT reductions led to a drop in performance. When methanogenic HRT was shortened to 3.5 days, methane yields decreased to 10 mL g VS_{added}^{-1}, probably due to the progressive washout of active methanogenic bacteria (Kim et al. 2012). Other studies digesting swine wastes also indicated that a minimum retention time is required to prevent the washout of methanogens. Kinyua et al. (2014) reported that an HRT of 14 days was insufficient for microorganisms to convert the substrate into VFAs. Methane yields were reduced as a consequence (100 mL g VS_{added}^{-1} when compared to 300 mL g VS_{added}^{-1} for 14 days and 21 days HRT, respectively).

Current trends and future developments

Beyond the optimization of AD parameters discussed in the last section, researchers have also tested other methods to enhance biogas production. The simultaneous digestion of more than one substrate (co-digestion) has been explored as a process intensification technique with swine effluents showing potential for the combined digestion with carbon wastes. Future AD systems for swine streams are also expected to improve by developing effective pretreatment methods. Novel practices of AD enhancement, such as using biological and inorganic additives, may also speed up digestion and stabilize biogas production. In this section, we briefly examine these aspects, along with providing a brief discussion of digestate management and the economic aspects of biogas utilization.

Co-digestion with carbon-rich substrates

Mono-digestion of swine-derived effluents does not always present satisfactory performance, even when digesting systems operate in optimized conditions. The high nitrogen content of these substrates has been identified as the main reason for problems due to the risk of accumulation of inhibitory compounds to toxic concentrations, especially ammonia (Riaño et al. 2011; Li et al. 2014; Rodriguez-Verde et al. 2014; Mao et al. 2017b). Co-digestion of swine wastes with carbon-rich organic materials has been widely tested to improve process stability and biogas yields. Combining these wastes is advantageous to counteract process inhibition by improving the C/N ratio and balancing nutrients. This combination also provides a more stable environment for microbial communities to develop (Riaño et al. 2011; Mao et al. 2017b). Another positive synergism is the possibility of operating at higher OLR, which can result in improved biogas productions.

Many studies have demonstrated the benefits of the co-digestion approach for the stabilization of swine wastes (Table 2). For example, Jiménez et al. (2015) reported higher methane yields from the co-digestion of swine manure, rice straw, and clay materials when compared with the digestion of swine manure alone. Also, in a study by Wu et al. (2010), digesting swine manure with three carbon-rich crop residues resulted in improved performance. Among the materials tested by these authors, the addition of corn stalks and oat straw performed better than wheat straw. When the C/N ratio was kept at 20:1, biogas production increased for all the co-substrates with cumulative methane volumes of 41.6 L, 35.0 L, and 16.6 L for corn stalks, oat straw, and wheat straw, respectively. As compared to the control, the values were an average of twelve times higher. Wang et al. (2009) and Mao et al. (2017b) also acknowledged the value of co-digesting agricultural by-products with swine wastes to increase AD efficiency. Wang et al. (2009) observed that 4.6 kg of straw added to 1000 kg of manure increased methane production by 10% in a continuous system when compared to the mono-digestion of swine manure; in turn, Mao et al. (2017b) reported higher cumulative methane production with a 70:30 swine manure/corn straw ratio (220 mL g VS_{added}^{-1}).

In addition, substrate chemical characteristics can also be improved by adding other types of residues to swine wastes. For instance, experiments by Riaño et al. (2011) suggested that winery wastewater is a good co-substrate for these digesting systems. Their results indicated that a 40/60% mixture of winery and swine wastes resulted in increased organic matter removal and higher methane yields (107 mL g COD_{added}^{-1} day^{-1} as compared to 27 mL g COD_{added}^{-1} day^{-1}). Banana stalks, apple wastes, and vegetable wastes have also been tested with excellent results (Kafle and Kim 2013; Tian et al. 2013; da Mazareli et al. 2016). Tian et al. (2013) reported optimal performances with a 50/50% mixture of banana stalks and swine wastes with methane yields being almost 2.65 times higher as compared with mono-digestion. Kafle and Kim (2013) observed that biogas production improved in co-digestion tests with apple wastes when using a mixture of 33:67% (VS basis) as opposed to SW mono-digestion. Finally, da Mazareli et al. (2016) found that the co-digestion of SW with vegetable wastes increased process stability and resulted in higher COD removals (over 85%).

