Syntrophy mechanism, microbial population, and process optimization for volatile fatty acids metabolism in anaerobic digestion

Zhang, Yao; Li, Chunxing; Yuan, Zengwei; Wang, Ruming; Angelidaki, Irini; Zhu, Gefu

Published in:
Chemical Engineering Journal

Link to article, DOI:
10.1016/j.cej.2022.139137

Publication date:
2023

Document Version
Peer reviewed version

The syntrophy mechanisms, microbial population, and process optimization for volatile fatty acids metabolism in anaerobic digestion

Yao Zhang[a,c#], Chunxing Li[b,e#], Zengwei, Yuan[b], Ruming Wang[d], Irini Angelidaki[e], Gefu Zhu[a,c*]

[a]*School of Environment and Nature Resources, Renmin University of China, Beijing 100872, China*

[b]*State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Nanjing 210023, China*

[c]*Key Laboratory of Energy Resource Utilization from Agriculture Residue, Ministry of Agriculture and Rural Affairs, China*

[d]*School of Environmental Science and Engineering, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai, 200240, China*

[e]*Department of Chemical and Biochemical Engineering, Technical University of Denmark, Kgs. Lyngby DK-2800, Denmark*

# These authors contribute to this work equally.

[*] Corresponding author, E-mail: zgf@ruc.edu.cn; Phone: 86-18610566239.

Abstract: The low efficiency and stability of anaerobic digestion (AD) has always hindered its wide application. The accumulation of volatile fatty acids (VFAs) cause acidification and inhibition of anaerobic digester. The conversion of some VFAs (propionate and butyrate) to acetate, CO_2, and H_2 is endergonic under standard conditions, which make it difficult to occur spontaneously in AD system. Thus, the degradation of VFAs is regarded as the rate-limiting step in AD. Studies proved that VFAs could be degraded through the syntrophic cooperation between bacteria and methanogens. Therefore, clear insight into the mechanism of syntrophic methanogenesis as well as the process optimization in syntrophic metabolism of VFAs are essential to improve the efficiency of AD. The present review summarized the roles of syntrophic bacteria and methanogens in AD process, containing metabolism pathways, biochemical reactions, microorganisms characteristics, substrate oxidation, and electron transfer in syntrophic methanogenesis. The main forms and mechanisms of interspecies electron transfer were detailly and deeply described. In addition, the process control and optimization of the syntrophic metabolism of VFAs were also summarized in the review for improving the efficiency of syntrophic methanogenesis. This review provides a deeper understanding of efficiency enhancement of syntrophic metabolism in AD system.

Keywords: Anaerobic digestion, Volatile fatty acids, Syntrophic metabolism, Methanogenesis, Interspecies electron transfer, Process optimization

1. Introduction

Anaerobic digestion (AD), as an important approach for disposal and energy recovery from organic waste/wastewater, has been widely used for its efficiency and economy. However, poor operational stability still limits the wide application of AD [1]. How to improve the stability and efficiency of AD system has always been a hot topic. Numerous studies focused on the optimization of AD under different process conditions. The main process parameters affecting AD are organic loading rate (OLR), pH, temperature, solid retention time (SRT), toxic compounds, and hydraulic retention time (HRT) [2]. The low OLR would decrease the methane yield since the starvation of microorganisms. However, too high OLR could result in organic overload [3]. Changes of pH would largely affect the activity of microorganisms, too acidic (pH＜3) or too alkaline (pH ＞ 12) are inhibitory to microorganisms involved in AD [4]. The temperature influences the activity of microorganisms (both bacteria and methanogen) [5]. SRT is a critical factor which maintains the balance and activity of the functional microorganisms in AD system [6]. In addition, the presence of toxic compounds in AD could lead to system failure in extreme cases. Thus, a great number of researches were focused on the techniques and modification of AD system [7, 8].

The AD process mainly depends on the mutual and syntrophic interaction of microorganisms. In order to optimize AD process, it is essential to understand the related biochemical reactions. AD is a microbial process mediated by cooperation of different microorganisms, which mainly includes four steps of hydrolysis, acidogenesis,

61 acetogenesis, and, methanogenesis [9]. In hydrolysis step, with the help of extracellular
62 hydrolytic enzymes, polysaccharides, proteins, lipids, and some other complex
63 polymers are hydrolyzed into sugars, amino acids, purines, pyrimidines, fatty acids,
64 glycerol, and some other monomers. For this process, researches have proposed that
65 the optimum temperature and pH are between 30~50 ℃ and between 5~7 [10]. The
66 produced monomers from hydrolysis are further degraded to volatile fatty acids (VFAs),
67 succinate, lactate, and ethanol by primary fermenting bacteria. In this process, the
68 generation of VFAs occurs when pH is higher than 5, pH lower than 5 benefits ethanol
69 production, and the reaction will stop when the pH lower than 4 [11]. The produced
70 acetate, H_2/CO_2, formate, and methanol can be directly used by methanogens for
71 methane generation. However, some other fermentation products represented by VFAs
72 cannot be directly utilized by methanogens, which need further fermentation by
73 secondary fermenting bacteria (syntrophic bacteria). Syntrophic bacteria are able to
74 converting the above fermentation products into acetate, formate or some other one-
75 carbon compounds for utilization by methanogens [12]. So, the metabolism and
76 conversion of VFAs are important in AD system, and their excess accumulation could
77 significantly destroy the process and inhibit methane generation [13, 14]. It was
78 commonly believed that methanogenesis is the rate limiting step in AD, however, the
79 syntrophic degradation of VFAs was regarded as another important rate limiting step in
80 AD. Therefore, in order to improve the efficiency of AD, it is essential to regard bacteria
81 and methanogens as a whole system to investigate the syntrophic methanogenesis of

VFAs.

Syntrophy is defined as a nutritional situation in which two or more organisms combine their metabolic capabilities to catabolize a substrate that cannot be catabolized by any of them alone [15]. In AD system, syntrophic interaction is known as the mutual thermodynamic dependence between bacteria and methanogens. Specifically, bacteria degrade available substrate into acetate, H_2, and/or formate, which are consumed by syntrophic partner of methanogens [16]. A typical example is the co-culture of two partners of strain S and strain M.o.H [17].

Strain S: $2CH_3CH_2OH + 2H_2O \rightarrow 2CH_3COO^- + 2H^+ + 4H_2$

$$(\Delta G^{\circ\prime} = +9.6\ kJ\ per\ mol\ of\ ethanol)$$

Strain M.o.H: $4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$

$$(\Delta G^{\circ\prime} = -131\ kJ\ per\ mol\ of\ methane)$$

Co-culture: $2CH_3CH_2OH + CO_2 \rightarrow 2CH_3COO^- + 2H^+ + CH_4$

$$(\Delta G^{\circ\prime} = -112\ kJ\ per\ mol\ of\ methane)$$

In the co-culture system, without hydrogenotrophic methanogens, bacteria cannot grow with ethanol as the substrate since the Gibbs free energy of this reaction is positive under standard conditions. According to the relationship between ΔG° and $\Delta G^{\circ\prime}$ under standard conditions:

$$\Delta G^{\circ} = \Delta G^{\circ\prime} + RTln\frac{[CH_3COO^-]^2[H_2]^4}{[CH_3CH_2OH]^2}$$

When the produced H_2 from the first reaction is consumed by methanogens, the Gibbs free energy of this reaction turns to negative and the reaction of oxidizing ethanol

happens [12]. Both acetate and hydrogen are the direct substrates of methanogens, their removal not only provide the condition for the growth of strain S., but also provide the carbon source and energy for methanogens. For bacteria, during the VFAs oxidation process, the Gibbs free energy of reactions are usually positive under standard conditions [18]. Thus, the reaction is favorable in thermodynamics only when methanogens keep the partial pressure of H_2 lower than 10^{-4} atm or more less [19]. Previous studies have shown that adding *Methanosarcina* in AD system could alleviate the inhibition to methane generation caused by the accumulation of acetate through coupling growth with syntrophic bacteria, and further improve the stability of the reactor [20]. Moertelmaier and coworker studied the metabolism and microbial population changes of VFAs in a modified anaerobic digester [21]. The results showed that acetate and propionate gradually accumulated in the initial stage of start-up, and the population of *Methanosaeta* remained unchanged, *Methanosarcina* decreased and *Methanomicrobiales* increased. During the degradation and accumulation of acetate and propionate, the number of propionate oxidizing bacteria increased gradually, accounting for 5.1% of the total number of bacteria. *Pelotomaculum* became the dominant bacterium, followed by *Smithella* and *Syntrophobacter*. The increase of the number of propionate oxidizing bacteria promoted the degradation of fatty acids in the system, which benefits the stability of the system. Thus, the syntrophic interaction between microorganisms plays a critical role in the oxidation of VFAs. It has been confirmed that interspecies electron transfer is mainly involved in the syntrophic

methanogenesis process [15]. In syntrophic methanogenic community, redox mediator which produced by the oxidation of VFAs via bacteria are transferred between bacteria and methanogens in a process called interspecies electron transfer [22]. The electrons are generally transferred by hydrogen or formic acid in syntrophic methanogenic community [23], which has been regarded as the forms of interspecies electron transfer for a long time. Interspecies H_2 transfer between bacteria and methanogens can decrease the partial pressure of H_2 to 10^{-4} atm and less, which makes the acetogenic reaction possible in thermodynamics [24, 25]. Except that, some electron shuttles like humic substances, quinones phenazines or insoluble biochemical compounds can also mediate the interspecies electron transfer [26, 27]. However, compared with H_2 and formate, these electron carriers have lower interspecies electron transfer efficiency due to their poor diffusion, which might be the limiting factor for methanogenesis [23]. Thus, the research on the optimization of syntrophic metabolism has become a hot topic in anaerobic methanogenesis. In recent years, direct interspecies electron transfer (DIET) via cytochrome, conductive pili, and other cellular structures have been reported as well [28], which shows higher efficiency than interspecies hydrogen/formate transfer [29]. Studies have shown that the efficiency and stability of AD systems are related to interspecies electron transfer. Many studies have shown that adding conductive materials (e.g., magnetite, biochar, and activated carbon) can accelerate methanogenic reactions and improve the efficiency of AD [30-33].

In order to comprehensively understand the syntrophic metabolism of VFAs and

improve the efficiency of AD system, the present review focuses on the interaction between syntrophic bacteria and methanogens during the process of syntrophic methanogenesis. In addition, the main forms and mechanisms of interspecies electron transfer in AD system is detailly analyzed. Lastly, the process control and optimization of VFAs syntrophic methanogenesis in AD system are summarized as well. These could give a significance on optimizing the metabolic ability of syntrophic bacteria and maintaining the stability of methanogenesis system.

2. Interspecies electron transfer in syntrophic community

During the macromolecular organic compounds conversion to methane process, electrons produced from the degradation of organic substrates and intermediate production by fermentation bacteria are utilized by methanogens to reduce CO_2 to CH_4 [34, 35]. Therefore, interspecies electron transfer plays an important role in all the involved reactions [22].

