





35 **Abstract**

36 Swine wastewaters with high levels of organic pollutants and antibiotics have become  
37 serious environmental concerns. Anaerobic technology is a feasible option for swine  
38 wastewater treatment due to its advantage in low costs and bioenergy production.  
39 However, antibiotics in swine wastewater have problematic effects on micro-organisms  
40 and the stability and performance of anaerobic processes. Thus, this paper critically  
41 reviews impacts of antibiotics on pH, COD removal efficiencies, biogas and methane  
42 productions as well as the accumulation of volatile fatty acids (VFAs) in the anaerobic  
43 processes. Meanwhile, impacts on the structure of bacteria and methanogens in  
44 anaerobic processes are also discussed comprehensively. Furthermore, to better  
45 understand the effect of antibiotics on anaerobic processes, detailed information about  
46 antimicrobial mechanisms of antibiotics and microbial functions in anaerobic processes  
47 is summarized in this review. Future research on deeper knowledge of the effect of  
48 antibiotics on anaerobic processes are suggested to reduce their adverse environmental  
49 impacts.

50 Keywords: Antibiotics, Anaerobic processes, Swine wastewater, Impacts, Micro-  
51 organisms

52 **1. Introduction**

53 In the last few decades, swine farming has changed from small family farms to  
54 large concentrated animal feeding operations (CAFOs) to increase pig production at  
55 lower cost (Moses and Tomaselli, 2017). According to the research conducted by the  
56 Worldwatch Institute, CAFOs now account for 55% of pork production worldwide.  
57 However, intensive pig production requires frequent use of antibiotics for disease  
58 control and growth promotion. Consequently, large amounts of wastewater with high  
59 concentrations of organic pollutants, antibiotics and other toxicants was produced by

60 CAFOs (Cheng et al., 2018). Anaerobic treatment technology has been widely used to  
61 treat swine wastewater (the concentrations of COD, NH<sub>3</sub>-N, TN and TP are 3000-  
62 15,000, 400-1400, 600-2100 and 100-250 mg/L, respectively) (Cheng et al., 2018). As a  
63 cost-effective technology, anaerobic treatment consumes less energy to remove  
64 pollutants and generate methane rich biogas (Guo et al., 2015b; Sui et al., 2017).  
65 Besides, the smaller amounts of sludge production in anaerobic processes also minimize  
66 the risks associated with discharging antibiotics and antibiotic resistance genes in sludge  
67 to the environment (Xiong et al., 2017).

68         However, we cannot ignore the possibility that antibiotics in swine wastewater  
69 could pose serious problems to micro-organisms in anaerobic processes. They could  
70 reduce the microbial activities or change microbial populations, influencing the  
71 pollutants removal and biogas production (Ji et al., 2013). Currently, some studies have  
72 confirmed that the introduction of antibiotics influences the performance of anaerobic  
73 systems (Loftin et al., 2005; Poels et al., 1984). Others conclude that the presence of  
74 antibiotics in the anaerobic processes could result in a changed microbial structure by  
75 shifting microorganisms to less sensitive ones to specific antibiotics or by developing  
76 strains with antibiotic resistant genes (Angenent et al., 2008; Shimada et al., 2008).

77         The most frequently detected classes of antibiotics in swine wastewater are  
78 sulfonamides, tetracyclines and macrolides, with the concentration of 324.4, 388.7 and  
79 72 µg/L, respectively (Cheng et al., 2018; Li et al., 2017; Tong et al., 2009; Wei et al.,  
80 2011). These antibiotics have different modes of action on microorganisms, like  
81 interfering with the functions of cell membranes, blocking protein synthesis and  
82 preventing nucleic acid synthesis (Walsh, 2003). In anaerobic processes, several  
83 microbial groups convert complex organic compounds to simple, chemically stabilized  
84 ones. The coaction of bacteria and methanogens is critical to high-rate and stable

85 anaerobic treatments (Aydin et al., 2015e; Town et al., 2014). The presence of  
86 antibiotics during the anaerobic treatment process could disrupt the balance. In this case,  
87 the accumulation of metabolic intermediates like VFAs would seriously inhibit the  
88 anaerobic treatment efficiency (Ji et al., 2013).

89       Considering the prevalence of anaerobic technologies for treating swine  
90 wastewater and the adverse effect of antibiotics on the environment, researchers started  
91 to investigate the performance of anaerobic treatment processes in removing antibiotics  
92 and their corresponding resistance genes (Cheng et al., 2018). In order to improve the  
93 treatment performance, the inhibition effect of antibiotics on the anaerobic treatment  
94 processes has aroused great concern. In this review paper, the impact of antibiotics on  
95 the treatment performance of anaerobic systems and the shift of microbial communities  
96 will be discussed respectively. It will help greatly to improve the stability and efficiency  
97 of the anaerobic treatment of swine wastewater with antibiotics, and reduce the  
98 emission of these antibiotics to the environment. Additionally, this review is helpful for  
99 understanding: firstly, the microbial relationships in anaerobic processes; and secondly,  
100 the impacts of antibiotics on the dynamics of anaerobic microbial communities.

### 101 **1.1 Antibiotic classes and antimicrobial mechanisms**

102       Based on the antimicrobial mechanisms, the classifications of antibiotics are  
103 summarized in Fig. 1 (Cheng et al., 2018; Kapoor et al., 2017). The classes of  
104 sulfonamides, tetracyclines and macrolides are bacteriostatic antibiotics. They limit the  
105 growth of microorganisms by interfering with their protein production, DNA  
106 replication, or other aspects of cellular metabolism, but do not necessarily kill them.  
107 Meanwhile,  $\beta$ -lactam antibiotics like amoxicillin and penicillin have the ability to kill  
108 microorganisms by inhibiting cell wall synthesis or inhibiting enzymes or protein  
109 translation (Kohanski et al., 2010). Nonetheless, there is not always a precise distinction

110 between bacteriostatic and bactericidal antibiotics. Some high concentrations of  
111 bacteriostatic antibiotics also may kill microorganisms (Ocampo et al., 2014).

112       Antibiotics with different modes of action are known to have various effects on  
113 micro-organisms in anaerobic treatment processes. In particular, sulfonamides inhibit  
114 the synthesis of folic acid required for synthesis of purines and nucleic acid by  
115 preventing the addition of para-aminobenzoic acid into the folate molecule through  
116 competing for the enzyme dihydropteroate synthetase. Tetracyclines and macrolides  
117 inhibit protein synthesis by reversibly binding to receptors on the 30S and 50S  
118 ribosomal subunit of microbes (Tenover, 2006). The ribosomes of archaea and bacteria  
119 are relatively dissimilar in terms of size and shape, and consist of between 50 and 70  
120 proteins depending on the species. They incorporate three rRNA molecules: 16S, 23S  
121 and 5S rRNA. Some archaeal r-proteins are also closer in characteristics to the  
122 eukaryote ribosomal proteins (Ramirez et al., 1993). All cells require folic acid, but as it  
123 cannot enter bacterial cells, they have to manufacture folic acid themselves.

124 Sulfamethoxazole (SMX) inhibits a critical enzyme, dihydropteroate synthase, and  
125 therefore restricts the growth of the bacteria (Hong et al., 1995). These antibiotics  
126 impact only on bacterial ribosomal proteins and bacterial cells and do not affect archaea  
127 ribosomal proteins and cells (Aydin et al., 2015c). Conversely,  $\beta$ -lactams are able to  
128 inhibit bacterial cell wall synthesis, and then lead to the death of bacterial cell due to  
129 osmotic instability or autolysis (Kohanski et al., 2010).

## 130 **1.2 Microbial functions in anaerobic processes**

131       Under the action of micro-organisms, anaerobic processes are divided into four  
132 phases: hydrolysis, acidogenesis, acetogenesis, and methanogenesis, as shown in Fig. 2.  
133 Micro-organisms in each phase co-operate with each other to convert organic materials  
134 to methane and CO<sub>2</sub> in a step-wise reaction (McInerney et al., 2009). Hydrolysis and

135 acidogenesis are the initial phases of anaerobic digestion. Hydrolytic bacteria degrade  
136 complex polymers like carbohydrates, proteins and fats into sugars, amino acids and  
137 long chain fatty acids, respectively. Subsequently, this phase is followed by the action  
138 of acidogens, which convert water-soluble chemical substances and end products of  
139 hydrolysis to short-chain organic acids (formic, acetic, propionic, butyric, and  
140 pentanoic), methanol, ethanol, CO<sub>2</sub>, and H<sub>2</sub> (Ali Shah et al., 2014). Acetate, H<sub>2</sub>, CO<sub>2</sub>,  
141 and methyl compounds can be directly used by methanogens, while other intermediates  
142 formed via acidogenesis, such as propionate, butyrate, isobutyrate, valerate, isovalerate,  
143 and ethanol, have to be further bio-degraded through syntrophic acetogenesis to form  
144 acetate, H<sub>2</sub>, and CO<sub>2</sub> before methanogens utilize them to produce methane  
145 (Venkiteshwaran et al., 2016).