These co-digestion studies hinted that system design might be a critical issue in this topic. The general goal is to maximize biogas production and offset the negative environmental value of waste streams. However, more long-term pilot-scale experiments with continuous systems are needed to assess the weaknesses and strengths of combining different substrates in terms of economic performance. Also, novel co-digestion feedstocks such as microalgae are currently being explored and may represent a low-cost treatment option for the recovery of energy and nutrients (N and P) (Miao et al. 2014; Wang et al. 2016). Wang et al. (2016), for example, reported that an integrated system co-digesting swine wastes and *Chlorella sp.* (10 ± 3% of algae by VS) resulted in a similar biogas production rate as digestion of swine manure alone, but with better digestate quality. Astals et al. (2015) furthered this idea and investigated the feasibility of a biorefinery based on the co-digestion of algae (*Scenedesmus sp.*) and

Table 2 Summary of the reviewed literature regarding the co-digestion of swine wastes with carbon-rich substrates to improve process performance

Feedstock mixture	Aim of co-digestion	Process conditions	Relevant results	References
Pig manure + rice straw + clay residues	Improve specific methanogenic activity	Batch. Laboratory scale. Mesophilic and (35 °C) thermophilic (55 °C)	SMA increased significantly	Jiménez et al. (2015)
Swine manure + corn stalks	Improve biogas and methane production	CSTR[a]. Laboratory scale (8 L). Mesophilic (37 °C)	Methane production increased 16 times	Wu et al. (2010)
Swine manure + oat straw			Methane production increased 14 times	
Swine manure + wheat straw			Methane production increased six times	
Swine manure + wheat straw	Improve methane productivity	CSTR. Laboratory scale (7 L). Thermophilic (55 °C). HRT = 15 days	Methane production increased by 10%	Wang et al. (2009)
Swine manure + corn straw	Effect of SM/CS ratio on methane production	Batch. Laboratory scale (1L). Mesophilic (37 °C)	Methane production and VS removal increased with increase in the SM/CS ratio	Mao et al. (2017a, b)
Swine manure + winery wastewater	Increase methane productivity	CSTR. Laboratory scale (7 L). Mesophilic (35 °C). HRT = 12 days. OLR = 0.85 gCOD L^{-1} day^{-1}	Methane production increased about four times	Riaño et al. (2011)
Swine manure + banana stalks	Improve biogas production	–, mesophilic (35 °C)	Methane yields increased about three times	Tian et al. (2013)
Swine manure + apple wastes (33%)	Improve biogas production	CSTR. Laboratory scale (5.5 L). Mesophilic and thermophilic. OLR = 5.0 g VS L^{-1}	Biogas yields increased by 16% and 48% for mesophilic and thermophilic temperatures	Kafle and Kim (2013)
Swine wastewater + vegetable wastes	Improve process stability and organic matter removal	HARFB[b]. Room temperature (21–24 °C). HRT = 1 day. OLR = 11 gCOD L^{-1} $day^{-1.}$	Methane production increased by 67%	da Mazareli et al. (2016)

[a]*CSTR* continuous stirred tank reactor

[b]*HARFB* high-rate horizontal anaerobic reactor with fixed bed

swine-derived wastes for energy (biogas), and lipid/protein production. Their experiments showed a positive synergy between swine wastes and raw algae, which resulted in higher methane yields than for raw algae mono-digestion (163 to 245 mL g VS_{added}^{-1}).

Based on this, the future trend in the treatment of swine wastes should be to assess the feasibility of integrating novel and traditional substrates in real AD systems under the concept of a biorefinery. Despite net energy recovery not showing yet clear improvements when compared to the conventional AD process, a complete assessment should be made considering several other aspects, primarily environmental. Additional factors such as product market valuation (not only biogas, but also nutrients), local discharge limits, and feedstock origin and abundance might also make a difference in terms of the overall benefit of these systems.

Pretreatment

Pretreatment processes in AD aim to promote physical or chemical modifications in substrate structure to facilitate the first stages of the digestion process, i.e., accelerate hydrolysis. Usually, SW contains complex particulate matter in the form of fibers that are resistant against dissolution and biodegradation. Therefore, including a pretreatment may be of interest to overcome this restriction.