2.1 Interspecies hydrogen/formic acid transfer

2.1.1 Interspecies hydrogen transfer

Interspecies H_2 transfer was firstly proposed in 1967, where two microorganisms species were found in cultures of *Methanobacillus omelianskii* [17]. After that, much research showed that syntrophic bacteria and methanogens can live in the syntrophic relationship to achieve interspecies electron transfer with H_2 as the electron carrier [17, 36]. The microorganisms with dehydrogenase/hydrogenases can continuously produce and consume hydrogen via the redox reaction between proton and electron ($2H^+ +$

166 $2e^- \rightleftharpoons H_2$). The product of this reaction depends on the redox potential of the substrates
167 related with hydrogenases. When there exists an electron donor with low redox
168 potential in the environment, the reaction would come to the direction of the hydrogen
169 generation [37]. As the main redox mediator in microbial metabolism, the redox
170 potentials of NAD^+/NADH and FADH/$FADH_2$ are -320 mV and -220 mV, respectively,
171 the redox potential of $Fd_{(ox)}/Fd_{(red)}$ was -398 mV or lower (-500 mV) [18, 38]. Among
172 them, only the process of $Fd_{(red)}$ reducing protons to produce hydrogen is exergonic, the
173 proton reduction reactions involving NADH and $FADH_2$ are endergonic under standard
174 conditions, which cannot happen spontaneously. In AD system, the standard redox
175 potential of most products produced by syntrophic bacteria through the oxidation of
176 small molecule organic matters (i.e., the oxidation from propionate to succinate) is
177 higher than that of H_2/H^+ ($E^{\circ\prime} = -414\ mV$). Methanogens that use hydrogen as
178 electron donor can utilize hydrogen to increase its standard redox potential. When the
179 concentration of hydrogen in the environment decreases from 101 kPa to 1 Pa, the
180 standard redox potential of hydrogen increases from -414 mV to -260 mV. While, the
181 standard redox potential of NADH is -320 mV, which is lower than that of hydrogen,
182 so electrons can transfer from NADH to H^+ or H^+/CO_2 [39]. Thus, hydrogenotrophic
183 methanogens consume hydrogen to maintain a lower hydrogen partial pressure in the
184 system, which leads to the increase of the redox potential of H_2/H^+. When the hydrogen
185 partial pressure in the system decreases to 1 Pa, the reaction of NADH reducing protons
186 to produce hydrogen can also be exergonic in thermodynamics [15].

There are usually two or more microorganisms involved in the process of syntrophic methanogenesis. Since hydrogen is the direct electron donor, interspecies H_2 transfer from syntrophic bacteria to methanogens is common in syntrophic methanogenesis community. And hydrogen seems to be an ideal electron carrier for its small size and easy to diffuse. Besides, many anaerobic microorganisms have hydrogenases, which makes it possible to catalyze the reversible conversion of hydrogen into protons and electrons [12]. In syntrophic methanogenesis community, the production of H_2 is often coupled with the transfer of redox intermediates like NAD^+/NADH, FAD/$FADH_2$, and $Fd_{(ox)}$/$Fd_{(red)}$ [15], but the marginal value of the minimum H_2 partial pressure varies from different bacteria to maintain their methane production. For methanogens containing cytochromatic C, the marginal value of H_2 partial pressure is more than 10 Pa, while that without cytochromatic C is less than 10 Pa [38]. Therefore, in the syntrophic methanogenesis community, syntrophic bacteria and methanogen can perceive the redox condition of the environment. The metabolism of syntrophic bacteria would be limited at high hydrogen partial pressure, meanwhile, the metabolism of methanogen is promoted [15]. During AD process, electrons produced from the degradation of organic substrates and intermediate production by fermentation bacteria are utilized by methanogens to reduce CO_2 to CH_4 [34, 35]. Interspecies H_2 transfer between bacteria and methanogens is used to control the concentration of H_2 to degrade organic substrate and methanogenesis.

Previous study proposed the model of anaerobic granules in UASB reactor, which

proved that the existed acetoclastic methanogen of *Methanosaeta* in the center of granules is surrounded by acetogens and hydrogenotrophic methanogens [40]. Thus, these granules can exist in syntrophic methanogenic community [41]. The outer layer of the granule was surrounded by fermentative microorganisms, which convert complex organic compounds into simple fermentation products as substrates for syntrophic methanogens [40]. The transmission of intermediate metabolites between microorganisms is mainly through diffusion. When there is physical aggregation between microbial cells, interspecific hydrogen transfer efficiency will be greatly improved [42]. The formation of granules would make it easier for interspecies H_2 transfer since it shortened the distance between cells in granules [43]. A wide range of hydrogenotrophic methanogens are found and cultured from anaerobic bioreactor [44]. In anaerobic bioreactors, the accumulation of VFAs influence the efficiency of AD. Therefore, efficient interspecies electron transfer is critical for the operation of reactor. Formation of granular sludge facilitates interspecies hydrogen transfer by reducing the distance between bacteria and methanogens.

2.1.2 Interspecies formate transfer

There is another method for interspecies electron transfer, which uses formate as the electron carrier. Formate can be produced in primary fermentation during the process of AD. Microorganisms that contain formate dehydrogenase can convert formate to H_2 and CO_2 [45, 46]. Apart from that, anaerobic bacteria can produce formate in the reduction of CO_2, and the low-potential electrons were provided by ferredoxins,

which could along with interspecies hydrogen transfer [47]. Accordingly, there are two pathways of interspecies formate transfer in methanogenesis: 1) Formate can make conversion to H_2 and CO_2 (HCO_3^-) by syntrophic bacteria with formate dehydrogenase and then convert to CH_4, 2) Formate can also be oxidized into CH_4 by hydrogenotrophic methanogen via formate hydrogenlyase [48, 49]. The oxidation reduction potential of redox couple formate /CO_2 is -432 mV which is similar to that of H^+/H_2 (-414 mV) [15]. In Thiele and Zeikus' study, interspecies formate transfer comes up to 90% in syntrophic community of *Desulfovibrio vulgaris* and *Methanobacterium formicicum* [50]. Some studies also showed that both interspecies formate and hydrogen transfer can be realized simultaneously in some syntrophic methanogenesis community. For instance, the syntrophic community of *Syntrophospora bryantii* and *Methanospirillum hungatei* own two pathways to produce methane due to that *Syntrophospora bryantii* can both produce hydrogen and formate in the degradation of butyrate [49, 51]. In addition, the co-culture of *Syntrophomonas wolfei* and *Methanospirillum hungatei* can also make butyrate degradation possible [52]. Besides, the study of *Syntrophobacher fumaorxidans* showed that *Syntrophpbacher fumaoxidans* can cooperate with the methanogen which utilize both hydrogen and formate as the syntrophic partner, but cannot with the methanogen which only utilize hydrogen [53].

2.1.3 Comparison between interspecies hydrogen transfer and interspecies formate transfer

Both interspecies hydrogen transfer and interspecies formate transfer are

spontaneous in syntrophic methanogenesis community in presence of methanogens. The standard redox potential of H^+/H_2 is -414 mV, which is similar to that of CO_2/formate (-420 mV), and the electron transfer components are similar as well. Therefore, there is no essential difference between these two electron transfer carriers in energetics. It was also indicated that interspecies formate transfer could replace interspecies hydrogen transfer [12]. Further research proposed that the choice of interspecies electron carrier is up to the difference between the activity of hydrogenase and formate dehydrogenase. In the butyrate-oxidizing syntrophic community with *Syntrophomonas wolfei* and *Syntrophobotulus glycolicus*, only hydrogen acts as electron carrier. In these syntrophic communities, hydrogenase shows activity, which is much higher than that of formate dehydrogenase. While in the propionate degradation syntrophic community of strain MPOB required both hydrogen and formate acting as interspecies electron carrier, since hydrogenase and formate dehydrogenase showed the same activity [54].

Besides, both interspecies hydrogen transfer and interspecies formate transfer follow Fick's law: $F = \frac{A \times D \times C}{d}$ (in which F = diffusion rate; A = surface area; D = molecular diffusion constant; C = concentration difference; d = distance). Accordingly, the efficiency of interspecies electron transfer is affected by the distance between microorganisms [15]. The aggregation of microorganisms is a common phenomenon in syntrophic community, which make it easier to attach themselves on carriers or to each other [55]. Besides, the aggregation between syntrophic bacteria and methanogens may

speed up the rate of interspecies electron transfer. Previous study showed that shorten the distance of interspecies via aggregation could make it easier for interspecies hydrogen transfer [43]. The diffusion rate of H_2 is 30 times that of formate, but the solubility of formate is much higher than that of H_2. Therefore, formate tends to form a higher concentration difference, which is about 1000 times higher than that of H_2 [56]. It is speculated that under the same diffusion rate, H_2 may be mainly involved in short distance interspecific transfer, while formate may be involved in long distance interspecific transfer [57].

2.2 Direct interspecies electron transfer

In recent years, some studies found that some anaerobic microorganisms could transfer electron directly via extracellular cytochrome and conductivity pili [35]. Reguera proposed that the pill of *G. sulfurreducens* could conduct electricity, and firstly named the polymeric protein growing around the cell as “conductive nanowires” [58]. Besides, they speculated that the exist of the conductive nanowires make bacteria free from the traditional pathway of electron transfer via direct contact with electron transfer, which make it possible for electron transfer in a long distance, and largely improve the efficiency of electron transfer. Actually, DIET was observed by Summers et al. in 2010 [59]. They co-cultured two *Geobacter* species of *Geobacter metallireducens* and *Geobacter sulfurreducens*. The former use ethanol as electron donor and the latter use ethanol as electron donor and fumarate as electron acceptor under anaerobic condition. To be more specific, *G. metallireducens* could oxidize ethanol without using fumarate

292 as the electron acceptor. On the contrary, *G. sulfurreducens* was able to reduce fumarate
293 to succinate but could not oxidize ethanol. Accordingly, interspecies electron transfer
294 is required between the two microorganisms in this system. Summers et al. showed that
295 without hydrogen or formate as electron carriers, the microorganism species aggregated
296 together for direct electrons transfer through a conductive medium. Furthermore, they
297 deleted the OmcS (a cytochrome C) gene to study the DIET between *Geobacter*
298 *metallireducens* and *Geobacter sulfurreducens*, resulting in the failure of interspecies
299 electron transfer in cell agglutination, which demonstrated the critical role of
300 cytochrome C in DIET. After that, Rotaru et al. [60] found that there existed direct
301 electron transfer between acetogenic *Geobacter* species and methanogenic
302 *Methanosarcina barkeri* through conductive pili or cytochrome. However, when the
303 gene encoding pili protein in *G. sulfurreducens* was deleted, the bacteria lost the ability
304 to reduce the insoluble electron receptor, indicating the important role of conductive
305 pili in electron transport [61]. Furthermore, Estevez-Canales et al. cultured *G.*
306 *sulfurreducens* with low iron medium to make sure the role of c-type cytochrome in
307 extracellular electron transfer. The concentration of cytochrome in the low iron
308 condition was 15 times lower than that in the normal condition. Although the cells
309 lacking cytochrome could reduce fumarate to succinate, they could not reduce ferric
310 citrate and transfer electrons to graphite electrode, suggesting that c-type cytochrome
311 played an important role in the extracellular electron transfer of G. *sulfurreducens* [61].
312 Up to now, the three main pathways of DIET are maintained as follow: (1) via

electron conducting pili; (2) via electron-transporting proteins associated with the outer membrane and comprising c-type cytochromes; (3) via non-biological conductive materials (CMs) [62-64].