146 As described in Fig.3 (Ariesyady et al., 2007; González-Fernández & García-  
147 Encina, 2009; Guo et al., 2015a; Lang et al., 2015; Sánchez-Andrea et al., 2014; Stone  
148 et al., 2009; Suhadolnik et al., 2017; van de Werken et al., 2008; Vanwonterghem et al.,  
149 2016; Wang et al., 2017a; Wang et al., 2016), bacteria species in *Firmicutes*,  
150 *Bacteroidetes*, *Thermotogae*, *Proteobacteria*, *Actinobacteria*, *Chloroflexi* and  
151 *Spirochaetes* phyla are the function bacteria in anaerobic processes (Ali Shah et al.,  
152 2014; Guo et al., 2015a; Venkiteshwaran et al., 2016; Yang et al., 2014). In particular,  
153 within the previously mentioned phyla, the genera of *Caldicellulosiruptor*, *Butyrivibrio*,  
154 *Acetivibrio*, *Anaerococcus*, *Clostridium*, *Spirochaeta*, *Halocella* and *Bacteroides* have  
155 the ability to degrade cellulose-, hemicellulose- and pectin-containing biomass to  
156 acetate, CO<sub>2</sub>, and hydrogen or ethanol (Blumer-Schuetz et al., 2008; van de Werken et  
157 al., 2008). The genera of *Bacteroides*, *Escherichia*, *Thermotoga* and *Halothermothrix*  
158 can convert particulate materials like carbohydrates, protein and animal fats into  
159 dissolved materials. The microbes in *Proteobacteria* phylum also play important roles in

160 anaerobic digestion process, because most of *them* are well known propionate, butyrate,  
161 and acetate-utilizing microbial communities (Ariesyady et al., 2007).

162 As is already known, the accumulation of VFAs can destroy methanogens activity  
163 and result in the failure of anaerobic processes. Thus, converting intermediary  
164 metabolites into acetate and other substrates used by methanogens is important to retain  
165 the balance of anaerobic processes. Some members belonging to the *Firmicutes*,  
166 *Proteobacteria* -and *Chloroflexi* phyla, like *Syntrophomonas*, *Streptococcus*,  
167 *Pelotomaculum*, *Syntrophobacter*, and *Smithella* are syntrophic bacteria. They can  
168 degrade various VFAs into acetates and hydrogen used by methanogenic bacteria  
169 (González-Fernández & García-Encina, 2009; Schink, 1997; Stone et al., 2009; Wang et  
170 al., 2017a). However, the release of hydrogen may be toxic to the microbial community  
171 at this stage, since the build-up of hydrogen partial pressure to more than  $10^{-4}$  atm will  
172 inhibit the destruction of propionate and butyrate intermediates (Venkiteshwaran et al.,  
173 2016). Therefore, a symbiosis between acetogenic bacteria and autotrophic methane  
174 bacteria which consume hydrogen to produce methane is necessary (Ali Shah et al.,  
175 2014).

176 The last phase of anaerobic processes is occupied by methanogenic archaea, which  
177 degrade the products of previous phases, such as acetic acid, H<sub>2</sub>, CO<sub>2</sub>, formate,  
178 methanol, methylamine, or dimethyl sulphide to methane (Ali Shah et al., 2014). During  
179 this phase, species mainly belong to four phylogenetic orders of methanogens, namely,  
180 *Methanomicrobiales*, *Methanobacteriales*, *Methanococcales*, and *Methanosarcinales*.  
181 They are known to be responsible for the production of methane (Demirel & Scherer,  
182 2008). There are mainly three recognized methanogenic pathways, these being  
183 acetoclastic, hydrogenotrophic and methylotrophic pathways (Vanwonterghem et al.,  
184 2016). The acetoclastic pathway is an extremely important one for methane production



185 (70%) in anaerobic digestion processes (Stams et al., 2012). It is executed by members  
186 of the genera *Methanosaeta* and *Methanosarcina*, which are known to use acetate for  
187 methanogenesis. *Methanosaeta* is a specialist that uses acetate exclusively,  
188 while *Methanosarcina* is a relative generalist genus that can utilize methanol,  
189 methylamine and acetate as well as hydrogen and carbon dioxide for methane  
190 production (Guo et al., 2015a). For hydrogenotrophic methanogenesis, members of  
191 *Methanospirillum*, *Methanoculleus* and *Methanoregula* genera can reduce CO<sub>2</sub>  
192 successively to methane with H<sub>2</sub> as the primary electron donor, and formate through a  
193 series of intermediates, including formyl, methylene, and methyl levels. To maintain the  
194 stability of anaerobic systems, these species play a crucial role in maintaining the very  
195 low partial pressures of H<sub>2</sub> necessary for the syntrophic communities of bacteria and  
196 archaea to function properly (Stams et al., 2012). Among the above three pathways,  
197 methylotrophic methanogens have the smallest number of species belonging to  
198 *Methanosphaera*, *Methanococcoides*, *Methanohalophilus* and *Methanolobus* genera  
199 (Vanwonterghem et al., 2016).

## 200 **2. Impacts of antibiotics on the performance of anaerobic processes**

201 Anaerobic processes are widely applied to the treatment of swine wastewater  
202 (Cheng et al., 2018). However, the inhibition effects of antibiotics on the performance  
203 of anaerobic treatment processes have been recently documented (Álvarez et al., 2010;  
204 Aydin et al., 2015c; Aydın et al., 2015). As reported earlier (Table 1), the antibiotics  
205 reveal a wide range of differences regarding the performance of anaerobic processes.  
206 This is due to the variations of antibiotics concentrations and types as well as the  
207 combination of different antibiotics (Mitchell et al., 2013; Ozbayram et al., 2015).

## 208 **2.1 Impact on pH**

209 The level of pH is an important process parameter during anaerobic treatment  
210 processes. It should be noted that both acidogenic and methanogenic micro-organisms  
211 have their optimal pH value. Failing to maintain the optimal range of pH (6.7-7.4) in the  
212 anaerobic reactor could break down the anaerobic systems (Chen et al., 2008; Lay et al.,  
213 1997). The presence of antibiotics in the anaerobic reactors could result in the  
214 accumulation of VFAs, which would cause a decrease in the pH value (Beneragama et  
215 al., 2013; Ma et al., 2013). For example, Shi et al. (2011) and Aydin et al. (2015b)  
216 indicated that the pH value in reactors with antibiotics decreased more than that in  
217 control reactors. In the pig slurry anaerobic digestion process, the pH of the reactor with  
218 chlortetracycline (CTC) was lower than that in the control reactor (Stone et al., 2009).

219 Additionally, it is notable that the pH value in the anaerobic reactor was sensitive  
220 to high concentrations of antibiotics. As reported by Miller et al. (2013), the pH value  
221 remained stable, which maintained between 7.4 and 7.6, with the addition of 1-5 mg/L  
222 SMX to the reactor, however, the value of pH decreased to 6.3 immediately in response  
223 to an increase in VFAs after 50 mg/L of SMX was added. Also demonstrated that the  
224 pH value did not decrease from neutral (6.8–7.2) to 5.9 until the concentration of SMX  
225 and tetracycline (TC) was up to 20 and 1.5 mg/L, respectively. Thus, the stability of  
226 anaerobic reactors could be effected by adding high concentrations of antibiotics.  
227 However, the sharp decrease of pH might only happen at the beginning of the anaerobic  
228 process with a short contact time between antibiotics and the anaerobic sludge, which  
229 would become stable as the reaction progressed. This has been confirmed by the report  
230 of Beneragama et al. (2013) and Shi et al. (2011), who stated that the presence of  
231 antibiotics (cefazolin and OTC) wielded no effect on the pH value during anaerobic  
232 digestion processes after a sharp decrease of pH in the beginning. As well, the study

233 about effects of antibiotics on anaerobic digestion of swine slurry showed similar  
234 results, since the pH in bioreactors with antibiotics did not show any difference with the  
235 control reactors at the end of the treatment cycle (Masse et al., 2000). The reason might  
236 be that the short contact time between high concentrations of antibiotic and the  
237 anaerobic sludge can promote the organic acid production (acidogens) which results in  
238 the accumulation of organic acids due to the failure of methanogens to convert the  
239 organic acids to methane (Ma et al., 2013). Such results demonstrate that anaerobic  
240 treatment processes are sufficiently buffered to minimize pH fluctuations and have  
241 undergone digestion without a failure.