Some pretreatment techniques have been developed to improve the methane yields of swine wastes. These methods work by increasing the solubility of the cell wall and releasing soluble COD, which then becomes readily available for microbial degradation (Vlyssides and Karlis 2004). In the reviewed literature (Table 3), the majority of studies reviewed have been focused on thermal and chemical pretreatment of SW. Wu et al. (2017) studied the thermal

Table 3 Summary of the reviewed literature regarding the pretreatment of swine wastes to enhance process performance

Pretreatment method	Process details	Mechanism	Relevant results	References
Thermal	70 °C for three days	Increased solubilization	Methane production increased by 40%	Wu et al. (2017)
Thermal	100 °C for one hour	Increased solubilization and biodegradation of hemicellulose and lignin	Methane production increased by 28%	Rafique et al. (2010)
Thermal + Chemical	70 °C for one hour + 5% v/v $Ca(OH)_2$	Increased solubilization via saponification and acid neutralization	Methane production increased 72%	
Chemical	5% v/v $Ca(OH)_2$	Increased solubilization via saponification and acid neutralization	Biogas production increased 12%, but methane production decreased	
Thermal (Steam explosion)	170 °C for 30 min	Increased solubilization via cell membrane degradation	Methane production increased by 44%	Ortega-Martinez et al. (2016)
Thermal + Chemical	0.0045 gNaOH gTS^{-1} for 18 min at 35 °C	Increased solubilization	Methane production decreased	
Thermal (Steam explosion)	170 °C for 30 min	Increased solubilization via cell membrane degradation	Methane production doubled	Ferreira et al. (2014)
Hydrothermal	170 °C for 30 min	Forming inhibitors	Methane production decreased	Huang et al. (2018)
	150 °C for 30 min	Increased solubilization	Methane production increased by 50%	
Thermal	170 °C for 30 min	Increased solubilization	Biogas production increased by 35% and COD removal 53%	González-Fernández et al. (2008)
Chemical	NaOH 6 wt.%	Increased solubilization via saponification and acid neutralization	Biogas production increased by 12%	Zhang et al. (2013)
Chemical	NaOH	Increased solubilization via saponification and acid neutralization	Biogas production increased by 13%	González-Fernández et al. (2008)
Chemical	HCl	Chemical oxidation	Biogas production decreased by 10%	González-Fernández et al. (2008)

treatment of swine manure at 70 °C for three days and found that the main organic components of the substrate were hydrolyzed. This pretreatment improved cumulative methane production by 39.5%. AD of swine wastes at higher temperatures has yielded conflicting results, probably due to the characteristics of the substrates. Rafique et al. (2010) reported that temperatures above 100 °C had no positive effects on the degradability of swine manure since these conditions significantly affect bacterial populations. However, other studies reported enhanced AD at higher temperatures with (Ferreira et al. 2014; Ortega-Martinez et al. 2016) and without steam explosion (González-Fernández et al. 2008; Huang et al. 2018). Ortega-Martínez et al. (2016) and Ferreira et al. (2014) observed that steam explosion at a temperature around 170 °C improved methane production. Improvements of 44% and 100% for retention times of 30 min were reported, respectively. At the same temperature but without steam explosion, Huang et al. (2018) observed benefits for the breakdown of soluble proteins, amino acids, and urea present in swine wastes, but with low methane yields possibly due to the accumulation of TAN and VFA. In fact, the poor performance might be the result of rapid hydrolysis and acidogenesis. Also, these authors found that pretreatment at 150 °C optimally loosened the structure of the organic matter to be digested, thus resulting in an increased cumulative methane yield when compared with the untreated waste (322 mL g VS_{added}^{-1}). Finally, González-Fernández et al. (2008) did not observe any instability when pretreating swine wastes at 170 °C. They revealed that thermal application before AD increased methane productivity by 35%.