DIET is regarded as an alternative way to interspecies hydrogen transfer or interspecies formate transfer in recent years since its high efficiency. Unlike the interspecies electron transfer using H_2 or formate as electron carriers, DIET does not need any electron carriers [35], which depends on outer membrane cytochromes, nano-line, electrically conductive pili, or the formation of multispecies aggregates [65]. Thus, the DIET shows higher energy efficiency than interspecies hydrogen or formic acid transfer [29, 66]. Although DIET has advantages over interspecific hydrogen/formate transfer in electron transfer efficiency and thermodynamics, the mechanism of its formation in the methanogenic system with more than two carbon VFAs as substrate is unclear [49]. *Geobacter* has the ability to degrade small molecular alcohols such as propanol and butanol, but cannot directly utilize VFAs such as propionate and butyrate. However, most of the interacting bacteria that have the ability to use propionate and butyrate do not have the cytochrome, conductive pili, and other structures of *Geobacter*. In addition to these theoretical contradictions, DIET is also susceptible to severe hydraulic conditions due to its dependence on physical contact. Therefore, finding alternative method to promote interspecific electron transfer efficiency is still the current research direction of optimizing AD process.

Since the DIET among methanogenesis was proposed, conductive materials have

been extensively reported to enhance methane production in AD system. Up to now, much work has shown that adding conductive materials like granular active carbon [67, 68], biochar [69], carbon fiber [70], magnetite [71], hematite [72], activated carbon, and nano-zero-valent iron [31] could promote the methanogenesis through improving the effectiveness of syntrophic interaction [73], interspecies electron transfer [74, 75]. The addition of conductive materials in AD system could promote the utilization of electrons for methanogenesis [64]. Li et al. [76] proved that the addition of stainless steel in AD system could enhance the ability of electron transfer to promote the methane production. Some studies also found that the maximum electron carrier flux via electrical conduction magnetite was around 10^6 times higher than that associated with the interspecies hydrogen transfer [29]. Besides, the addition of conductive materials also improves the rate of substrate metabolism and methanogenesis [68, 77]. According to previews studies, interspecies electron transfer can also take place between syntrophic bacteria and methanogens or with the aid of conductive materials, which can largely improve the efficiency of methanogenesis [62, 78, 79]. The addition of conductive materials would make the syntrophic interaction more efficient by capturing more electrons. Previous study suggested that adding some specific conductive materials could change the methanogenic pathway from interspecies hydrogen transfer to DIET for greater efficiency [80]. Besides, Yin's study believed the continuously addition of Fe_3O_4 in syntrophic methanogenic community could induce acetoclastic methanogens to hydrogenotrophic methanogens and promote the syntrophic interaction

between bacteria and methanogens [81]. In addition, adding conductive materials in AD system is favorable for the changes in environment such us temperature, pH, H_2, and pressure [82-85].

2.3 Interspecies electron transfer mediated by electron shuttle

Except the above two extracellular electron transfer mechanisms, electron shuttles can also conduct extracellular electron transfer like DIET under anaerobic conditions. Take the syntrophic community of G. *metallireducens* and G. *sulfurreducens* for example, when anthraquinone-2, 6-disulfonate sodium (AQDS) was present, the interspecies hydrogen/formate transfer process was replaced, and the rate of methane production increased significantly [86]. Electron shuttle was also known as redox mediator, which acted as electron carriers to participate in redox reactions reversibly [87]. According to its source, electron shuttles can be divided into endogenous electron shuttles and exogenous electron shuttles. The endogenous electron shuttles are produced by microorganisms themselves i.e., flavin and black pigment, while, the exogenous electron shuttles are natural substances or artificially synthesized materials such as sulfur compounds and humus. Electron shuttle-mediated extracellular electron transfer generally involves the following reactions: the oxidized form of electron shuttles accept electrons from the oxidization of organic matter and become the reduced form of electron shuttles, then the reduced form of electron shuttles transfer electrons to extracellular electron acceptors and return to the oxidized form of electron shuttles. Through the two steps, electron shuttles can be reversibly oxidized and reduced. A

typical example is the flavin dinucleotide (FAD) synthesized by extracellular respiratory bacteria, which can be hydrolyzed into electron shuttle flavin mononucleotide (FMN) after transmembrane into periplasmic space. However, FMN can obtain electrons directly from cytochrome MtrC to become reduced FMN_{red}, which changes back to oxidized FMN_{ox} after contacting extracellular electron receptors (such as iron and manganese oxides, humus, etc.) to realizes electron transfer [88, 89]. Under anaerobic condition, interspecies electron transfer can be also mediated by humus or humic acids [90, 91]. Subsequent electron spin resonance spectroscopy (ESR) and nuclear magnetic resonance (NMR) studies confirmed that quinone groups in humus were the main electron acceptance sites, which conducted electron transfer [92, 93]. Researches confirmed that using humic acid or anthraquinoe-2,6-disulfonate (AQDS) and its redox form AHQDS as electron acceptor could oxidize substrates by diverse anaerobic microorganisms. For example, several microorganisms were found to reduce humic acids or anthraquinone-2,6-disulfonate (AQDS) using hydrogen as electron donor (e.g., the halorespiring bacterium, *Desulfitobacterium* PCE1, the sulfate-reducing bacterium, *Desulfovibrio* G11 and the methanogenic archaea, *Methanospirillum hungatei* JF1) or lactate (*Desulfitobacterium dehalogenans* and *Desulfitobacterium* PCE1) [23, 26]. Reduced humic acid can also act as electron donor to reduce some oxidized substrates. Based on this principle, riboflavin and quinones have been widely used in decolorization and denitrification of azo dyes, reduction of polybiphenyl and heavy metals such as Fe (III), Mn (IV), Cr (VI), U (VI) to promote

the process of low biological conversion [26, 94, 95].

Unlike the concentration of hydrogen/formate, which depend on the microbial metabolic activity, the concentration of electron shuttles can be easily manipulated artificially. Different from the solid conductive material required for the formation of DIET mechanism, the water-soluble nature of the electron shuttle also facilitates the addition for engineering applications. Given the above advantages, since quinone respiratory bacteria were ubiquitous in anaerobic sludge, interspecies electron transfer mediated by electron shuttle is expected to become the next hot research direction in strengthening microbial interaction.

3. Overview of syntrophic community in anaerobic digestion systems

3.1 Microorganisms involved in syntrophic community

The bacteria in syntrophic systems are not limited to a distinct phylogenetic group of microorganisms, but occur throughout the microbial world. Bacteria grow in pure culture used sugars, amino acids, and organic acids as the substrates. These substrates can be oxidized into carbonyl compounds such as pyruvate or acetaldehyde as the electron acceptor for the oxidation of NADH. The oxidation of NADH couple with the reduction of carbonyl compounds is energetically favorable. When the H_2 concentration decreases to 10 Pa with the help of methanogens, the redox potential of hydrogen changes from -410 mV to -260 mV (formate similarly), which makes the reaction energetically favorable. Thus, when methanogens are active in the culture, syntrophic microorganisms will no longer oxidize substrate into ethanol, lactate, propionate or

418 butyrate [34]. Some bacteria like *Syntrophomonas wolfei*, *Syntrophus aciditrophicus,*
419 and *Pelotomaculum thermopropionicum* can neither utilize alternate electron acceptors
420 nor oxidize substrate to ethanol, lactate, propionate [96-98]. They must rely on the
421 reduction of protons or CO_2, forming hydrogen or formate, respectively, to make this
422 process possible. Some bacteria are able to produce methane by themselves, the ability
423 of the substrate oxidation in pure culture will be better than that in coculture [99].
424 Bacterial of *Firmicutes*, *Deltaproteobacteria*, *Synergistetes* can participate in
425 syntrophic methanogenesis processes [34]. There are three types of bacteria in
426 *Firmicutes* have been found to accomplish syntrophic metabolism, which belong to
427 *Syntrophomonadaceae*, *Desulfotomaculum*, and *Thermoanaerobacteraceae*,
428 respectively [100-102]. In AD system, bacteria oxidize VFAs, succinate, alcohol,
429 lactate, and some other compounds to acetate, H_2, and formate, which are finally
430 converted into methane by methanogens [103]. However, the oxidization of some VFAs
431 like propionate, butyrate is endergonic under standard conditions. It can happen
432 spontaneously only with the partial pressure of H_2 lower than 10^{-4} atm. Previous
433 research firstly shown that *Syntropgomonas wolfei* could oxidize VFAs in a coculture
434 with a hydrogenotrophic and/or formate-consuming microorganisms [98, 104, 105].
435 Syntrophic microbial degradation of propionate by *Syntrophobacher fumaroxidans* and
436 butyrate by *Syntrophomonas* (*Syntrophospora*) *bryantii* can occur only if the
437 methanogenic partners utilize both hydrogen and formate [57].

438 Apart from bacteria, methanogenic archaea also play a critical role in syntrophic

community. Methanogenic archaea are strictly anaerobic microorganisms belonging to the *Euryarchaeota*, which cannot use oxygen as electron acceptor [106-108]. Methanogen can only utilize some specific substrates like acetate, H_2, and methyl-group containing compounds for methane generation. Accordingly, they can be divided into three groups including hydrogen trophic methanogens, acetate trophic methanogens, and methyl trophic methanogens. Although the limited substrates, they still process great phylogenetic and ecological diversity. Methanogens are generally divided into five orders based on their phylogeny and phenotypic properties: *Methanobacteriales*, *Methanopyrales*, *Methanomicrobiales*, *Methanosarcinales*, and *Methanopyrales* [36]. *Methanobacterials*, *Methanomicrobials*, and *Methanosarcinales* often appear in AD system [15]. Most methanogens are hydrogenotrophic type of methanogenic, which can reduce CO_2 to methane with H_2 or formate as electron donor. In addition, there are still some acetoclastic methanogens, which utilize acetate as substrate. Two genera of the order *Methanosarcinales*: *Methanothrix* (fam. *Methanosaeta*) and *Methanosarcina* (fam. *Methanosarcinaceae*) belong to the group of acetoclastic methanogens. Members of the genus *Methanothrix* are strictly acetoclastic. While, members of the genus *Methanosarcina* can produce methane not only from acetate and H_2/CO_2, but also directly from methanol and other methyl compounds. Methanogens that utilize methylated compounds belong to methylotrophic methanogens. This group includes members of the orders *Methanobacteriales*, *Methanosarcinales*, and *Methanomassiliicocales*, family *Methanosarcinaceae*. For some methyl-reducing

methanogens cannot dismutate methyl compounds, they can reduce methyl group to methane using H_2 and/or formate as electron donors [14]. More information of methanogens are summarized in Table 2. Methanogens are abundant in environment, which is lack of O_2 and other electron acceptors like NO_3^-, Fe^{3+}, and SO_4^{2-}. In methanogenic culture, complex organic matters are degraded into CH_4 with the help of different groups of anaerobic microorganisms. The presence of methanogen in AD maintains the H_2 partial pressure to make it possible for VFAs degradation by syntrophic bacteria.

3.2 The interaction among syntrophic community

In AD, syntrophic bacteria and methanogens live in syntrophic coculture. Both of them utilize the metabolic abilities from their syntrophic partner in order to solve energy problem, degrade complex compounds [15]. The phenomenon of syntrophic metabolism in methanogenic environment was firstly proposed by [17]. Their research found that in *Methanobacillus omelianskii* culture, the cooperation of two bacterial species can convert ethanol to acetate and methane. Before that, *Methanobacillus omelianskii* was used to be known as a strictly anaerobic bacterium, which can oxidize ethanol to acetate, and the energy release from this process is used for the growth of bacterium [17]. The research of Bryant demonstrated that *Methanobacillus omelianskii* was a syntrophic association of two microorganisms. One of these was *Methanobacterium bryantii* strain M.o.H, which synthesized methane from hydrogen and CO_2, the other one was species S, which degraded ethanol to acetate and hydrogen.