## 242 **2.2 Impact on COD removal efficiencies**

243 The COD removal efficiency could be affected by the presence of antibiotics in  
244 anaerobic treatment processes, which is significantly related to the concentrations of  
245 antibiotics (Aydin et al., 2015b). Previous research concluded that removing efficiencies  
246 of COD in anaerobic treatment processes can be significantly inhibited by high  
247 concentrations of antibiotics. For example, the COD removal efficiency only decreased  
248 from  $97.8 \pm 2.5\%$  to  $92.9 \pm 1.3\%$  as the addition of SMX from 1 to 40 mg/L. However,  
249 the ASBR system began to collapse when 45 mg/L of SMX was added with only  $25.0 \pm$   
250  $1.1\%$  of COD elimination (Cetecioglu et al., 2016). Sponza and Demirden (2007) also  
251 reported that the COD removal efficiencies in an upflow anaerobic sludge blanket  
252 reactor (UASB) reactor only varied from 89% to 82% when the sulfamerazine  
253 concentration rose from 10 to 65 mg/L. Once the concentration increased to 90 mg/L,  
254 only 68% of COD could be removed.

255 Meanwhile, tetracycline antibiotics reveal similar inhibition trends with  
256 sulfonamide antibiotics in the anaerobic processes. Cetecioglu et al. (2013) indicated  
257 that TC had no effect on the removal efficiency of COD in an ASBR reactor with the

258 concentration  $\leq 5.7$  mg/L, while adding higher dose of TC (8.5 mg/L) in the reactor  
259 resulted in a significant inhibition of the overall COD removal efficiencies (only 9%)  
260 compared with that in the control reactor ( $\geq 96\%$ ), and the metabolic activity of the  
261 biomass could not be reactivated at the end of the operation. Xiong et al. (2017) also  
262 reported that the COD concentration in the effluent of the anaerobic process was not  
263 affected in the presence of tetracycline with the concentration less than 150  $\mu\text{g/L}$ , but  
264 increased in greater amounts when adding 20 mg/L of TC.

265 Frequently, however, antibiotics are present as mixtures in the environment. The  
266 effects of mixtures differ from the individual compounds, due to the antagonistic and/or  
267 synergistic effects among them (Ozbayram et al., 2015). For instance, Aydin et al.  
268 (2015c), Aydin et al. (2014) and Aydin et al. (2016) indicated that the combination of  
269 antibiotics (erythromycin-tetracycline-sulfamethoxazole (ETS), sulfamethoxazole-  
270 tetracycline (ST), erythromycin-sulfamethoxazole (ES) and erythromycin-tetracycline  
271 (ET)) had more serious inhibition than the individual antibiotics on the COD utilization  
272 and methane production. As well, the study also indicated that the joint inhibition of  
273 combined antibiotic on the performance of anaerobic treatment was more serious at  
274 higher concentrations (Aydin et al., 2015b; Aydin et al., 2015c). In particular, Aydin et  
275 al. (2015b) concluded that the COD removal efficiency was not significantly affected if  
276 the added concentration of SMX and TC mixtures was less than 10 mg/L, whereas, the  
277 performance of the reactor declined substantially after 30 mg/L of the SMX and TC  
278 mixture was added to the reactor. The authors also concluded that less than 17 mg/L of  
279 ETS did not exert a noticeable effect on the overall COD removal efficiency, while the  
280 effluent soluble COD concentration would increase to more than 2000 mg/L ( $82 \pm 21.2$   
281 mg/L in control reactor), corresponding to an overall COD reduction of only 10% when  
282 46 mg/L of ETS were added. What is worse, such inhibition cannot be balanced since

283 the metabolic activity of the biomass could not be reactivated to induce noticeable  
284 substrate utilisation.

285 As discussed above, it is notable that the inhibitory influence of antibiotics on the  
286 COD removal efficiencies of the anaerobic process resulted in the accumulation of  
287 soluble COD in reactors. This is most likely due to the presence of antibiotic  
288 stimulated activities of fermentative or acid-forming bacteria in the anaerobic reactors  
289 which produce more soluble COD. In addition, the utilization of the soluble COD  
290 might decrease due to the inhibition of antibiotics on the interrelated activities of  
291 hydrogen producing acetogenic bacteria, methane producing aceticlastic methanogens,  
292 and or a combination of all these processes (Stone et al., 2009).

### 293 **2.3 Impact on biogas and methane production**

294 Biogas and methane generation is the inherent outcome of COD removal under  
295 anaerobic conditions, their production is parallel to the effluent COD concentration of  
296 anaerobic treatment processes. As the terminal end-products produced from  
297 biodegradable materials, biogas and methane production is good indicators of digestive  
298 performance (Boe et al., 2010). However, the effect of antibiotics on methane and COD  
299 might be different. As reported earlier, the addition of TC started to inhibit biogas  
300 production with the dosing of 1.65 mg/L in an ASBR despite achieving complete COD  
301 removal. Lu et al. (2016) also reported that the presence of TC (250 µg/L) in an  
302 anaerobic baffled reactor (ABR) had less influence on the degradation of organic matter  
303 but had a strong influence on biogas generation and the accumulation of VFAs. This  
304 might be the reason that the methanogenesis process was sensitive to TC presence, but  
305 the acidogenesis process was insensitive, so that only part of the substrate is utilized for  
306 methane production.

307 Similarly, there is a wide range of inhibition from complete biogas inhibition to  
308 slight biogas enhancement based on the antibiotic types and concentrations. For  
309 example, Sanz et al. (1996) indicated that the presence of penicillin and TC in swine  
310 slurries reduced methane production by 35% and 25%, respectively. However, other  
311 antibiotics in slurries from pigs, including tylosin, lyncomycin, sulfamethazine, and  
312 carbadox, did not significantly affect methane production. In addition, higher  
313 concentrations of antibiotics showed more serious influence on methane production.  
314 This is explained by their bactericidal characteristics that kill bacteria instead of  
315 inhibiting bacterial growth at high concentrations, which is very different to  
316 bacteriostatic characteristics at lower concentrations (Bauer et al., 2014; Shi et al.,  
317 2011). According to the report by Cetecioglu et al. (2015) and Mohring et al. (2009),  
318 biogas production was not affected significantly by adding sulfonamide antibiotics (less  
319 than 18 mg/L) in the anaerobic system; however, the inhibition became noticeable with  
320 the concentration of SMX higher than 45 mg/L.

321 Compared to other types of antibiotics, CTC and OTC showed a relatively serious  
322 effect on the biogas and methane production in anaerobic digesters (Wang et al., 2016;  
323 Yin et al., 2016). As reported by Sun et al. (2012), CTC was the most significant  
324 inhibitor among the antibiotics of CTC, amoxicillin, florfenicol, and sulfamethazine. In  
325 contrast, doxycycline (DC) showed less activity and resulted in a 25%-45% reduction  
326 on methane production with the concentration of 10-100 mg/L. Stone et al. (2009)  
327 stated that the CTC (27mg/L) reactor inhibited the production rate of methane and  
328 resulted in 27.8% reduction of total production, although total methane production  
329 increased in all anaerobic reactors of swine slurry. Sanz et al. (1996) also revealed that  
330 CTC was a powerful inhibitor for acetoclastic methanogenic bacteria with 50%  
331 reduction of methane at 40 mg/L of CTC concentration. Similar to CTC,

332 oxytetracycline (OTC) indicates a significant effect on methane production, and  
333 inhibition increased with the growing concentration of OTC (Ince et al., 2013). For  
334 instance, Arikan et al. (2006) revealed that OTC could reduce methane production by  
335 27% even at 3.1 mg/L in the anaerobic digestion system. Xin et al. (2014) also indicated  
336 that the generation rate of methane and total production of biogas during the anaerobic  
337 digestion process decreased with the concentrations of OTC stepwise increasing from 0,  
338 20, 50 to 80 mg/L. As well, the methane production fell by 56%, 60% and 62% at OTC  
339 and CTC concentrations of 10, 50 and 100 mg/L during the anaerobic digestion of pig  
340 slurry, respectively (Álvarez et al., 2010). Similarly, a highly significant decrease in  
341 methane production in the presence of OTC (around 3 mg/L) was also observed in two-  
342 phase and single-phase digesters of cattle manure, with 43% and 52% lower than  
343 control digesters, respectively. Interestingly, higher methane yields and OTC reductions  
344 were achieved with two-phase configuration, due to much higher cell activity was  
345 observed than that in single-phase digester (Akyol et al., 2016). This could be explained  
346 that CTC and OTC are effective against both gram-negative and gram-positive  
347 organisms by inhibiting bacterial protein synthesis through binding to the 30S ribosomal  
348 subunit. Therefore, at high CTC and OTC concentrations, they would impose a  
349 significant inhibition effect on the activity of microorganisms in anaerobic treatment  
350 processes (Beneragama et al., 2013; Wang et al., 2016).