Chemical pretreatments, particularly alkaline, have also been tested at laboratory scale to improve AD. Zhang et al. (2013) found that NaOH treatment before co-digestion of banana stems and swine wastes positively influenced cumulative biogas production. The difference became significant at a dose of 6 wt.% (24,082 mL after 40 days, 12% higher as compared to the control) (Zhang et al. 2013). Similarly, González-Fernández et al. (2008) enhanced methane productivity by 13% (239 mL g COD_{added}^{-1}) by treating SW with NaOH for 24 h at 32 °C (González-Fernández et al. 2008). Finally, mechanical screening techniques have also been explored as pretreatment, but efforts with swine manure solids have proved unattractive with methane productivity remaining unchanged (González-Fernández et al. 2008).

Since most pretreatments are energy-consuming or have other incurred costs, several aspects should be considered when transitioning the technologies to real systems. Energy balance must be assessed to guarantee that the process is economically feasible. Another issue to consider is the use of chemicals and pathogens removal (Ariunbaatar et al. 2014). Future research should naturally focus on the optimization of pretreatment with an associated economic analysis based on pilot-scale experiments. Alternative pretreatment methods should also attract some interest, either in combination or stand-alone. A recent study by Yu et al. (2017) reported promising results when alkaline microwave pretreatment was used to pretreat swine wastes. Other methods, such as electrochemical technologies, have also been found to positively affect AD performance. However, most studies found in the literature have been focused on substrates other than swine wastes (Yu et al. 2014; Wang et al. 2015; Ye et al. 2016). As such, future research should also be performed to assess whether electrochemical methods may be an encouraging option to enhance the anaerobic biodegradability of SW.

Process enhancement using additives

Another topic of current interest in AD research is the utilization of inorganic and biological additives to improve process performance (Table 4). Inorganic additives comprise zeolites, which can promote anaerobic biomass immobilization on surface particles, and also capture ammonia, conductive oxides, and heavy metals (Romero-Güiza et al. 2016).

Regarding SW, zeolites have been applied with promising results to increase methane production. Lin et al.

Table 4 Summary of the reviewed literature regarding AD process enhancement via the use of inorganic and biological additives

Additive		Process conditions		Relevant results	References
		Additive concentration	AD experiments		
Inorganic	Natural zeolite	60 g L^{-1}	Batch (1000 mL). Laboratory scale. Mesophilic conditions (35° C)	Biogas production increased by 20%	Lin et al. (2013)
Inorganic	Natural zeolite	40 g L^{-1}	BMP (500 mL). Mesophilic conditions (37° C)	Biogas production increased by 35%	Wijesinghe et al. (2018)
	Sodium Zeolites			Biogas production increased by 17%	
Inorganic	Natural zeolite	8.0 g L^{-1}	Batch (1200 mL). Laboratory scale. Thermophilic conditions (55 °C)	Methane production increase by 16%	Kotsopoulos et al. (2008)
Inorganic	Iron oxide–zeolite	5.0 wt.%	Batch (1000 mL). Laboratory scale. Mesophilic conditions (15 °C)	Methane production doubled	Lu et al. (2018)
			Batch (1000 mL). Laboratory scale. Mesophilic conditions (25 °C)	Methane production increased by 50%	
			Batch (1000 mL). Laboratory scale. Mesophilic conditions (35 °C)	Methane production increased by 34%	
Inorganic	Graphene oxide	500 mg L^{-1}	BMP. Mesophilic conditions (37 °C)	Methane production decreased	Zhang et al. (2017)
Biological	Lignocellulolytic microbial consortium	1.5:1 mg VSS g VS_{added}^{-1}	BMP. Mesophilic conditions (37 °C)	Methane production increased by 55%	Tuesorn et al. (2013)
Biological	Microorganism isolated from WWTP	–	Batch (50 mL). Laboratory scale. Mesophilic conditions (35 °C)	Methane production increased by 45%	Pessuto et al. (2016)

(2013) demonstrated that a natural zeolite dose of 60 g L^{-1} is an effective way of increasing biogas production. They observed enhanced performances of approximately 20% (356 mL g VS_{added}^{-1}). In another study, Wijesinghe et al. (2018) improved biogas production by adding natural and sodium zeolites at a dose of 40 g L^{-1}. These authors reported enhancements of 35%, and 17% for both zeolites, respectively, in comparison with control digesters. Both studies were conducted at mesophilic conditions.