When these two microorganisms grow respectively, neither of them can produce methane. However, when they grew together with ethanol as the substrate, the hydrogen produced from species S was utilized by strain M.o.H to produce methane.

Previous study discussed two kinds of syntrophic interactions: facultatively syntrophic interaction and obligately syntrophic interaction. As for facultatively syntrophic interaction, some substrates can be degraded by syntrophic bacteria, methanogens do not participate in this process, but they take part in the growth and metabolic of bacteria. For obligately syntrophic interaction, neither bacteria nor methanogen can degrade specific organic substrates individually. Their cooperation plays a critical effect for their growth and substrates degradation [22]. The distance between cells may affect the degradation of organic compounds and the growth rate of microorganisms, which lead to the formation of compact aggregates [50, 109, 110].

4. Process optimization for VFAs syntrophic metabolism in anaerobic digestion system

4.1 Optimization of process parameters in anaerobic digestion

As mentioned above, the main process parameters affecting AD process are OLR, HRT, pH, temperature, and SRT. OLR represents the volume of the organic matter introduced into the digester per unit volume of the anaerobic digester [111]. Nkuna et al. [112] proposed that if the OLR of AD system can reach to the substrate conversion efficiency of 75%, the AD system would keep stability. HRT represents the time of the organic material remains resident in the reactor. Studies have revealed that OLR and

HRT related shocks result in differing responses from AD microbial communities, which in turn contributes to varying levels of system tolerance to the differing stresses [113]. Thus, keep the balance between OLR and HRT could maintain the stability of AD system. To achieve economic viability, AD system should be optimized to produce maximal methane yield at the highest OLR and lowest HRT [114]. Low pH in AD system is led by the accumulation of VFAs during acidogenesis, which is harmful to methanogens. Adding some alkaline substance could avoid the accumulate of VFAs in system [2]. Besides, co-digestion can also realize the control of pH. Lisboa and Lansing demonstrated that the co-digestion of food waste with manure could increase the buffer capacity of the system and minimize the effects of pH changes on process stability [115]. The temperature of AD system can affect the rate of reactions. AD process could be carried out in psychrophilic (＜20 ℃), mesophilic (30~38 ℃), and thermophilic (50~65 ℃) three temperature ranges [116]. At thermophilic temperatures, the system always needs more energy input, higher degree of imbalance and sensitivity to toxic inhibitors. So, most AD systems are operated within the mesophilic temperature range even though the thermophilic range could improve the rate of process [2]. Ahring et al. demonstrated that when the temperature of a continuous stirred tank reactor (CSTR) with cattle manure feedstock increased from 55 to 65 ℃, the production of methane decreased from 65% and 71% to 45% [117]. SRT represents the average resident time of microbial cells within the AD system and is a significant factor maintaining the balance and activity of the functional microbial groups in AD system [6]. Chen et al.

observed that extended SRT contributed to elevated release of recalcitrant compounds and reduced methane yield during the thermophilic AD of wasted activated sludge [118]. While the traditional opinion is that maintaining high SRT could avoid the influence of OLR change and toxic substrate [2]. This is because the ability of some microorganisms could increase at thermophilic temperature (65 ℃), and some microorganisms require increased SRT to withstand the effects of low SRT [118]. The study reflected that the optimization of AD system should consider the synergic nature of AD.

In recent years, mathematical modeling has made a lot of progress in the environmental field [119, 120]. A great number of researches focus on using mathematical model to optimize operational parameters in AD system. In order to demonstrate the kinetics of AD process, several mathematical models have been developed. Alwan [7] used a simulation model to optimize the continuous biochemical reactor, and the study showed that the maximum biomass concentration (80.57 g/L) could be achieved when the feed substrate concentration was 197.5 g/L and the dilution rate was 197.57 g/L. Alwan also used global stochastic optimization to improve the operability of lab-scale spouted bed [8]. Georgia et al. [121] proposed a simple AD model for food industry waste, assuming that the organic matter consists of refractory and readily degradable COD, which can be used in different anaerobic digesters through different Monod kinetic consumption for methane production. Cai et al. carried out a Box-Behnken design-response surface methodology (RSM), correlation analysis, and two kinetic models to determine the effect and linkages of the parameters related to

ammonia inhibition in AD [122]. Wang et al. applied several machine learning algorithms in regression and classification models on digestion performance to identify determinant operational parameters and predict methane production, which reveal the great potential of using machine learning algorithms to predict AD performance [6].

4.2 New process for syntrophic methanogenesis enhancement

Since 1890s, the first anaerobic digester used to treat domestic sewage appeared in the UK. Up to now, the technology of anaerobic biological treatment has experienced more than 100 years of development and undergone a number of technology innovation. Anaerobic digester, as the first generation of anaerobic biological treatment equipment, was not widely used due to its low efficiency and burden. Up to the middle of the 20th century, there appeared the second generation of anaerobic reactors such as Anaerobic Contact Process (ACP), Anaerobic Filter (AF), Up-flow Anaerobic Sludge Bed (UASB), which realized the separation of the SRTand HRT, which greatly improved the organic loading and processing efficiency. In the 1990s, a new generation of anaerobic reactors, represented by up-flow anaerobic fluidized bed (AFB) [123], anaerobic expansive granular sludge bed (EGSB) [124] and internal circulating reactor (IC) [125], emerged. The new anaerobic reactor strengthened the mass transfer process between sludge and sewage, thus further improved the treatment efficiency. In recent years, researchers have proposed a variety of new reactors, such as anaerobic baffle reactor (ABR) [126], anaerobic intermittent reactor (ASBR) [127], and anaerobic membrane bioreactor (AnMBR) [128], which provide a new direction for the development of

anaerobic wastewater treatment technology.

From the perspective of products, AD could be divided into the production of VFAs, hydrogen, and methane [129-131]. Every process performs specific reaction and has its own optimum process parameters. In order to produce VFAs, bioreactors could either be designed to produce VFA as the primary product [132] or as a by-product [133]. Several bioreactors such us packed bed biofilm column reactor [134], anaerobic leach bed reactors [129], two-stage thermophilic anaerobic membrane bioreactor [135], CSTR [136], and continuous flow fermentation reactors [137] have shown promising results for the production and separation of VFAs. For hydrogen production, anaerobic down-flow structured bed reactor [138], UASB [139], CSTR [140], continuously external circulating bioreactor [141] have been proposed to improve the production of hydrogen. The design and equipment of bioreactors would largely affect the process of methane production. Several bioreactors like dry AD [131], field scale plug flow reactors [142], UASB [143], CSTR [140], induced bed reactors (IBR) [144], and AnMBR [145] were shown to produce methane in different substrates.

Although anaerobic biological treatment technology has accumulated a large number of research results and application experience in basic theory, reactor design, and process operation, there are still some bottlenecks limiting the efficiency and stability of AD system [146, 147]. For instance, the low organic content could be a key limiting factor when use raw waste water as substrate to produce methane. When the content of organic matter in sewage is low, functional microorganisms like

methanogens cannot get enough substrate for growth and metabolism and suffer from hunger for a long time which leads to the activity of sludge far below the optimal value [148]. For some granular sludge-based reactors (i.e., UASB), long-term low strength wastewater conditions can affect the granulation process and even lead to granular sludge disintegration [149]. Besides, AD process is very sensitive to the inhibitory factors in sewages [146, 147, 150]. For example, the different functional microorganisms involved in AD process have different pH optima, modify the system pH so as to cater for the needs of the individual microbial groups would be a method to optimize AD process. Lin et al. proposed a two-stage AD system for methane generation from swine manure. One of the major benefits of the system was preventing pH fluctuations from detrimentally affecting the individual microbial groups and resulting in system collapse since the acidogenic and methanogenic phases were separated [151]. Thus, bioreactors with two-stage AD system enable the simultaneous production of biohydrogen and methane. Besides, temperature and other environment condition can also be separate specifically to the microorganisms on each step [139, 143].

According to the previous studies, introducing bioelectrochemical system (BES) and conductive material into anaerobic system shown positive impact on methane generation [63, 152]. Since Pham et al. [153] firstly proposed the idea of coupling the BES system with AD system, researchers have made a series of attempts on the development, application, and regulation of the combined AD system. For example,

607 Tartakovsky et al. [154] demonstrated that the UASB reactor equipped with electrodes
608 for water electrolysis significantly improved the methane production and system
609 stability. Zou et al. [155] proposed a technique of an ABR combined with MECs to
610 enhance the stability and efficiency of ABR. For the new process, the COD removal
611 rate and methane content were up to 98% and 98% respectively. Moreover, Zamalloa
612 et al. [156] compared the biogas production and biogas quality of a 20 L lab-scale septic
613 tank coupled with a microbial electrolysis cell (MEC) and a common septic tank. The
614 result showed that the biogas production of MEC-septic tank system is 5 time as much
615 as the common one, the emission of phosphorous and H_2S was also lower than the
616 common septic tank. In conclusion, many experimental phenomena proved introducing
617 BES in anaerobic system can significantly improve the methane production and system
618 stability. [27, 28, 114, 115, 118]

619 **4.3 Metabolic regulation of main microorganisms in syntrophic methanogenesis**
620 **process**

621 During methanogenesis, intermediates are consumed by methanogens to produce
622 methane. As is mentioned before, methanogens were confined to several specific
623 substrates. Acetoclastic methanogenesis from acetate accounts for approximately 2/3 of
624 the methane production, with hydrogenotrophic methanogenesis accounting for
625 approximately the remaining 1/3 of the methane production. Besides, methanogenesis
626 from methanol, methylamines, and formate has also been observed [157]. Due to the
627 complexity of microorganism species and metabolic pathways in AD system,

628 understanding the diversity of microorganism species and the rules of community
629 succession caused by the changes of environmental factors are critical for studying the
630 structure and function of microbial community and revealing the degradation
631 mechanism. Microbial communities in anaerobic digestor were influenced by
632 environmental parameters, such as added inoculum, temperature, pH, HRT, feedstock
633 type, OLR, impurities, VFAs, C/N ratio, reactor design, and stirring intensity [158].
634 Wang et al. [159] studied the composition and evolution of microorganism communities
635 in the AD of grass silage by metagenomics, established and analyzed a clone library,
636 concluding that under different environmental conditions, the community composition,
637 the structure, the abundance of functional flora and the dominant flora would change
638 correspondingly. Duan et al. [160] also conducted metagenomic analysis to reveal the
639 nonylphenol-shaped acidification and methanogenesis during sludge AD. Their study
640 found that aceticlastic methanogenesis was replaced by the combination of aceticlastic
641 and hydrogenotrophic methanogenesis when improve the concentration of noylphenol.
642 The effective operation of AD system largely depends on the enrichment of functional
643 microorganisms and the structure of microbial community in the activated sludge AD.
644 Adding some specific microorganisms to increase the activity and diversity of activated
645 sludge will achieve better results. Bioaugmentation is the process of increasing the rate
646 of biodegradation by adding dominant strains [161]. Bioaugmentation is widely used
647 in wastewater treatment, which can improve the substrate degradation. Mohan et al.
648 [162] used bioaugmentation to treat synthetic wastewater containing benzyl alcohol,

649 the degradation of benzyl alcohol increased from 0.98 mg/min to 1.90 mg/min.
650 Maqbool et al. [163] demonstrated that bioaugmentation with hydrocarbon degrading
651 bacterial culture in UASB could accelerate the car wash wastewater treatment.