351 As for tylosin, its inhibition behavior was only obvious at higher concentrations. It  
352 may be attributed to its limited spectrum of activity toward gram-negative bacteria,  
353 although tylosin is active against many gram-positive bacteria (Sanz et al., 1996). In an  
354 anaerobic sequencing batch reactor (ASBR), Angenent et al. (2008) and Shimada et al.  
355 (2008) both indicated that tylosin at 1.6 and 1.67 mg/L had negligible effects on the  
356 total methane production and propionate degradation, though the rate of both decreased.

357 Nonetheless, a reduction of methane production occurred after its concentration  
358 increased to 167 mg/L, because its high inhibition on propionate- and butyrate-  
359 oxidizing syntrophic bacteria and fermenting bacteria resulted in unfavourable effects  
360 on methanogenesis. The inhibition of high concentrations of tylosin on the anaerobic  
361 treatment process was also confirmed by Mitchell et al. (2013), who wrote that tylosin  
362 at a concentration of less than 100 mg/L did not show any inhibition on the total  
363 production of biogas, while as the concentration increased stepwise to 130, 260, 520  
364 and 913 mg/L, biogas production reduced by 10%, 20%, 30% and 38%, respectively.  
365 However, penicillin showed inhibitions on the methane production at all concentrations  
366 evaluated since this type of antibiotics can inhibit the cell wall synthesis (Rodríguez et  
367 al., 2017). Specifically, masse et al. (2000) found that bioreactors with penicillin or TC  
368 experienced a significant decrease of methane production (35% and 25%, respectively),  
369 with respect to the control ones. Gartiser et al. (2007) also discovered that amoxicillin, a  
370 comparable penicillin antibiotic, lowered biogas production by 10% and 20% with 12.3  
371 and 95.9 mg/L amoxicillin added in the reactor.

372 Again, the combination of antibiotics demonstrated higher inhibition on the  
373 methane production than the individual antibiotic. For example, Álvarez et al. (2010)  
374 reported the significant inhibition of anaerobic digestion in the swine slurry containing a  
375 combination of CTC and OTC at concentrations of 10, 50 and 100 mg/L; methane  
376 production reduced by 56%, 60% and 62%, respectively. Aydin et al. (2015b) also  
377 reported that the biogas and methane production were almost completely inhibited by  
378 the combined antibiotics of ERY, TC and SMX. The research conducted by Aydin et al.  
379 (2015a), Aydin et al. (2015b), and Ozbayram et al. (2015) showed the synergistic and  
380 antagonistic effect between different antibiotics. In the mixture of ETS, SMX can have  
381 an antagonistic effect on ERY and TC, however, the synergistic effect was observed in



382 almost all antibiotic mixtures that included TC as a component (Aydin et al., 2015b;  
383 Aydin et al., 2015).

384 However, some reports concluded that antibiotics had no or only a limited effect  
385 on the production of biogas even at high concentrations (Chelliapan et al., 2011;  
386 Mitchell et al., 2013). As described previously, SMX did not inhibit biogas production  
387 at 6-100 mg/L (Gartiser et al., 2007). The author also stated that the reactor with  
388 sulfamethazine and ampicillin up to 280 mg/L and 350 mg/L exhibited no impact on  
389 total biogas production compared with the control reactor, although they did inhibit  
390 biogas production rates during early stages of anaerobic digestion. Similarly, Lallai et  
391 al. (2002) demonstrated that OTC (125 and 250mg/L) had no varied effect on methane  
392 production in the anaerobic digestion of swine slurry in comparison to the control value.  
393 They concluded that both the acid-forming and methane-forming microbes were not  
394 affected by the presence of OTC. Chelliapan et al. (2011) also found negligible biogas  
395 inhibition with 100–800 mg/L tylosin in an up-flow anaerobic stage reactor (UASB).  
396 The reason for the above varied results may be attributed to the differences in the  
397 histories of sludge used, the acclimatization period, the microbial structures and the  
398 operational conditions. Álvarez et al. (2010) reported that the less inhibitive behavior of  
399 sulfonamides was due to their reduced antibacterial activity resulting from the large size  
400 of fresh inoculum and the complexity of the sludge (inherently including endogenous  
401 protein-degradation products). These organic compounds prevented sulfonamides from  
402 causing bacteriostasis on susceptible bacteria. It is known that methanogens are  
403 responsible for producing methane in an anaerobic treatment process. As advances are  
404 made in the anaerobic digestion process, the methanogen populations become more  
405 established, which leads to more methane being produced.

406           Moreover, according to the review paper by Chen et al. (2008), higher  
407 concentrations of antibiotics could be tolerated after a period of adaptation by microbes  
408 in anaerobic processes. Nevertheless, certain concentrations of antibiotics would exert  
409 some pressure on methanogens at the beginning, so methanogens had to undergo an  
410 acclimatization phase (Beneragama et al., 2013). Acclimatization is a selection and  
411 multiplication of specialized microorganisms capable of biodegrading or co-  
412 metabolizing the compounds or only surviving in the presence of relevant compounds  
413 after a certain adaptation time. Hence, the acclimatization phase was considered vital  
414 for the biodegradation of antibiotics and their impact on the performance of anaerobic  
415 treatment processes (Wang et al., 2017b). As a result, the antibiotics might inhibit the  
416 initial methane production, but this inhibition could be recover following the  
417 acclimatization period. This has been confirmed by Masse et al. (2000), the inhibition of  
418 antibiotics on methane production decreased because the reactors exhibited patterns of  
419 recovery or acclimation as time progressed.

420           The historical sludge and the acclimatization phase might therefore be potentially  
421 influential factors in the impact of antibiotics on methane production, and less inhibition  
422 on methane production was noted in sludge including a historical sludge with multiple-  
423 antibiotic used before (Huang et al., 2014). For instance, the absence of inhibition of  
424 antibiotics on the production of methane and treatment efficiency of anaerobic  
425 processes, reported by Loftin et al. (2005) and Dreher et al. (2012), might be the  
426 consequence of the acclimation experienced by their microbial consortia, since it has  
427 adapted to the presence of antibiotics. García-Sánchez et al. (2016) also revealed that  
428 for biomass that had no contact with an antibiotic, the presence of tylosin inhibited the  
429 generation of methane even at concentrations as small as 0.01 mg/L. Whereas, in the  
430 digesters acclimating the presence of tylosin at a concentration of 0.01 - 0.065 mg/L,

431 methanogenesis was not inhibited and the generation of methane improved. It may  
432 imply that the microorganisms have developed not only a resistance to the antibiotic but  
433 also the ability to metabolize it.

434       The increasing concentrations of antibiotics do not always cause the elevated  
435 inhibition of methane production; conversely, the use of an appropriate dose of  
436 antibiotics could aid in enhancing methane production due to their metabolism by  
437 microorganisms (Sponza & Demirden, 2007). The authors indicated a relatively high  
438 concentration of sulfamethazine could increase the total amount of methane production.  
439 In the UASB reactor, with the addition of 10 and 90 mg/L of sulfamerazine, the daily  
440 methane gas production was recorded as 1558 ml and 2000-2275 ml, respectively,  
441 although the maximum methane percentage decreased from 76% to 60%. Yin et al.  
442 (2016), Lu et al. (2014) and Yin et al. (2015) also demonstrated the beneficial role of  
443 CTC, OTC, cefalexin and colistin sulfate in methane production in anaerobic treatment.  
444 These results might be attributed to the utilization of antibiotics as co-substrates  
445 together with glucose-COD, which were used as primary carbon and energy sources for  
446 micro-organisms in the anaerobic processes (Sponza & Demirden, 2007).