Interestingly, Kotsopoulos et al. (2008) improved digestion with much lower zeolite doses between 8 and 12 g L^{-1}. In one of the reported experimental sets, they reported methane yields of 1629, 2637, and 2714 mL for zeolite doses of 0, 8, and 12 g L^{-1}, respectively. Their findings also included higher BOD_5 and VS removals. The conflicting results might be due to the different swine wastes used. Another important difference is that Kotsopoulos et al. (2008) performed their tests under thermophilic conditions. In both cases, the zeolites might act as an efficient surface to immobilize functional microbes, thus enabling the enhancement of the process due to increased biomass availability.

Other new zeolite-based additives have also been recently tested (Yamada et al. 2015; Lu et al. 2018). Yamada et al. (2015) found that particles of conductive iron oxides could accelerate the flow of electrons between microbial species in AD, thus facilitating microbial interspecies electron transfer. In fact, this electric syntropy may be critical in methanogenesis due to the syntrophic interactions between VFA-oxidizing bacteria and hydrogenotrophic methanogens (Yamada et al. 2015). Lu et al. (2018) furthered this idea and added zeolites coated with iron oxide minerals to the digestion of swine wastes (5 wt.%). Their experiments resulted in a remarkable increase in cumulative methane yields at different temperatures. Specifically, methane productions of 126.97, 285.08, and 437.85 mL g VS_{added}^{-1} were reported at 15 °C, 25 °C, and 35 °C, respectively. These values were 221.52%, 50.68%, and 33.84% higher when compared with digesters without zeolites (Lu et al. 2018).

Also, zeolites are known to keep favorable ammonia conditions, thus decreasing the likelihood of inhibition. Coincidentally, Wijesinghe et al. (2018) observed that natural and sodium zeolites at a dose of 100 g L^{-1} reduced ionized ammonia in the media by 50% and 52%, respectively, compared to the treatment without any addition.

Naturally, further research is expected with the mixing of novel additives in substrates. For instance, Zhang et al. (2017) investigated the role of graphene oxide as an AD additive with the potential to boost methane yields due to enhanced electron shuttle effects. However, they reported reduced methane yields with graphene oxide concentrations up to 500 mg L^{-1}. Despite the unpromising results, more experiments with novel additives should compose a future research focus in terms of process intensification techniques.

On the other hand, biological additives include bioaugmentation, i.e., the addition of mixed cultures of microorganisms with specific capabilities to facilitate particulate organic matter degradation and improve methanogenesis. These capabilities include high hydrolytic features or high methanogenic activity in stressful conditions (Romero-Güiza et al. 2016; De Vrieze and Verstraete 2016). Concerning the enhancement of biogas production from SW with the addition of external groups of microorganisms, two studies should be highlighted. Firstly, Tuesorn et al. (2013) showed the potential of a symbiotic biomass-degrading consortium to improve the production of biogas from swine wastes rich in fiber. The microbial consortium used in their study was isolated from the microflora in sugarcane bagasse compost. It comprised a multi-species lignocellulolytic enzyme system, which included extracellular cellulases of *clostridia,* hemicellulases, and a *β-glucanase* from *Clostridium*, *Bacillus*, and *Thermobacillus*. The addition of the microbial consortium enhanced biogas and methane production as compared to the control, with better results under mesophilic conditions. In particular, a 55% increase in accumulated methane (180 mL g VS_{added}^{-1}) was reported at 37 °C with a microbial consortium/swine manure ratio of 1.5:1 mg VSS g VS^{-1} (Tuesorn et al. 2013). Secondly, Pessuto et al. (2016) used microorganisms isolated from sewage sludge to improve the digestion of swine wastes. Before microorganisms addition, a biogas production of 30 mL g TS^{-1} was observed after 100 days. After the addition, biogas volume improved to 44 mL g TS^{-1}, which corresponded to an increase of more than 45%.

These biological additives compose a promising strategy for process intensification due to low cost, and more studies should emerge in the literature as process understanding increases. Nonetheless, practical application at full-scale plants should be far away in the future as risks related to the performance of specific microbial cultures continue to be high. This situation may imply unacceptable economic risks for farmers and project promoters as a consequence.