652 In addition, it is essential to know interactions between microorganisms through
653 quorum sensing (QS) for the system optimization in syntrophic methanogenesis. QS is
654 defined as the cell-to-cell communication occurred in microbial community in order to
655 collectively control and tune biological behaviors to cope with changes of cell density
656 and the surrounding environment [164, 165]. Microorganisms can produce and release
657 signal molecules, when the concentration of these signal molecules comes to an extreme
658 value, they will combine with the receptors and then activate the expression of target
659 genes to modify relevant phenotypes [166]. The phenomenon of QS was first
660 investigated in bacterium *Vibrio fischeri*, which proved the importance of QS in
661 bioluminescence [167, 168]. After that, numerous studies proposed that many microbial
662 processes were controlled by QS, such us the production of secondary metabolite [169],
663 the production of antibiotics [170], the formation of biofilm [171], the secretion of
664 virulence factors [172] and so on. Through QS, microorganisms are able to operate like
665 an organ and adapt to the environment actively [173].

666 At present, five kinds of QS signal molecules, N-acyl-homoserine lactones
667 (AHLs), Auto-inducer-2 (AI-2), Autoinducing peptides (AIPs), methyl dodecenoic acid,
668 and quinolone have been discovered [174-176]. QS signal system can be divided into
669 different types according to the different signal molecules [177]. For example, Gram-

negative bacteria mainly use LuxR/I mediated by acyl-homoserine lactones (AHLs) [178]; the target of signal Autoinducer 2 (AI-2) is Gram-negative or Gram-positive bacteria [179]; Gram-positive bacteria generally use autoinducing peptide (AIP) as self-inducer to regulate the development of gene receptors through a two-component signaling system [180]; in entero-hemorrhagic E.coli, there exists autoinducer 3 (AI-3) which regulate the expression of virulence factors; the QS signal system of methanogenic is similar to Gram-negative, thus belongs to LuxR/I system [181]. Studies have shown that AHLs and AIPs are the intraspecific QS signaling molecules of Gram-negative (G^-) and Gram-positive (G^+) bacteria respectively, while, AI-2 is the self-inducer of bacterial secretion and mediates microbial interspecific behavior [182]. Up to now, more than 100 proteobacteria have been identified to secrete AHLs. These microorganisms mainly synthesize AHLs through three types of enzymes, which are proteins belonging to the family of LuxI, LuxM, and HdtS, respectively. Most AHL synthases are proteins belonging to the LuxI family [183].

Currently, QS has been increasingly applied in wastewater treatment to promote the aggregation activity and the environmental resistance of activated sludge [184, 185]. Some research showed that the addition of AHLs could promote the chitinase activity in activated sludge, which indicated that QS system could mediate the secretion of extracellular enzymes and improve the efficiency of aerobic activated sludge process [186]. *Pseudomonas aeruginosa* can degrade aromatic compounds and secrete two kinds of QS signaling molecules under aerobic conditions, the addition of endogenous

691 signal molecules could promote the degradation of phenol [187]. All these studies
692 indicate that QS can be used as a control approach to improve the efficiency of
693 wastewater treatment.

694 As for AD, since both hydrogen and acetate producing bacteria and methanogens
695 are G^- bacteria, and most acid producing fermentation bacteria are G^- bacteria as well,
696 the current research on QS in methane fermentation system mainly focuses on AHLs.
697 Up to now, a series of AHLs have been detected in anaerobic sludge [188], and the
698 methanogenic bacteria in anaerobic granular sludge can release and utilize AHLs by
699 themselves, in order to optimize the methanogenic metabolic process of VFAs [189].
700 Some studies have shown that in UASB reactor, AHLs can promote the growth of
701 *Methanothrix*, which accelerates the formation of sludge and maintains the stability and
702 efficiency of reactor [181]. The addition of some specific AHLs can accelerate the
703 degradation of organic substrates and the production of methane. Exogenous AHLs
704 plays an important role in regulating the concentration of bacterial extracellular
705 polymers and the structure of microbial community [190, 191]. Du et al. [192]
706 constructed a microbial QS database of anaerobic methanogen through metagenomic
707 and bioanalytical analysis to investigate the functional quorum sensing genes in AD
708 reactor, which provided guidance for studying QS in AD.

709 **5. Future perspective**

710 AD has been widely used in waste/wastewater treatment with additional energy
711 recovery [193]. After hydrolysis, acidogenesis, and acetogenesis steps, complex

712 organic substrates are converted to methane by methanogens [192]. Unlike aerobic
713 process, sequential reactions of degradation are essential, thus syntrophic interactions
714 between microorganisms via interspecies electron transfer is needed [14]. Syntrophic
715 interactions between bacteria and methanogens are the basis to maintain an efficient
716 AD system [23]. The microorganisms involved in syntrophic methanogenesis and
717 mechanism of interspecies electron transfer between microorganisms are summarized
718 in the present work. Study on optimizing syntrophic metabolic is an emerging topic on
719 anaerobic microbiology recently. Many studies focus on adding conductive materials
720 or nanoparticles to enhance syntrophic metabolic in AD. Therefore, how to enhance
721 syntrophic metabolic ability via regulating the synthesis capability of electronic carriers
722 need further investigation. Besides, the relationship between the two main electron
723 carriers of hydrogen and formate and the differences between them for the contribution
724 of product need more consideration as well. Recently, many studies focused on
725 bioaugmentation technology, adding conductive materials during AD, external power
726 supply for anaerobic reactor, two-phase AD reactor and coupling process to improve
727 the efficiency of AD. Lastly, the application of omics analysis i.e. metagenomics and
728 metatranscriptomics can provide deeper physiological and biochemical information by
729 predicting the specific metabolic pathways and functional genes, which promote
730 microbial AD process and develop high efficient AD technology.

731 **6. Conclusions**

732 Methanogenesis has always been considered as a rate-limiting step in AD. With
733 the deepen understanding of the metabolic process of syntrophic bacteria, it was found

734 that the syntrophy conservation of VFAs was also an important rate-limiting step.
735 Therefore, it is essential to take syntrophic bacteria and methanogen as a whole for
736 analysis to improve the AD efficiency. The nature of syntrophic metabolism of VFAs
737 mediated by anaerobic microorganisms is interspecies electron transfer. Traditional
738 interspecies electron transfer uses hydrogen/formate as electron carriers. The formation
739 of hydrogen and formate is hindered by thermodynamics, and their diffusion is
740 restricted by Frick's law. Thus, the transfer of matter and energy between syntrophic
741 microorganisms and methanogens are mainly limited by the process of interspecific
742 electron transfer. Seeking a more efficient interspecies electron transfer method with
743 higher thermodynamic barriers than interspecies hydrogen/formate transfers has
744 gradually become a hot research topic. Along with the research of microorganism
745 extracellular respiration, the mechanism of DIET and electron shuttle has been studied
746 and presented higher advantage. Previous works mainly focus on process optimization.
747 However, due to the complexity of microorganisms involved in AD, process
748 optimization would not easily achieve. Understanding the microorganisms and
749 biochemical reactions among AD would accelerate the syntrophic methanogenesis,
750 which benefits the efficiency and stability of the system.
751

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Grant numbers: 52070176, 21876167, 51678553), Interdisciplinary platform for ecological civilization, Major Innovation & Planning Interdisciplinary Platform for the "Double-First Class" Initiative, Renmin University of China, and the Opening Project of the key Laboratory of Energy Utilization of Agricultural Wastes of the Ministry of Agriculture and Villages (KLERUAR2020-01).

References

[1] P. Shanmugam, N. Horan, Optimising the biogas production from leather fleshing waste by co-digestion with MSW, Bioresour. Technol. 100 (2009) 4117-4120. http://doi.org/10.1016/j.biortech.2009.03.052

[2] B. P., Basics of Anaerobic Digestion Process. In: Anaerobic Technology in Pulp and Paper Industry, SpringerBriefs in Applied Sciences and Technology, Springer, Singapore, 2017.

[3] K. Kundu, S. Sharma, T.R. Sreekrishnan, Influence of Process Parameters on Anaerobic Digestion Microbiome in Bioenergy Production: Towards an Improved Understanding, BioEnergy Res. 10 (2016) 288-303. http://doi.org/10.1007/s12155-016-9789-0

[4] M.A. Khan, H.H. Ngo, W.S. Guo, Y. Liu, L.D. Nghiem, F.I. Hai, L.J. Deng, J. Wang, Y. Wu, Optimization of process parameters for production of volatile fatty acid, biohydrogen and methane from anaerobic digestion, Bioresour. Technol. 219 (2016) 738-748. http://doi.org/10.1016/j.biortech.2016.08.073

[5] A. Roopnarain, H. Rama, B. Ndaba, M. Bello-Akinosho, E. Bamuza-Pemu, R. Adeleke, Unravelling the anaerobic digestion 'black box': Biotechnological approaches for process optimization, Renewable Sustainable Energy Rev. 152 (2021). http://doi.org/10.1016/j.rser.2021.111717

[6] L. Wang, F. Long, W. Liao, H. Liu, Prediction of anaerobic digestion performance and identification of critical operational parameters using machine learning algorithms, Bioresour. Technol. 298 (2020) 122495. http://doi.org/10.1016/j.biortech.2019.122495

[7] G. M. Alwan, Simulation and Optimization of a Continuous Biochemical Reactor, J. Chem. Eng. Process Technol. 04 (2012). http://doi.org/10.4172/2157-7048.1000142

[8] G.M. Alwan, Improving Operability of Lab-Scale Spouted Bed Using Global Stochastic Optimization, J. Eng. Res. Appl. 5 (2015) 136-146.