#### 447 **2.4 Impact on the accumulation of VFAs**

448       VFAs, as short chain fatty acids that include formate, acetate, propionate,  
449 butyrate etc., are intermediate and or end products of the anaerobic process. Their  
450 accumulation is important for the performance of anaerobic systems (Lins et al.,  
451 2015). One research group observed that adding SMX, ERY, and TC at 15 - 20, 1.5,  
452 and 1.5 mg/L concentrations, respectively, could accumulate 400 - 600 mg/L of VFAs  
453 (Aydin et al., 2015d). Similarly, Cetecioglu et al. (2015) and Miller et al. (2013) stated  
454 that increasing SMX concentration to 45 mg/L and 50 mg/L could result in the  
455 accumulation of VFAs and the decrease of pH, alkalinity in anaerobic processes.

456 Stone et al. (2009) indicated the VFAs accumulation in the CTC (27mg/L) treatment  
457 reactor was the greatest about 37.4% - 47.0% more than the tylosin and control reactors,  
458 and the acetate concentration in the CTC treatment (12,269 mg/L) was greater than  
459 either the tylosin (7687 mg/L) or the no-antimicrobial control (6498 mg/L) treatments.  
460 This might be the reason that the generation of acetate through soluble organic  
461 fermentation is efficient, however, the utilization of acetate by either homoacetogenic  
462 bacteria or aceticlastic methanogens was inhibited by CTC. As well, both propionate  
463 and butyrate concentrations in the CTC and tylosin (1.67 mg/L) treatment reactor were  
464 greater than those in control, implying that CTC and tylosin inhibited propionate and  
465 butyrate degraders, such as the genera of *Pelotomaculum*, *Psychrobacter* and  
466 *Streptococcus* (as shown in Table 2). Similarly, Sanz et al. (1996) also indicated the  
467 butyrate degrading bacteria were affected by CTC at low concentration, and died as the  
468 concentration of CTC increased above 100 mg/L. This resulted in a greater  
469 accumulation of short-chained VFAs during the anaerobic digestion compared to a no-  
470 antimicrobial control system. The anaerobic reactors with OTC (30, 60, 90 mg/L) also  
471 exhibited an increasing trend in the accumulation of total VFAs compared to the control  
472 reactor (Beneragama et al., 2013).

473 The accumulation of VFAs showed a positive correlation with antibiotic  
474 concentrations. This has been confirmed by Aydin et al. (2015a), who revealed that the  
475 VFAs concentration increased in a linear manner with the increase of antibiotics  
476 concentration. Xiong et al. (2017) also indicated that TC had no impact on the  
477 accumulation of VFAs with the concentrations less than 150 µg/L, while a significant  
478 accumulation of VFAs and increase in propionate were observed in the reactor  
479 subjected to the highest concentration of TC (20 mg/L). The accumulation of VFAs

480 may result from the stimulation of the acidogenesis and/or the subsequent inhibition of  
481 VFAs degradation as well as methanogenic activities (Beneragama et al., 2013).

482 As well, the effect of a combination of antibiotics in swine wastewater is higher  
483 than the effect of the individual antibiotic. For example, Cetecioglu et al.  
484 (2012) examined the individual inhibitory effects of antibiotics on the ASBR process.  
485 They indicated that the VFA accumulation started from 25 mg/L within the tetracycline  
486 dosage and 250 mg/L within the SMX and ERY dosage. However, Aydin et al. (2015a)  
487 found VFAs started to accumulate even in the presence of very low concentration of  
488 antibiotic mixtures (1 mg/L of ET–ST). Their results indicate that antibiotic  
489 combinations have an effect on acetate, propionate and butyrate degradation pathways,  
490 leading to the accumulation of VFAs and soluble microbial products, which results in a  
491 decrease in the total methane production.

492 In summary, different classes of antibiotics reflect different impacts on the  
493 performance of anaerobic treatment processes related to their mode of actions.  
494 Generally, lower dose of antibiotics shows less impact on the stability, the removal  
495 efficiencies and the production of biogas and methane in anaerobic processes. In  
496 contrast, relatively higher concentrations of antibiotics cause toxic effects on  
497 microorganisms in the anaerobic process, affect the substrate removal and the biogas  
498 production, and even result in a total collapse of the reactors. The various combinations  
499 of antibiotics increase inhibition effects over the individual antibiotics. Additionally, the  
500 synergistic and antagonistic effects are also identified in the reactor within the antibiotic  
501 mixtures.

### 502 **3. Impact of antibiotics on the microbial communities in anaerobic processes**

503       The anaerobic system contains a complex microbial culture, and the conversion of  
504 organic compounds to methane is carried out by various microbial communities in  
505 acidogenic and methnogenic processes (Town et al., 2014). There is a significant  
506 correlation between the microbial community (both bacterial and archaeal) and the  
507 performance of anaerobic reactors such as COD removal efficiency, biogas production,  
508 and VFAs accumulation. Failure to maintain the stability of these microorganisms  
509 would result in a decrease in the performance and stability of anaerobic reactors (Aydin  
510 et al., 2015e; Beneragama et al., 2013; Cetecioglu et al., 2016). Based on all of the  
511 above, we can conclude that the presence of antibiotics has a negative effect on biogas  
512 and methane production, yet appears to have a positive effect on the accumulation of  
513 VFAs. This indicates that the methanogenesis process is sensitive to the exposure to  
514 antibiotics, while the acidogenesis process is not. As reported, the decrease in methane  
515 and biogas production was closely related to the disappearance of the acetoclastic  
516 methanogens represented by the *Methanosarcinales* order in the reactor with antibiotics  
517 (Aydin et al., 2015e). Venkiteshwaran et al. (2016) and Aydin et al. (2015) did report  
518 the change in the composition and diversity of the microbial community was linked to  
519 the performance of anaerobic reactors. Hence, in anaerobic treatment processes, a  
520 relatively higher balance and diversity in their bacterial communities resulted in a  
521 higher biogas and methane production. Nonetheless, the existence of antibiotics in  
522 anaerobic systems may change the structure of the microbial community (Xin et al.,  
523 2014; Table 2). The reason is that antibiotics in general, even those broad-spectrum  
524 medications, have their selective effects on various groups of microbes. As a result,  
525 the selective antibiotic effects alter the relative abundance of microbial species, and

526 subsequently interfere with the interactions among different species (Wang et al.,  
527 2017b).

### 528 **3.1 Impact on the bacteria in anaerobic processes**

529 During the initial phase of anaerobic treatment processes, hydrolytic and  
530 fermentative bacteria, belonging to *Bacteroidetes*, *Firmicutes*, *Thermotogae*,  
531 *Actinobacteria* and *Spirochaetes* phyla (in Fig. 3) showed positive correlation with the  
532 presence of antibiotics. For example, Cetecioglu et al. (2016) demonstrated that  
533 *Clostridium* species in the *Firmicutes* phylum which were known to produce lactic acid,  
534 ethanol and volatile fatty acids, became dominant in the ASBR with SMX. As well, the  
535 number of *Acinetobacter* species increased along with the rising concentration of SMX  
536 (Cetecioglu et al., 2016). This may explain why *Acinetobacter* species were reported as  
537 the hosts of sulfonamide resistance genes. Xiong et al. (2017) also found the changes in  
538 microbial communities in anaerobic treatment processes when exposed to different  
539 concentrations of TC. In the anaerobic reactor with 20 mg/L of TC, the relative  
540 abundance of bacteria belonging to *Bacteroidetes*, *Spirochaetes* and *Firmicutes* phyla  
541 increased significantly in comparison with the reactor with 150 µg/L of TC (Xiong et  
542 al., 2017). Specifically, the VFAs producing bacteria increased significantly in the  
543 reactor with the high concentration of TC, including *Clostridium aurantibutyricum*,  
544 *Microbacter margulisiae*, *Porphyromonas pogonae*, *Treponema zuelzeriae* and  
545 *Proteiniphilum acetatigenes* (propionate-producing bacteria). Similarly,  
546 *Spirochaetaceae*, in the phylum *Spirochaetes*, could ferment glucose to acetate, ethanol,  
547 and small amounts of lactate which present an obviously increasing trend during the  
548 anaerobic digestion reactors with the addition of CTC (Wang et al., 2017a). The  
549 positive effect of antibiotics on some bacteria might relate to the contact time between

550 antibiotics and biomass. This was demonstrated by faster growth kinetics and a better  
551 adaption rate of antibiotics and the bacteria (Aydin et al., 2015c; Ma et al., 2013).