Digestate management

The sustainability of AD depends on managing the resulting digestate. Currently, most digestate from agricultural wastes such as SW is mechanically separated into liquid and solid fractions and used as biofertilizer (Dahlin et al. 2015; Monlau et al. 2015; Logan and Visvanathan 2019). This land application has clear economic and environmental benefits, but has also raised several concerns by accumulation of nutrients and heavy metals in agriculture soil (Monlau et al. 2015; Ni et al. 2017). Digestate management also faces handling and storage issues due to the lack of arable land for spreading and increasing transportation costs (Dahlin et al. 2015; Monlau et al. 2015).

Based on this, non-agricultural techniques of digestate processing have been proposed to solve this issue. Liquid SW digestate was found to support the growth of some microalgae species due to the chemical composition of the digested effluent (Deng et al. 2017; Koutra et al. 2018; Wang et al. 2019). Deng et al. (2017) demonstrated that the nutrients and organic acids present in SW were a suitable carbon substrate to grow *Chlorella vulgaris*. Their results showed that *C. vulgaris* completely degraded ionized ammonia and rapidly reduced TN by 70%. TP and COD were also notably reduced, with removal percentages around 50% and 80%, respectively. Koutra et al. (2018) furthered this idea by testing different microalgae strains on a digestate derived from agro-industrial wastes, including SW. They found that *C. vulgaris, Parachlorella kessleri,* and *Acutodesmus obliquus* were the microalgae most suitable for the valorization of the digestate. These three strains grew well on the digestate and also presented good results concerning nutrients removal. FAN removal was almost complete, while TP removal exceeded 80%. Their results hinted that selecting the appropriate microalgae species is fundamental to couple microalgae technology and AD. Other experiments by Wang et al. (2019) showed that microalgae cultivation could be combined with other processes (flocculation, struvite precipitation, and adsorption by activated carbon) to provide a complete process flow for the treatment of SW digestate. These microalgae studies (Deng et al. 2017; Koutra et al. 2018; Wang et al. 2019) indicated that microalgal biomass cultivation might be a future option for digestate post-treatment.

On the other hand, a growing body of the literature has also examined the use of liquid digestate recirculation for methane production (Ni et al. 2017; Pezzolla et al. 2017). Ni et al. (2017) demonstrated that digestate recirculation under low OLRs (5 g VS $L^{-1}d^{-1}$) improved methane production and system stability. However, recirculation also led to an intensified accumulation of heavy metals (e.g., Pb, Mn, Cu, and Zn), particularly under high OLRs. Pezzolla et al. (2017) also reported positive effects on biogas production from digestate recirculation. Overall, they concluded that the recirculation process improved the agronomical and environmental qualities of the final digestate. However, these results should be read with caution as concentrations of heavy metal elements were not reported.

Solid SW digestate has most often been tested as a raw material for energy and value-added products. Thermochemical processes are commonly used methods to achieve conversion. The initial work on the topic focused on digestate combustion. However, Pedrazzi et al. (2015) described difficulties during the combustion process due to ash sintering even with digestate and wood biomass mixtures. More recently, Barbanera et al. (2018) used thermogravimetric analysis (TGA) to study the combustion of SW solid digestate and wood gasification char in different blends. They concluded that co-combustion could provide an interesting option for the valorization of both residues. However, the findings do not validate the utilization of digestate as fuel in real conditions. Gasification of digestate is also recently gaining interest. Antoniou et al. (2019) obtained a syngas with medium lower heating value (2.88 MJ Nm^{-3}) in experiments with SW digestate in a laboratory-scale downdraft fixed-bed gasifier, thus demonstrating the potential of the technology. Finally, digestate from swine wastes was also evaluated as a potential feedstock for preparing biochars. Several experiments by Hung et al. (2017) suggested that the biochars obtained from SW digestate may be used as a biofertilizer, biosorbent, or soil amendment, but were not suitable as solid fuels in the industrial sector. The resulting biochars showed good mesoporosity and low higher heating value, thus justifying these claims.

Economic aspects of biogas utilization

Biogas utilization in agro-industrial farms results in economic benefits by allowing owners to reduce expenses in waste management and energy demand. However, a complete economic analysis of AD projects must incorporate the outcomes from expenditures and revenues to permit an investment decision.