[9] J.S. Zhang, W.Q. Zhao, H.W. Zhang, Z.J. Wang, C.F. Fan, L.H. Zang, Recent achievements in enhancing anaerobic digestion with carbon-based functional materials, Bioresour. Technol. 266 (2018) 555-567. http://doi.org/10.1016/j.biortech.2018.07.076

[10] J.N. Meegoda, B. Li, K. Patel, L.B. Wang, A Review of the Processes, Parameters, and Optimization of Anaerobic Digestion, Int. J. Environ. Res. Public. Health 15 (2018). http://doi.org/10.3390/ijerph15102224

[11] A. Anukam, A. Mohammadi, M. Naqvi, K. Granström, A Review of the Chemistry of Anaerobic Digestion: Methods of Accelerating and Optimizing Process Efficiency, Processes 7 (2019). http://doi.org/10.3390/pr7080504

[12] B. Schink, Energetics of syntrophic cooperation in methanogenic degradation, Microbiol. Mol. Biol. Rev. 61 (1997) 262-280. http://doi.org/10.1128/.61.2.262-280.1997

[13] X.F. Pan, I. Angelidaki, M. Alvarado-Morales, H.G. Liu, Y.H. Liu, X. Huang, G.F. Zhu, Methane production from formate, acetate and H-2/CO2; focusing on kinetics and microbial characterization, Bioresour. Technol. 218 (2016) 796-806. http://doi.org/10.1016/j.biortech.2016.07.032

[14] A.N. Nozhevnikova, Y.I. Russkova, Y.V. Litti, S.N. Parshina, E.A. Zhuravleva, A.A. Nikitina, Syntrophy and Interspecies Electron Transfer in Methanogenic Microbial Communities, Microbiology 89 (2020) 129-147. http://doi.org/10.1134/s0026261720020101

[15] A.J.M. Stams, C.M. Plugge, Electron transfer in syntrophic communities of anaerobic bacteria and archaea, Nat. Rev. Microbiol. 7 (2009) 568-577. http://doi.org/10.1038/nrmicro2166

[16] Y. Zhang, J. Li, F. Liu, H. Yan, J. Li, X. Zhang, Reduction of Gibbs free energy and enhancement of Methanosaeta by bicarbonate to promote anaerobic syntrophic butyrate oxidation, Bioresour. Technol. 267 (2018) 209-217. http://doi.org/10.1016/j.biortech.2018.06.098

[17] M.P. Bryant, E.A. Wolin, M.J. Wolin, R.S. Wolfe, Methanobacillus omelianskii, a symbiotic association of two species of bacteria, Archiv fur Mikrobiologie 59 (1967) 20-31. http://doi.org/10.1007/bf00406313

[18] R.K. Thauer, K. Jungermann, K. Decker, Energy-Conservation in Chemotropic Anaerobic Bacteria, Bacteriol. Rev. 41 (1977) 100-180. http://doi.org/10.1128/mmbr.41.1.100-180.1977

[19] N. Muller, P. Worm, B. Schink, A.J.M. Stams, C.M. Plugge, Syntrophic butyrate and propionate oxidation processes: from genomes to reaction mechanisms, Environ. Microbiol. Rep. 2 (2010) 489-499. http://doi.org/10.1111/j.1758-2229.2010.00147.x

[20] F.A. Shah, Q. Mahmood, M.M. Shah, A. Pervez, S.A. Asad, Microbial Ecology of Anaerobic Digesters: The Key Players of Anaerobiosis, Sci. World J. (2014) 1-21. http://doi.org/10.1155/2014/183752

[21] C. Moertelmaier, C. Li, J. Winter, C. Gallert, Fatty acid metabolism and population dynamics in a wet biowaste digester during re-start after revision, Bioresour. Technol. 166 (2014) 479-484. http://doi.org/10.1016/j.biortech.2014.05.085

[22] A.J.M. Stams, F.A.M. de Bok, C.M. Plugge, M.H.A. van Eekert, J. Dolfing, G. Schraa, Exocellular electron transfer in anaerobic microbial communities, Environ. Microbiol. 8 (2006) 371-382. http://doi.org/10.1111/j.1462-2920.2006.00989.x

[23] G. Martins, A.F. Salvador, L. Pereira, M. Madalena Alves, Methane Production and Conductive Materials: A Critical Review, Environ. Sci. Technol. 52 (2018) 10241-10253. http://doi.org/10.1021/acs.est.8b01913

[24] B.E. Logan, S.E. Oh, I.S. Kim, S. Van Ginkel, Biological hydrogen production measured in batch anaerobic respirometers, Environ. Sci. Technol. 36 (2002) 2530-2535. http://doi.org/10.1021/es015783i

[25] Y. Liu, M. Gu, Q. Yin, J. Du, G. Wu, Thermodynamic analysis of direct interspecies electron transfer in syntrophic methanogenesis based on the optimized energy

distribution, Bioresour. Technol. 297 (2020). http://doi.org/10.1016/j.biortech.2019.122345
[26] F.J. Cervantes, F.A.M. de Bok, D.D. Tuan, A.J.M. Stams, G. Lettinga, J.A. Field, Reduction of humic substances by halorespiring, sulphate-reducing and methanogenic microorganisms, Environ. Microbiol. 4 (2002) 51-57. http://doi.org/10.1046/j.1462-2920.2002.00258.x
[27] V. Hernandez-Montoya, L.H. Alvarez, M.A. Montes-Moran, F.J. Cervantes, Reduction of quinone and non-quinone redox functional groups in different humic acid samples by Geobacter sulfurreducens, Geoderma 183 (2012) 25-31. http://doi.org/10.1016/j.geoderma.2012.03.007
[28] S. Peth, R. Horn, F. Beckmann, T. Donath, J. Fischer, A.J.M. Smucker, Three-dimensional quantification of intra-aggregate pore-space features using synchrotron-radiation-based microtomography, Soil Sci. Soc. Am. J. 72 (2008) 897-907. http://doi.org/10.2136/sssaj2007.0130
[29] C.C. Viggi, S. Rossetti, S. Fazi, P. Paiano, M. Majone, F. Aulenta, Magnetite Particles Triggering a Faster and More Robust Syntrophic Pathway of Methanogenic Propionate Degradation, Environ. Sci. Technol. 48 (2014) 7536-7543. http://doi.org/10.1021/es5016789
[30] G. Baek, J. Kim, K. Cho, H. Bae, C. Lee, The biostimulation of anaerobic digestion with (semi)conductive ferric oxides: their potential for enhanced biomethanation, Appl. Microbiol. Biotechnol. 99 (2015) 10355-10366. http://doi.org/10.1007/s00253-015-6900-y
[31] R. Wang, C. Li, N. Lv, X. Pan, G. Cai, J. Ning, G. Zhu, Deeper insights into effect of activated carbon and nano-zero-valent iron addition on acidogenesis and whole anaerobic digestion, Bioresour. Technol. 324 (2021). http://doi.org/10.1016/j.biortech.2021.124671
[32] W. Wang, D.J. Lee, Valorization of anaerobic digestion digestate: A prospect review, Bioresour. Technol. 323 (2021) 124626. http://doi.org/10.1016/j.biortech.2020.124626
[33] H.T.T. Nguyen, M.T. Noori, B. Min, Accelerating anaerobic digestion process with novel single chamber microbial electrochemical systems with baffle, Bioresour. Technol. 359 (2022) 127474. http://doi.org/10.1016/j.biortech.2022.127474
[34] J.R. Sieber, M.J. McInerney, R.P. Gunsalus, Genomic Insights into Syntrophy: The Paradigm for Anaerobic Metabolic Cooperation, in: S. Gottesman, C.S. Harwood, O. Schneewind (Eds.) Annu. Rev. Microbiol.2012, pp. 429-452.
[35] C.-D. Dube, S.R. Guiot, Direct Interspecies Electron Transfer in Anaerobic Digestion: A Review, in: G.M. Guebitz, A. Bauer, G. Bochmann, A. Gronauer, S. Weiss (Eds.) Biogas Science and Technology2015, pp. 101-115.
[36] Y. Liu, W.B. Whitman, Metabolic, phylogenetic, and ecological diversity of the methanogenic archaea, in: J. Wiegel, R.J. Maier, M.W.W. Adams (Eds.) Incredible Anaerobes: From Physiology To Genomics To Fuels2008, pp. 171-189.
[37] P.M. Vignais, B. Billoud, Occurrence, classification, and biological function of

hydrogenases: An overview, Chem. Rev. 107 (2007) 4206-4272. http://doi.org/10.1021/cr050196r
[38] R.K. Thauer, A.-K. Kaster, H. Seedorf, W. Buckel, R. Hedderich, Methanogenic archaea: ecologically relevant differences in energy conservation, Nat. Rev. Microbiol. 6 (2008) 579-591. http://doi.org/10.1038/nrmicro1931
[39] C. Wallrabenstein, B. Schink, Evidence of reversed electron transport in syntrophic butyrate or benzoate oxidation by Syntrophomonas wolfei and Syntrophus buswelli, Arch. Microbiol. 162 (1994) 136-142.
[40] F.A. Macleod, S.R. Guiot, J.W. Costerton, Layered Strucyure of Bacterial Aggregates Produced in an Upflow Anaerobic Sludge Bed and Filter Reactor, Appl. Environ. Microbiol. 56 (1990) 1598-1607. http://doi.org/10.1128/aem.56.6.1598-1607.1990
[41] W.M. Wu, M.K. Jain, E.C. Demacario, J.H. Thiele, J.G. Zeikus, Microbial Composition and Characterization of Prevalent Methanogens and Acetogens Isolated from Syntrophic Methanogenic Granules, Appl. Microbiol. Biotechnol. 38 (1992) 282-290. http://doi.org/10.1007/bf00174484
[42] M.S.A. Blackwell, R.C. Brookes, N. de la Fuente-Martinez, H. Gordon, P.J. Murray, K.E. Snars, J.K. Williams, R. Bol, P.M. Haygarth, Phosphorus Solubilization and Potential Transfer to Surface Waters from the Soil Microbial Biomass Following Drying-Rewtting and Freezing-Thawing, in: D.L. Sparks (Ed.) Adv. Agron.2010, pp. 1-35.
[43] M. Felchner-Zwirello, J. Winter, C. Gallert, Interspecies distances between propionic acid degraders and methanogens in syntrophic consortia for optimal hydrogen transfer, Appl. Microbiol. Biotechnol. 97 (2013) 9193-9205. http://doi.org/10.1007/s00253-012-4616-9
[44] S. McHugh, M. Carton, T. Mahony, V. O'Flaherty, Methanogenic population structure in a variety of anaerobic bioreactors, FEMS Microbiol. Lett. 219 (2003) 297-304. http://doi.org/10.1016/s0378-1097(03)00055-7
[45] S. Leonhartsberger, I. Korsa, A. Bock, The molecular biology of formate metabolism in enterobacteria, J. Mol. Microbiol. Biotechnol. 4 (2002) 269-276.
[46] P. Sinha, S. Roy, D. Das, Role of formate hydrogen lyase complex in hydrogen production in facultative anaerobes, Int. J. Hydrogen Energy 40 (2015) 8806-8815. http://doi.org/10.1016/j.ijhydene.2015.05.076
[47] B. Schink, D. Montag, A. Keller, N. Mueller, Hydrogen or formate: Alternative key players in methanogenic degradation, Environ. Microbiol. Rep. 9 (2017) 189-202. http://doi.org/10.1111/1758-2229.12524
[48] R.G. Sawers, Formate and its role in hydrogen production in Escherichia coli, Biochem. Soc. Trans. 33 (2005) 42-46. http://doi.org/10.1042/bst0330042
[49] L. Shen, Q. Zhao, X. Wu, X. Li, Q. Li, Y. Wang, Interspecies electron transfer in syntrophic methanogenic consortia: From cultures to bioreactors, Renewable Sustainable Energy Rev. 54 (2016) 1358-1367. http://doi.org/10.1016/j.rser.2015.10.102

[50] J.H. Thiele, J.G. Zeikus, Control of Interspecies Electron Flow during Anaerobic-Digestion – Significance of Formate Transfer Versus Hydrogen Transfer During Syntrophic Methanogenesis in Flocs, Appl. Environ. Microbiol. 54 (1988) 20-29. http://doi.org/10.1128/aem.54.1.20-29.1988

[51] X. Dong, G. Cheng, A.J.M. Stams, Butyrate Oxidation by Syntrophospora-Bryantii in Coculture with Different Methanogens and in Pure Culture with Pentenote as Electron-Acceptor, Appl. Microbiol. Biotechnol. 42 (1994) 647-652.