552 Yet, a negative relationship between antibiotics and the VFAs degrading bacteria  
553 in anaerobic processes was found by previous researchers. Aydin et al. (2016) indicated  
554 that *Bacteroidetes*, *Acinetobacter* and *Proteobacteria* were negatively affected by  
555 different antibiotic combinations present in the anaerobic reactor compared to the  
556 control samples. This is despite the fact that the population of the *Firmicutes* in the  
557 reactor did not significantly change in comparison to the control reactor according to  
558 antibiotic concentration (3.0 mg/L). Xin et al. (2014) and Akyol et al. (2016) also  
559 reported that the total microbial diversity decreased with the addition of OTC in the  
560 anaerobic digestion reactor, and a higher concentration of OTC would decrease the  
561 relative abundance of *Gammaproteobacteria* in *Proteobacteria* phylum and result in the  
562 disappearance of *Sphingobacteriaceae* in *Bacteroidetes* phylum, although the genus  
563 *Flavobacterium* revealed great resistance to the increase in antibiotic loadings, and  
564 existed throughout the entire digestion process. Bacterial phyla, including  
565 *Proteobacteria*, *Cloacimonetes*, *Ignavibacteriae*, and *Chloroflexi*, showed the  
566 significantly less number in the reactor with high concentrations of TC than those in  
567 the control reactor (Xiong et al., 2017). According to one study about the acute effects  
568 of antibiotics on syntrophic butyrate and propionate-oxidizing bacteria in ASBR,  
569 antibiotics could cause inhibitory effects on butyrate and propionate degradation  
570 bacteria, including the species of *Syntrophomonas*, *Syntrophospora*,  
571 *Syntrophobacter* and *Pelotomaculum* (Aydin et al., 2015a). As a result, the utilization of  
572 VFAs by bacteria is affected by the addition of antibiotics, and the propionic acid  
573 utilization is much more affected than butyric acid utilization.



### 574 **3.2 Impact on methanogens in anaerobic processes**

575 During the methnogenic phase, the impact of antibiotics on the structures of  
576 acetogenotrophic, hydrogenotrophic and methylotrophic methanogens could  
577 dominate the performance of the whole anaerobic treatment processes. As reported,  
578 the presence of antibiotics in the anaerobic reactors had adverse effects on  
579 acetogenotrophic methanogens but positive effects on the abundance of  
580 hydrogenotrophic methanogens. For instance, Xiong et al. (2017), Cetecioglu et al.  
581 (2013) and Wang et al. (2017a) concluded that the long-term exposure of TC and CTC  
582 to the anaerobic reactor had negative effects on the relative abundances of  
583 acetogenotrophic methanogens like *Methanothrix*, *Methanoculleus*, and  
584 *Methanobacterium* genus. However, an increase in the relative abundance of  
585 *Methanomassiliicoccus* and *Methanoculleus* (hydrogenotrophic methanogens) was  
586 observed. Similarly, the abundance of hydrogenotrophic methanogens, especially  
587 *Methanobacterium species*, and *methanogenic archeons* in the ASBR with the high  
588 concentration of SMX (40 mg/L) also became dominant through the operation, while  
589 the acetoclastic methanogens disappeared in the last phase (Cetecioglu et al., 2016; Shin  
590 et al., 2010). The order *Methanomicrobiales*, which utilizes hydrogen or formate as  
591 electron acceptors to produce methane, is the most abundant methanogenic group in the  
592 anaerobic digestion processes with high concentrations of OTC (Wang et al., 2016).

593 In addition, Aydin et al. (2015e) and Cetecioglu et al. (2015) also reported that the  
594 total number of methanogenic population was not affected in the processes with long-  
595 term exposure to high concentrations and combined antibiotics, probably due to the shift  
596 of the major pathway to hydrogenotrophic methanogenesis. Hydrogenotrophic  
597 methanogens were higher in substrate utilization rate, growth rate and cell yield to  
598 exposed toxic substances compared to acetoclastic methanogens (Aydin et al., 2015c).

599 The reason is that the inhibition effect of antibiotics on the acetoclastic methanogens  
600 makes homoacetogenic bacteria more competitive to transfer acetate to H<sub>2</sub> and CO<sub>2</sub>, and  
601 this procedure would provide a substrate for hydrogenotrophic methanogens to produce  
602 methane. Consequently, *homoacetogenesis* coupled with *hydrogenotrophic*  
603 *methanogenesis* enables the microbial community to maintain the system's stability  
604 (Aydin et al., 2015a; Cetecioglu et al., 2016). The research reported by Aydin et al.  
605 (2015c) also confirmed that antibiotic combination did have a dramatic effect on the  
606 acetoclastic methanogens present in the ST and ETS reactors. In particular, as the  
607 ancestral form of methane production, hydrogenotrophic methanogenesis was  
608 reported to be the most widespread in all methanogenic orders (Baptiste et al., 2005).

609 Methanogenic archaea show more tolerance than bacteria when exposed to  
610 antibiotics which inhibiting protein synthesis like tetracyclines and macrolides (Aydin  
611 et al., 2015). The reason is that archaeal ribosomes have a heterogeneous protein  
612 composition, enabling them to adapt to harsh environmental conditions in comparison  
613 with bacterial ribosomes (Hilpert et al., 1981). Furthermore, sulfonamides are  
614 bacteriostatic inhibitors designed to prevent bacterial infections and therefore reveal a  
615 more obvious effect on bacteria than archaea (Aydin et al., 2015e). Thus, both bacteria  
616 and methanogenic archaea can be affected by the presence of antibiotics according to  
617 the classes and concentrations of antibiotics. Previous research revealed that even  
618 though some species in the anaerobic process were negatively affected by higher  
619 antibiotic concentrations, the surviving species continued the degradation of substrate  
620 and the production of methane (Cetecioglu et al., 2016).

#### 621 **4. Future perspectives**

622 Anaerobic processes are widely applied to treat wastes from swine farms. So far,  
623 studies mainly focused on the occurrence, fate, and removal of antibiotics from swine

624 wastewaters through anaerobic processes (Cheng et al., 2018). However, the toxic  
625 effect of antibiotics on micro-organisms in anaerobic processes should be given more  
626 attention considering the important role of micro-organisms in these processes. In this  
627 review, we can see the complicity of microbial communities responding to antibiotics.  
628 Hence, it is important and necessary to study the impact of antibiotics on the treatment  
629 process and microorganisms in anaerobic processes, to understand the fate of antibiotics  
630 in anaerobic processes, to know the removal mechanism of antibiotics by the anaerobic  
631 treatment, and to improve their removal efficiencies from wastewater.

632 To date, there are still large gaps in our knowledge on the impact of antibiotics on  
633 anaerobic processes. Only a few types of antibiotics, such as SMX, TC, CTC, etc., were  
634 studied previously, whilst studies on other antibiotics in swine wastewater are very  
635 limited. Furthermore, these analyses mainly focused on the potential inhibiting  
636 mechanisms of individual antibiotic. However, we should not ignore the fact that  
637 antibiotics do not appear in swine wastes individually, but rather together with many  
638 other types of antibiotics and toxic pollutants (hormones and/or heavy metals) (Zhang et  
639 al., 2017b). The current studies on the impact of the coexistence of different types of  
640 antibiotics and metals on the anaerobic treatment processes only touch the tip of the  
641 iceberg; much more investigation should be done in the future due to their synergistic  
642 and or antagonistic effects (Aydin et al., 2015c; Guo et al., 2012; Zhang et al., 2017a).  
643 In addition, only a few studies focused on the impact of the metabolites of antibiotics on  
644 micro-organisms in anaerobic processes, and noted their potential toxic effects  
645 (Baumann et al., 2015). Thus, further studies are needed to obtain more comprehensive  
646 data about the impact of the combination of many antibiotics as well as their  
647 metabolites on microbial communities in anaerobic treatment processes.

648 Furthermore, the proliferation and dissemination of antibiotic resistance genes in  
649 the anaerobic swine wastewater treatment process are the most serious threats of  
650 antibiotics to the environment and people's health. Therefore, to reduce the threats of  
651 antibiotic resistance genes in anaerobic treatment processes, more studies are urgently  
652 required to explore the relationship between antibiotics, microorganisms and antibiotic  
653 resistance genes.