Previous studies have assessed the feasibility of biogas projects using swine wastes (Table 5). Most of these studies are based on discounted cash flow (DCF) methodology to calculate financial indicators such as net present value (NPV) and internal rate of return (IRR). The biogas plants reviewed include different farm structures, digester designs, and substrates. As such, the techno-economic assessments presented also differ significantly. Generally, the AD systems reviewed treat a few thousand tons of waste yearly (less than 15,000 t a^{-1}) and have medium installed capacities (smaller than 250 kW). Cucchiella et al. (2019) recently proposed that the minimum size for a biogas plant to reach profitability is 200 kW. Their study applied an economic model based on DCF to evaluate biogas plants using swine wastes in the context of Italy. Also, co-digestion plants are common and have higher electricity production when compared with mono-digestion systems. Dennehy et al. (2017) compared the feasibility of mono- and co-digestion systems in Ireland and found that co-digesting food waste in an integrated pig farm had the highest revenue-generating potential under optimal market conditions. Total capital (CAPEX) and operating costs (OPEX) for such a process were €M 1.2 and 0.3 €M a^{-1}. Digestate construction and digestate management dominated these expenditures, respectively.

Two of the studies analyzed different economic scenarios for real biogas projects. Ruiz et al. (2018) assessed the economic performance of a thermophilic biogas plant built in Spain by Biogas Cell Fuel S.L. The installation treats 26,000

Table 5 Summary of the reviewed literature regarding the economic analysis of biogas plants digesting swine wastes

Reference	Farm/waste volume	Co-digestion/co-substrate	Real project	Generator	Methane yield	Annual electricity yield	Electricity price/subsidies	CAPEX	OPEX	Financial indicators	Year
Nolan et al. (2012)	500 sows/10,500 m^3 a^{-1}	Yes/grass silage	No	66 kW_{el}	995 m^3 d^{-1}	39.2 kWh m^{-3} feedstock	0.15 € kWh^{-1}/FIT	8437 € kW_{el}^{-1}	2.76 € m^{-3} feedstock a^{-1}	–	2012
Gebrezgabher et al. (2010)	50 farms/67,500 t a^{-1}	Yes/poultry manure, maize, food waste	Yes	1590 kW_{el}	289 m^3 d^{-1}	222.3 kWh t^{-1} feedstock	0.16 €/kWh^{-1}/FIP	4245 € kW_{el}^{-1}	3.14 € t^{-1} feedstock a^{-1}	NPV: 4195 M€; IRR: 21%	2010
Orive et al. (2016)	Pig farm/8750 t a^{-1}	No	No	62 kW_{el}	426 m^3 d^{-1}	48.57 kWh t^{-1} feedstock	0.13 € kWh^{-1}/unclear	4850 € kW_{el}^{-1}	16.3 € t^{-1} feedstock a^{-1}	NPV: 135,701 €; IRR 11%	2016
		Yes/olive pomace		262 kW_{el}	1785 m^3 d^{-1}	195.5 kWh t^{-1} feedstock		3382 € kW_{el}^{-1}	16.5 € t^{-1} feedstock a^{-1}	NPV: 782,493 €; IRR 14%	2016
Dennehy et al. (2017)	521 pigs/10,950 t a^{-1}	Yes/food waste	No	269 kW_{el}	1970 m^3 d^{-1}	215.5 kWh t^{-1} feedstock	0.15 € kWh^{-1}/FIT	4803 € kW_{el}^{-1}	17.8 € t^{-1} feedstock a^{-1}	NPV: 1.69 M€; IRR: 20%	2017
Li et al. (2015)	Rice–wine–pig farm/29,340 t a^{-1}	No	No	93 kW_{el}	500 m^3 d^{-1}	27.67 kWh t^{-1} feedstock	0.06 € kWh^{-1}/No	5296 € kW_{el}^{-1}	0.29 € t^{-1} feedstock a^{-1}	NPV: 10,000 €; IRR: 6%	2015
Ruiz et al. (2018)	Multi-waste plant/26,000 t a^{-1}	Yes/cow slurry, sewage sludge and food wastes	Yes	250 kW_{el}	3900 m^3 d^{-1}	76.92 kWh t^{-1} feedstock	0.05 € kWh^{-1}/No	11,617 € kW_{el}^{-1}	9.23 € t^{-1} feedstock a^{-1}	NPV: 474,375 €; IRR: 10%	2018

t a^{-1} of biowaste materials (including pig wastes) and is economically viable in the conditions described. Gebrezgabher et al. (2010) studied the Green Power biogas project, which is located in the northern part of the Netherlands and produces electricity and heat. They concluded that the plant is in good economic situation, earning a profit before tax of €M 1.0 and showing a positive NPV of €M 4.0.