[52] M.J. McInerney, M.P. Bryant, R.B. Hespell, J.W. Costerton, Syntrophomonas-Wolfei Gen-Nov Sp-Nov, an Anaerobic, Syntrophic, Fatty-acid Oxidizing Bacterium, Appl. Environ. Microbiol. 41 (1981) 1029-1039. http://doi.org/10.1128/aem.41.4.1029-1039.1981

[53] X.Z. Dong, A.J.M. Stams, Evidence for H_2 and Formate Formation during Syntrophic Butyrate and Propionate Degradation, Anaerobe 1 (1995) 35-39. http://doi.org/10.1016/s1075-9964(95)80405-6

[54] J.E. Schmidt, B.K. Ahring, Interspecies Electron Transfer During Propionate and Butyrate Degradation in Mesophilic, Granular Sludge, Appl. Environ. Microbiol. 61 (1995) 2765-2767. http://doi.org/10.1128/aem.61.7.2765-2767.1995

[55] P. Bossier, W. Verstraete, Triggers for microbial aggregation in activated sludge?, Appl. Microbiol. Biotechnol. 45 (1996) 1-6. http://doi.org/10.1007/s002530050640

[56] M. Zhang, L. Zang, A review of interspecies electron transfer in anaerobic digestion, IOP Conference Series: Earth and Environmental Science, IOP Publishing, 2019, pp. 042026.

[57] F.A.M. de Bok, C.M. Plugge, A.J.M. Stams, Interspecies electron transfer in methanogenic propionate degrading consortia, Water Res. 38 (2004) 1368-1375. http://doi.org/10.1016/j.watres.2003.11.028

[58] G. Reguera, K.D. McCarthy, T. Mehta, J.S. Nicoll, M.T. Tuominen, D.R. Lovley, Extracellular electron transfer via microbial nanowires, Nature 435 (2005) 1098-1101. http://doi.org/10.1038/nature03661

[59] Z.M. Summers, H.E. Fogarty, C. Leang, A.E. Franks, N.S. Malvankar, D.R. Lovley, Direct Exchange of Electrons Within Aggregates of an Evolved Syntrophic Coculture of Anaerobic Bacteria, Science 330 (2010) 1413-1415. http://doi.org/10.1126/science.1196526

[60] A.-E. Rotaru, P.M. Shrestha, F. Liu, T. Ueki, K. Nevin, Z.M. Summers, D.R. Lovley, Interspecies Electron Transfer via Hydrogen and Formate Rather than Direct Electrical Connections in Cocultures of Pelobacter carbinolicus and Geobacter sulfurreducens, Appl. Environ. Microbiol. 78 (2012) 7645-7651. http://doi.org/10.1128/aem.01946-12

[61] M. Estevez-Canales, A. Kuzume, Z. Borjas, M. Fueeg, D. Lovley, T. Wandlowski, A. Esteve-Nunez, A severe reduction in the cytochrome C content of Geobacter sulfurreducens eliminates its capacity for extracellular electron transfer, Environ. Microbiol. Rep. 7 (2015) 219-226. http://doi.org/10.1111/1758-2229.12230

[62] D.R. Lovley, Syntrophy Goes Electric: Direct Interspecies Electron Transfer, in: S. Gottesman (Ed.) Annu. Rev. Microbiol.2017, pp. 643-664.
[63] J.-H. Park, H.-J. Kang, K.-H. Park, H.-D. Park, Direct interspecies electron transfer via conductive materials: A perspective for anaerobic digestion applications, Bioresour. Technol. 254 (2018) 300-311. http://doi.org/10.1016/j.biortech.2018.01.095
[64] Y. Wu, S. Wang, D. Liang, N. Li, Conductive materials in anaerobic digestion: From mechanism to application, Bioresour. Technol. 298 (2020). http://doi.org/10.1016/j.biortech.2019.122403
[65] M. Morita, N.S. Malvankar, A.E. Franks, Z.M. Summers, L. Giloteaux, A.E. Rotaru, C. Rotaru, D.R. Lovley, Potential for Direct Interspecies Electron Transfer in Methanogenic Wastewater Digester Aggregates, Mbio 2 (2011). http://doi.org/10.1128/mBio.00159-11
[66] Q. Cheng, D.F. Call, Hardwiring microbes via direct interspecies electron transfer: mechanisms and applications, ENVIRON SCI-PROC IMP 18 (2016) 968-980. http://doi.org/10.1039/c6em00219f
[67] S. Kato, K. Hashimoto, K. Watanabe, Microbial interspecies electron transfer via electric currents through conductive minerals, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 10042-10046. http://doi.org/10.1073/pnas.1117592109
[68] F. Liu, A.-E. Rotaru, P.M. Shrestha, N.S. Malvankar, K.P. Nevin, D.R. Lovley, Promoting direct interspecies electron transfer with activated carbon, Energy Environ. Sci. 5 (2012) 8982-8989. http://doi.org/10.1039/c2ee22459c
[69] S. Chen, A.-E. Rotaru, P.M. Shrestha, N.S. Malvankar, F. Liu, W. Fan, K.P. Nevin, D.R. Lovley, Promoting Interspecies Electron Transfer with Biochar, Sci. Rep. 4 (2014). http://doi.org/10.1038/srep05019
[70] S. Barua, B.S. Zakaria, B.R. Dhar, Enhanced methanogenic co-degradation of propionate and butyrate by anaerobic microbiome enriched on conductive carbon fibers, Bioresour. Technol. 266 (2018) 259-266. http://doi.org/10.1016/j.biortech.2018.06.053
[71] Y. Jing, J. Wan, I. Angelidaki, S. Zhang, G. Luo, iTRAQ quantitative proteomic analysis reveals the pathways for methanation of propionate facilitated by magnetite, Water Res. 108 (2017) 212-221. http://doi.org/10.1016/j.watres.2016.10.077
[72] S. Kato, K. Hashimoto, K. Watanabe, Methanogenesis facilitated by electric syntrophy via (semi)conductive iron-oxide minerals, Environ. Microbiol. 14 (2012) 1646-1654. http://doi.org/10.1111/j.1462-2920.2011.02611.x
[73] C.Q. Wang, Y. Liu, S. Jin, H. Chen, X.Y. Xu, Z.R. Wang, B. Xing, L. Zhu, Responsiveness extracellular electron transfer (EET) enhancement of anaerobic digestion system during start-up and starvation recovery stages via magnetite addition, Bioresour. Technol. 272 (2019) 162-170. http://doi.org/10.1016/j.biortech.2018.10.013
[74] A.F. Salvador, G. Martins, M. Melle-Franco, R. Serpa, A.J.M. Stams, A.J.

Cavaleiro, M.A. Pereira, M.M. Alves, Carbon nanotubes accelerate methane production in pure cultures of methanogens and in a syntrophic coculture, Environ. Microbiol. 19 (2017) 2727-2739. http://doi.org/10.1111/1462-2920.13774

[75] G. Baek, J. Kim, J. Kim, C. Lee, Role and Potential of Direct Interspecies Electron Transfer in Anaerobic Digestion, Energies 11 (2018). http://doi.org/10.3390/en11010107

[76] Y. Li, Y. Zhang, Y. Yang, X. Quan, Z. Zhao, Potentially direct interspecies electron transfer of methanogenesis for syntrophic metabolism under sulfate reducing conditions with stainless steel, Bioresour. Technol. 234 (2017) 303-309. http://doi.org/10.1016/j.biortech.2017.03.054

[77] S. Chen, A.-E. Rotaru, F. Liu, J. Philips, T.L. Woodard, K.P. Nevin, D.R. Lovley, Carbon cloth stimulates direct interspecies electron transfer in syntrophic co-cultures, Bioresour. Technol. 173 (2014) 82-86. http://doi.org/10.1016/j.biortech.2014.09.009

[78] A.-E. Rotaru, P.M. Shrestha, F. Liu, M. Shrestha, D. Shrestha, M. Embree, K. Zengler, C. Wardman, K.P. Nevin, D.R. Lovley, A new model for electron flow during anaerobic digestion: direct interspecies electron transfer to Methanosaeta for the reduction of carbon dioxide to methane, Energy Environ. Sci. 7 (2014) 408-415. http://doi.org/10.1039/c3ee42189a

[79] D.R. Lovley, Happy together: microbial communities that hook up to swap electrons, ISME J. 11 (2017) 327-336. http://doi.org/10.1038/ismej.2016.136

[80] Z. Zhao, Y. Zhang, Y. Li, Y. Dang, T. Zhu, X. Quan, Potentially shifting from interspecies hydrogen transfer to direct interspecies electron transfer for syntrophic metabolism to resist acidic impact with conductive carbon cloth, Chem. Eng. J. 313 (2017) 10-18. http://doi.org/10.1016/j.cej.2016.11.149

[81] Q. Yin, S. Yang, Z. Wang, L. Xing, G. Wu, Clarifying electron transfer and metagenomic analysis of microbial community in the methane production process with the addition of ferroferric oxide, Chem. Eng. J. 333 (2018) 216-225. http://doi.org/10.1016/j.cej.2017.09.160

[82] M.O. Fagbohungbe, B.M.J. Herbert, L. Hurst, H. Li, S.Q. Usmani, K.T. Semple, Impact of biochar on the anaerobic digestion of citrus peel waste, Bioresour. Technol. 216 (2016) 142-149. http://doi.org/10.1016/j.biortech.2016.04.106

[83] F. Lu, C. Luo, L. Shao, P. He, Biochar alleviates combined stress of ammonium and acids by firstly enriching Methanosaeta and then Methanosarcina, Water Res. 90 (2016) 34-43. http://doi.org/10.1016/j.watres.2015.12.029

[84] W. Yan, N. Shen, Y. Xiao, Y. Chen, F. Sun, V.K. Tyagi, Y. Zhou, The role of conductive materials in the start-up period of thermophilic anaerobic system, Bioresour. Technol. 239 (2017) 336-344. http://doi.org/10.1016/j.biortech.2017.05.046

[85] D. Wang, Y. Han, H. Han, K. Li, C. Xu, H. Zhuang, New insights into enhanced anaerobic degradation of Fischer-Tropsch wastewater with the assistance of magnetite, Bioresour. Technol. 257 (2018) 147-156.