## 654 **5. Conclusion**

655 The key conclusions in this review article are as follows:

- 656 - Higher doses and combined antibiotics revealed more inhibition effects on  
657 anaerobic processes than that of lower concentration and individual antibiotics.
- 658 - The VFAs degrading bacteria and acetoclastic methanogens were more sensitive  
659 to antibiotics than other hydrolytic bacteria, VFAs producing bacteria, and  
660 hydrogenotrophic methanogens in the anaerobic processes.

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#### 954 **Figure Captions**

955 Fig. 1 Classes and antimicrobial mechanisms of antibiotics in swine wastewater

956 Fig. 2 Phases of the anaerobic digestion process

957 Fig.3 Microorganisms ((a) bacteria; (b) methanogens) and their functions in anaerobic  
958 process



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960 **Table Captions**

961 Table 1 Impact of antibiotics on the performance of anaerobic treatment processes

962 Table 2 Impact of antibiotics on the microbial communities in anaerobic processes

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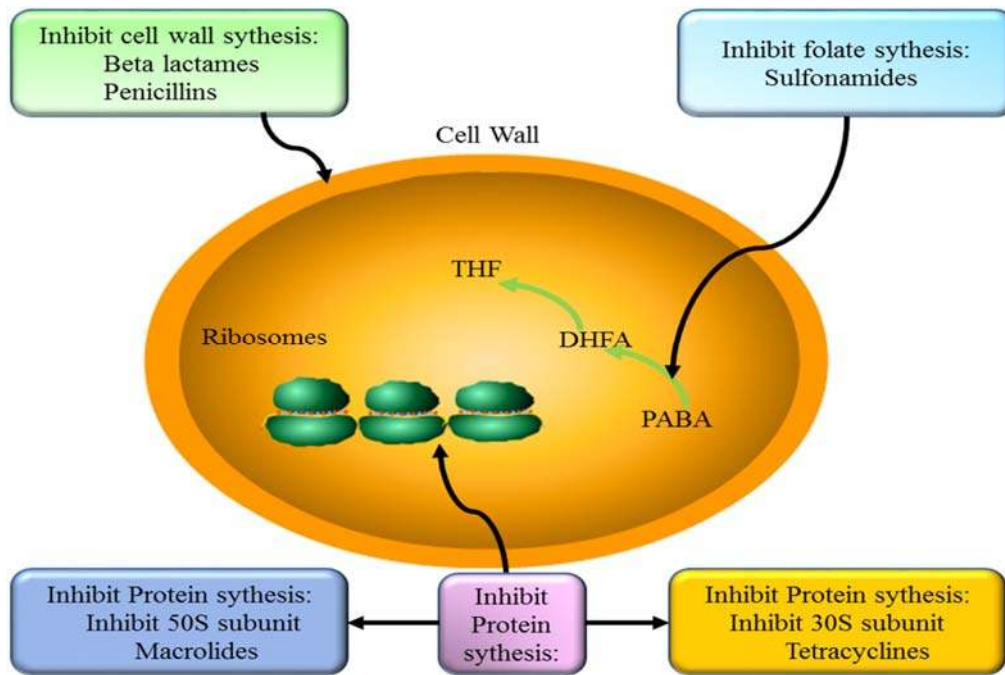
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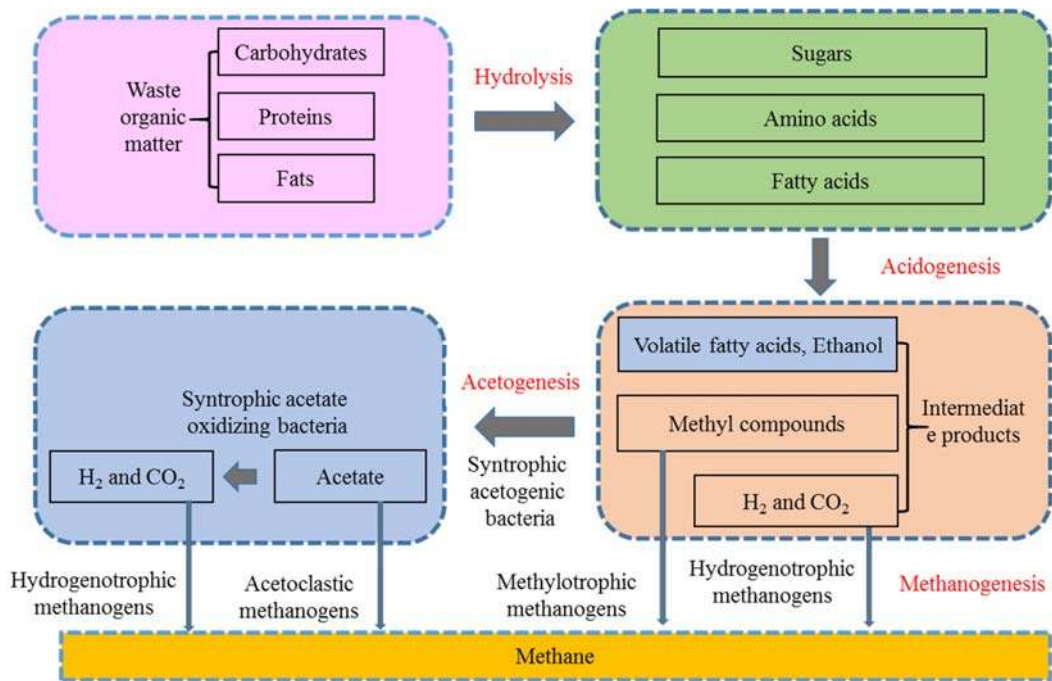
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Fig. 1 Classes and antimicrobial mechanisms of antibiotics in swine wastewater



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Fig. 2 Phases of the anaerobic digestion process

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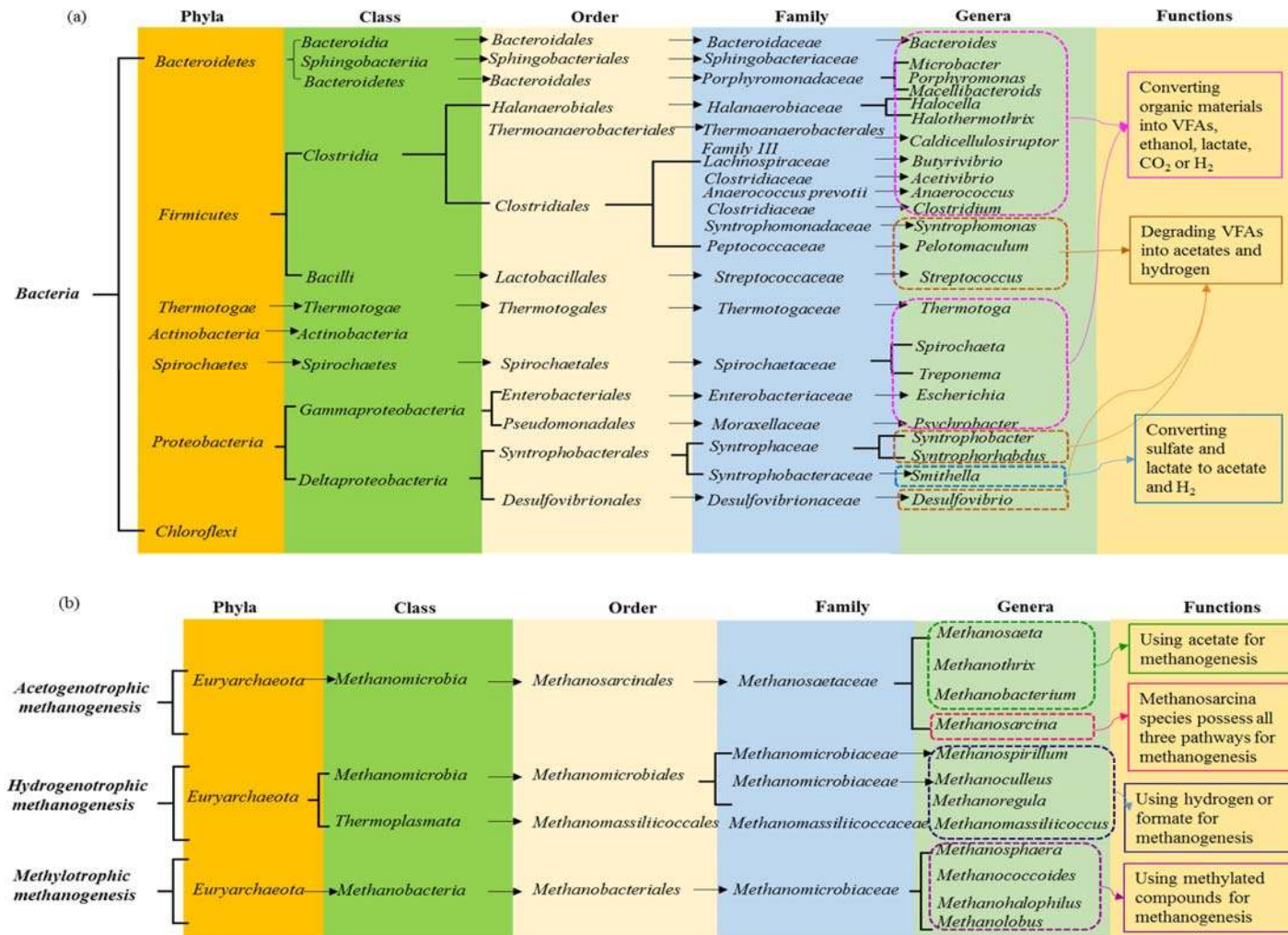