Nevertheless, the economic profitability of biogas projects remains an open question. High capital investment and maintenance still compose a barrier for AD use since capital costs are the main contributor to energy generation costs (Vasco-Correa et al. 2018). De Clercq et al. (2017) investigated the economic performance of a biogas plant in south China designed to produce 15,000 Nm^3 d^{-1} of biogas, and their results showed poor economic performance due to high CAPEX and OPEX. In the reviewed studies, the CAPEX and OPEX figures ranged between €3382 – €11,617 kW_{el}^{-1} for CAPEX and €0.29 – €17.8 t^{-1} of feedstock a^{-1} for OPEX. Economic incentives can thus have an impact on the economic feasibility of biogas projects. These incentives have been focused mainly on electricity generation by offering above market feed-in-tariffs (FIT) or a feed-in-premium (FIP). Nolan et al. (2012) verified that the break-even point feed-in tariff for a farm with 500 pigs was € 0.27 kWh^{-1}, instead of the € 0.15 kWh^{-1} currently proposed in Ireland. Gutierrez et al. (2016) reached a similar conclusion for biogas adoption in Mexico, i.e., biogas from the AD of pig wastes is only profitable with subsidies.

Based on this discussion, economic assessment is crucial to select the pathway for biogas utilization. Biogas combined heat and power is currently the most robust technology, but biomethane offers novel market opportunities as compared with raw biogas. However, some key barriers to market development include biogas upgrading and lack of an external market. Other interesting alternatives are the use of raw biogas as fuel for farm vehicles and the production of second-generation fuels. Hydrogen particularly stands out since it can be produced by biogas reforming or other processes. Electrooxidation, for example, can work as the pretreatment or post-treatment of the SW generated on farm, thus complementing waste treatment. The use of biogas or derived fuels will undoubtedly increase in livestock sector industries such as pig farms as more pilot-scale projects demonstrate the technology. Besides technological innovations, future research should focus on energy balance, environmental, and economic assessment to ensure the viability of these novel solutions.

Conclusion

AD has emerged as an attractive pathway to stabilize wastewaters with high organic loads, such as swine wastewater. The degradation of organic materials by the action of microorganisms produces biogas, a valuable fuel with many applications. When compared with other treatments, the possibility for energy recovery is the main feature of AD. Cost-effectiveness, relatively low energy requirements, and higher odor control are other advantages. In the context of SW treatment, AD research activities have been focused on studying microbial dynamics (functional and metabolic insights), process performance (optimization), and stability (inhibition and toxicity). The microbiology of AD has been increasingly used to improve process understanding at the fundamental level. Researchers have also been experimenting with different operating conditions by changing process parameters to optimize performance and limit inhibition. Nonetheless, more on-farm studies are lacking to optimize the process in real conditions.

On the other hand, there is a strong need to improve biogas yields and enhance the economy of biogas plants. Methods of process enhancement include co-digestion of SW with carbon-rich feedstocks to balance the C/N ratio and improve performance. The development of effective pretreatment techniques is also likely to be a future research topic before full-scale implementation of AD systems. Using specific microbial populations and inorganic additives, for example, are two techniques which may significantly speed up digestion and stabilize biogas production. However, pretreatment methods could be unsustainable in terms of environmental footprints, even if they enhance performance. As such, energy balance and economic feasibility studies are two concepts of great importance within the field. Innovative practices of digestate management will also gain emphasis, but only if the economy of the processes is favorable.

Economic analysis should be the primary driving force to increase the use of biogas in the livestock sector in farms worldwide. While traditional pathways of biogas utilization such as electricity and heat production are established technologies, novel pathways such as on-farm biomethane production will play an important role in the future as a renewable fuel. Also, the possibility of in situ hydrogen production, by either wastewater electrooxidation or biogas reforming, is also expected to attract more attention in future research. If well developed, these AD-based processes may significantly contribute to solving the challenging energy and environmental problems currently faced by pig farms.

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