Fig.3 Microorganisms ((a) bacteria; (b) methanogens) and their functions in anaerobic processes

Table 1 Impact of antibiotics on the performance of anaerobic treatment processes

Antibiotic	Concentrations (mg/L)	Effects			References
		COD removal efficiencies	Biogas/methane production	VFAs accumulation (mg/L)	
Sulfamethoxazole	1–45	96.8 ± 2.6%–25.0 ± 1.1%	Biogas: 1004 ± 129 mL/d–96 mL/d	Propionic acid : not detected–438; Acetic acid: not detected–342	(Cetecioglu et al., 2016)
	6–100	–	No inhibition	–	(Gartiser et al., 2007)
Sulfamerazine	10–90	88%–68%	Methane content: 76%–60%	200–600	(Sponza & Demirden, 2007)
	≤280	–	No impact on total biogas production	–	(Mitchell et al., 2013)
Sulfamethazine	5.0–160	–	Inhibited	Accumulated	(Sun et al., 2012)
Tetracycline	1.65–5.7–8.5	96%–9%–0%	Biogas: 951–853–71 mL/day	Acetic acid: not detected–457; Propionic	(Cetecioglu et al., 2013)

				acid: not detected– (750–385); Butyric acid: 4–20; Valeric acid: 14–70	
	0.15–20	Decreased	Methane: 160–110 mL	propionic acid: 0.7–44	(Xiong et al., 2017)
	550 mg/kg in pig diet	No statistically affected	25% reduction of methane	Not statistically affected	(Masse et al., 2000)
Chlortetracyclin	500 mg/kg in pig slurry	Decreased	Increased	Accumulated	(Wang et al., 2017a)
	28	Decreased	27.8% reduction of methane	Accumulated	(Stone et al., 2009)
	40	–	50% reduction of methane	–	(Sanz et al., 1996)
Oxytetracycline	60, 100, 140 mg/kg in pig slurry	–	Reduce biogas production by 9.9, 10.4, and 14.1%	–	(Wang et al., 2016)
	30, 60, 90	–	79.1, 70.3, 68.6% of the control values	Increased	(Beneragama et al., 2013)

	125, 250	–	No inhibition	–	(Lallai et al., 2002)
	3.1	No significant effect	Reduce methane production by 27%	–	(Arikan et al., 2006)
Oxytetracycline					
–					
Chlortetracycline	10, 50 and 100	–	Reduced methane production by 56, 60 and 62%	–	(Álvarez et al., 2010)
Tylosin	130, 260, 520, 913	–	Reduced biogas production by 10, 20, 30, 38%	–	(Mitchell et al., 2013)
		Negligible effects			
	1.67–167	to completely inhibited	Negligible effects to completely inhibited	Accumulated	(Shimada et al., 2008)
Amoxicillin	60, 120	–	75% and 68% of the control	–	(Lallai et al., 2002)
	12.3, 95.9	–	Reduced biogas production by 10% and 20%	–	(Gartiser et al., 2007)

Penicillin	16 mg/kg used in pig diets	No statistically affected	35% reduction of methane production	not statistically affected	(Masse et al., 2000)
Erythromycin– Tetracycline (ET)	(0.1 + 0.1)– (3+3)	90%–12%	Production rate decreased by more than 97%		(Aydin et al., 2016)
combinations					
Sulfamethoxazo le –Tetracycline	20 + 1.5 12–43	Decreased 80%–10%	– Biogas production: 1247 mL/day–0	–	(Aydin et al., 2015c) (Aydin et al., 2015b)
Sulfamethoxazo le – Erythromycin – Tetracycline	2.5 + 2.5 + 25 18– 46	Decreased 75%–10%	– Biogas production: 1247 mL/day–0; methane yield dropped from 0.32 L/g COD removed to 0	– Acetic acid : 50–1000; Butyric acid: 140–710	(Aydin et al., 2015c) (Aydin et al., 2015b)



Table 2 Impact of antibiotics on the microbial communities in anaerobic processes

Antibiotic	Concentrations (mg/L)	Microbies	Effects	References
Sulfamethoxazole	1–45	<i>Clostridium</i>	Decreased	(Cetecio glu et al., 2016)
		<i>Acinetobacter</i>	Increased	
		<i>Acetoclastic methanogenic</i>	Disappeared	
		<i>Methanobacterium,</i>	Higher than	
		<i>Methanogenic archeons</i>	acetoclastic methanogens	
Tetracycline	1.65–8.5	<i>Bacteroidetes,</i>	Increased	(Xiong et al., 2017)
		<i>Spirochaetes,</i>		
		<i>Syntrophomonas,</i>		
		<i>Clostridium</i>		
		<i>aurantibutyricum,</i>		
		<i>Microbacter</i>		
		<i>margulisiae,</i>		
		<i>Porphyromonas</i>		
		<i>pogonae, Treponema</i>		
		<i>zuelzerae,</i>		
<i>Proteiniphilum</i>	Decreased			
<i>acetatigenes,</i>				
<i>Proteobacteria,</i>				
<i>Cloacimonetes,</i>				
		<i>Ignavibacteriae,</i>		

		<i>Chloroflexi</i> ,		
		<i>Syntrophomonas</i> ,		
		<i>unclassified</i>		
		<i>Syntrophobacterace</i>		
		<i>ae</i> ,		
		<i>Syntrophobacter</i>		
		<i>wolinii</i> ,		
		<i>Methanomassiliicocc</i>		
		<i>us</i> , <i>Methanoculleus</i>		
		<i>acetate-utilizing</i>		
		<i>Methanothrix</i> ,	Stable	
		<i>Methanoculleus</i> ,		
		<i>Methanobacterium</i>		
Chlortetracyc		<i>Spirochaetaceae</i> ,		
line		<i>Acinetobacter</i> ,		
		<i>Pseudomonas</i> ,	Increased	
		<i>Comamonadaceae</i> ,		
	0.5 g/kg (pig	<i>Methanomassiliicocc</i>		(Stone et
	slurry)	<i>us</i>		al., 2009;
		<i>Syntrophomonas</i>		Wang et
		<i>spp.</i> , <i>Syntrophospora</i>	Decreased	al.,
		<i>spp.</i> ,		2017a)
		<i>Syntrophobacter spp</i>		
		<i>Methanosaetaceae</i> ,	Decreased	
28		<i>Methanosarcinaceae</i>		

Oxytetracycline		<i>Sphingobacteriaceae</i>	Disappeared	
	20, 50, and 80	<i>Gammaproteobacteria</i>	Decreased	(Stone et al., 2009;
	60, 100,	<i>Flavobacterium</i>	Increased	Xin et
	140mg/kg (pig manure)	<i>Methanomicrobiales</i>	Decreased	al., 2014)
Tylosin		<i>Syntrophomonas</i>		(Shimada et al.,
	1.67	<i>spp., Syntrophospora</i>	Decreased	2008)
		<i>spp.</i>		
Erythromycin		<i>Firmicutes</i>	Stable	
-tetracycline		<i>Bacteroidetes,</i>		
(ET)		<i>Acinetobacter,</i>	Decreased	(Aydin et
combinations	0.1–4	<i>Proteobacteria</i>		al., 2016)
		<i>Actinobacteria,</i>		
		<i>Fusobacterium</i>	Increased	
Sulfamethoxazole -	20 + 1.5	<i>Methanosarcinales</i>	Decreased	
Tetracycline				(Aydin et
Sulfamethoxazole -				al.,
				2015c)
Erythromycin	2.5 + 2.5 + 25	<i>Methanosarcinales</i>	Decreased	
- Tetracycline				