http://doi.org/10.1016/j.biortech.2018.02.084
[86] J.A. Smith, K.P. Nevin, D.R. Lovley, Syntrophic growth via quinone-mediated interspecies electron transfer, Front. Microbiol. 6 (2015). http://doi.org/10.3389/fmicb.2015.00121
[87] C.-C. Hsueh, C.-C. Wu, B.-Y. Chen, Polyphenolic compounds as electron shuttles for sustainable energy utilization, Biotechnol. Biofuels 12 (2019) 1-26.
[88] E. Marsili, D.B. Baron, I.D. Shikhare, D. Coursolle, J.A. Gralnick, D.R. Bond, Shewanella Secretes flavins that mediate extracellular electron transfer, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 3968-3973. http://doi.org/10.1073/pnas.0710525105
[89] H. von Canstein, J. Ogawa, S. Shimizu, J.R. Lloyd, Secretion of flavins by Shewanella species and their role in extracellular electron transfer, Appl. Environ. Microbiol. 74 (2008) 615-623. http://doi.org/10.1128/aem.01387-07
[90] L. Huang, I. Angelidaki, Effect of humic acids on electricity generation integrated with xylose degradation in microbial fuel cells, Biotechnol. Bioeng. 100 (2008) 413-422. http://doi.org/10.1002/bit.21786
[91] A. Thygesen, F.W. Poulsen, B. Min, I. Angelidaki, A.B. Thomsen, The effect of different substrates and humic acid on power generation in microbial fuel cell operation, Bioresour. Technol. 100 (2009) 1186-1191. http://doi.org/10.1016/j.biortech.2008.07.067
[92] D.R. Lovley, J.D. Coates, E.L. BluntHarris, E.J.P. Phillips, J.C. Woodward, Humic substances as electron acceptors for microbial respiration, Nature 382 (1996) 445-448. http://doi.org/10.1038/382445a0
[93] X.Q. Lu, W.D. Johnson, J. Hook, Reaction of vanadate with aquatic humic substances: An ESR and V-51 NMR study, Environ. Sci. Technol. 32 (1998) 2257-2263. http://doi.org/10.1021/es970930r
[94] A.T. Stone, J.J. Morgan, Reduction and Dissolution of Manganese(III) and Manganese(IV) Oxides by Organics .1. Reaction with Hydroquinone, Environ. Sci. Technol. 18 (1984) 450-456. http://doi.org/10.1021/es00124a011
[95] D.R. Lovley, J.L. Fraga, J.D. Coates, E.L. Blunt-Harris, Humics as an electron donor for anaerobic respiration, Environ. Microbiol. 1 (1999) 89-98. http://doi.org/10.1046/j.1462-2920.1999.00009.x
[96] M.J. McInerney, L. Rohlin, H. Mouttaki, U. Kim, R.S. Krupp, L. Rios-Hernandez, J. Sieber, C.G. Struchtemeyer, A. Bhattacharyya, J.W. Campbell, R.P. Gunsalus, The genome of Syntrophus aciditrophicus: Life at the thermodynamic limit of microbial growth, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 7600-7605. http://doi.org/10.1073/pnas.0610456104
[97] T. Kosaka, S. Kato, T. Shimoyama, S. Ishii, T. Abe, K. Watanabe, The genome of Pelotomaculum thermopropionicum reveals niche-associated evolution in anaerobic microbiota, Genome Res. 18 (2008) 442-448. http://doi.org/10.1101/gr.7136508
[98] J.R. Sieber, D.R. Sims, C. Han, E. Kim, A. Lykidis, A.L. Lapidus, E. McDonnald,

L. Rohlin, D.E. Culley, R. Gunsalus, M.J. McInerney, The genome of Syntrophomonas wolfei: new insights into syntrophic metabolism and biohydrogen production, Environ. Microbiol. 12 (2010) 2289-2301. http://doi.org/10.1111/j.1462-2920.2010.02237.x

[99] P.S. Beaty, M.J. McInerney, McInerney, Growth of Syntrophomonas-Wolfei in Pure Culture on Crotonate, Arch. Microbiol. 147 (1987) 389-393. http://doi.org/10.1007/bf00406138

[100] M. Friedrich, B. Schink, Electron-Transport Phosphorylation Driven by Glyoxylate Respiration with Hydrogen as Electron-Donor in Membrane-Vesicles of a Glyoxylate-Fermenting Bacterium, Arch. Microbiol. 163 (1995) 268-275. http://doi.org/10.1007/bf00393379

[101] R.K. Nilsen, T. Torsvik, T. Lien, Desulfotomaculum thermocisternum sp nov, a sulfate reducer isolated from a hot North Sea oil reservoir, Int. J. Syst. Bacteriol. 46 (1996) 397-402. http://doi.org/10.1099/00207713-46-2-397

[102] M. Westerholm, S. Roos, A. Schnurer, Syntrophaceticus schinkii gen. nov., sp nov., an anaerobic, syntrophic acetate-oxidizing bacterium isolated from a mesophilic anaerobic filter, FEMS Microbiol. Lett. 309 (2010) 100-104. http://doi.org/10.1111/j.1574-6968.2010.02023.x

[103] A.J.M. Stams, D.Z. Sousa, R. Kleerebezem, C.M. Plugge, Role of syntrophic microbial communities in high-rate methanogenic bioreactors, Water Sci. Technol. 66 (2012) 352-362. http://doi.org/10.2166/wst.2012.192

[104] D.A. Amos, M.J. McInerney, Poly-Beta-Hydroxyalkanoate in Syntrophomonas-Wolfei, Arch. Microbiol. 152 (1989) 172-177. http://doi.org/10.1007/bf00456097

[105] M.J. McInerney, C.G. Struchtemeyer, J. Sieber, H. Mouttaki, A.J.M. Stams, B. Schink, L. Rohlin, R.P. Gunsalus, Physiology, ecology, phylogeny, and genomics of microorganisms capable of syntrophic metabolism, in: J. Wiegel, R.J. Maier, M.W.W. Adams (Eds.) Incredible Anaerobes: From Physiology To Genomics To Fuels2008, pp. 58-72.

[106] S. Fetzer, R. Conrad, Effect of Redox Potential on Methanogenesis by Methanosarcina-Barkeri, Arch. Microbiol. 160 (1993) 108-113. http://doi.org/10.1007/bf00288711

[107] A.J. Horne, D.J. Lessner, Assessment of the oxidant tolerance of Methanosarcina acetivorans, FEMS Microbiol. Lett. 343 (2013) 13-19. http://doi.org/10.1111/1574-6968.12115

[108] A.Y. Kallistova, A.Y. Merkel, I.Y. Tarnovetskii, N.V. Pimenov, Methane formation and oxidation by prokaryotes, Microbiology 86 (2017) 671-691. http://doi.org/10.1134/s0026261717060091

[109] R. Conrad, T.J. Phelps, J.G. Zeikus, Gas Metabolism Evidence in Support of the Juxtaposition of Hydrogen-Producing and Methanogenic Bacteria in Sewage-Sludge and Lake-Sediments, Appl. Environ. Microbiol. 50 (1985) 595-601. http://doi.org/10.1128/aem.50.3.595-601.1985

[110] S. Ishii, T. Kosaka, K. Hori, Y. Hotta, K. Watanabe, Coaggregation facilitates

interspecies hydrogen transfer between Pelotomaculum thermopropionicum and Methanothermobacter thermautotrophicus, Appl. Environ. Microbiol. 71 (2005) 7838-7845. http://doi.org/10.1128/aem.71.12.7838-7845.2005

[111] R. Nkuna, A. Roopnarain, R. Adeleke, Effects of organic loading rates on microbial communities and biogas production from water hyacinth: a case of mono- and co-digestion, J. Chem. Technol. Biotechnol. 94 (2019) 1294-1304. http://doi.org/https://doi.org/10.1002/jctb.5886

[112] R. Nkuna, A. Roopnarain, C. Rashama, R. Adeleke, Insights into organic loading rates of anaerobic digestion for biogas production: a review, Crit. Rev. Biotechnol. 42 (2022) 487-507. http://doi.org/10.1080/07388551.2021.1942778

[113] K. Kundu, S. Sharma, T.R. Sreekrishnan, Changes in microbial communities in a hybrid anaerobic reactor with organic loading rate and temperature, Bioresour. Technol. 129 (2013) 538-547. http://doi.org/10.1016/j.biortech.2012.11.118

[114] J. Antonio Magdalena, S. Greses, C. Gonzalez-Fernandez, Anaerobic degradation of protein-rich biomass in an UASB reactor: Organic loading rate effect on product output and microbial communities dynamics, J. Environ. Manage. 274 (2020). http://doi.org/10.1016/j.jenvman.2020.111201

[115] M. Sol Lisboa, S. Lansing, Characterizing food waste substrates for co-digestion through biochemical methane potential (BMP) experiments, Waste Manag. 33 (2013) 2664-2669. http://doi.org/10.1016/j.wasman.2013.09.004

[116] J. McKennedy, O. Sherlock, Anaerobic digestion of marine macroalgae: A review, Renewable Sustainable Energy Rev. 52 (2015) 1781-1790. http://doi.org/10.1016/j.rser.2015.07.101

[117] B.K. Ahring, A.A. Ibrahim, Z. Mladenovska, Effect of temperature increase from 55 to 65 degrees C on performance and microbial population dynamics of an anaerobic reactor treating cattle manure, Water Res. 35 (2001) 2446-2452. http://doi.org/10.1016/s0043-1354(00)00526-1

[118] Y. Chen, K. Xiao, X. Jiang, N. Shen, R.J. Zeng, Y. Zhou, Long solid retention time (SRT) has minor role in promoting methane production in a 65 degrees C single-stage anaerobic sludge digester, Bioresour. Technol. 247 (2018) 724-729. http://doi.org/10.1016/j.biortech.2017.09.153

[119] S. Kudo, D.I. Allasiw, K. Omi, M. Hansen, Translocal learning approach: A new form of collective learning for sustainability, Resour. Environ. Sustain. 2 (2020). http://doi.org/10.1016/j.resenv.2020.100009

[120] R. Salemdeeb, R. Saint, W. Clark, M. Lenaghan, K. Pratt, F. Millar, A pragmatic and industry-oriented framework for data quality assessment of environmental footprint tools, Resour. Environ. Sustain. 3 (2021). http://doi.org/10.1016/j.resenv.2021.100019

[121] G. Antonopoulou, M. Alexandropoulou, C. Lytras, G. Lyberatos, Modeling of Anaerobic Digestion of Food Industry Wastes in Different Bioreactor Types, Waste Biomass Valorization 6 (2015) 335-341. http://doi.org/10.1007/s12649-015-9362-7

[122] Y. Cai, D. Gallegos, Z. Zheng, W. Stinner, X. Wang, J. Proter, F. Schafer, Exploring the combined effect of total ammonia nitrogen, pH and temperature on anaerobic digestion of chicken manure using response surface methodology and two kinetic models, Bioresour. Technol. 337 (2021) 125328. http://doi.org/10.1016/j.biortech.2021.125328

[123] R.J. Frankin, W.A.A. Koevoets, W.M.A. Vangils, A. Vanderpas, Application of the Biobed(r) Upflow Fluidized-Bed Process for Anaerobic Wastewater Treatment, Water Sci. Technol. 25 (1992) 373-382. http://doi.org/10.2166/wst.1992.0169

[124] L.A. Nunez, B. Martinez, Anaerobic treatment of slaughterhouse wastewater in an Expanded Granular Sludge Bed (EGSB) reactor, Water Sci. Technol. 40 (1999) 99-106. http://doi.org/10.2166/wst.1999.0396

[125] W. Driessen, P. Yspeert, Anaerobic treatment of low, medium and high strength effluent in the agro-industry, Water Sci. Technol. 40 (1999) 221-228. http://doi.org/10.1016/s0273-1223(99)00629-0

[126] W.P. Barber, D.C. Stuckey, The use of the anaerobic baffled reactor (ABR) for wastewater treatment: A review, Water Res. 33 (1999) 1559-1578. http://doi.org/10.1016/s0043-1354(98)00371-6

[127] S.W. Sung, R.R. Dague, Laboratory Studies on the Anaerobic Sequencing Batch Reactor, Water Environ. Res. 67 (1995) 294-301. http://doi.org/10.2175/106143095x131501

[128] D.C. Stuckey, Recent developments in anaerobic membrane reactors, Bioresour. Technol. 122 (2012) 137-148. http://doi.org/10.1016/j.biortech.2012.05.138

[129] D. Cysneiros, C.J. Banks, S. Heaven, K.-A.G. Karatzas, The effect of pH control and 'hydraulic flush' on hydrolysis and Volatile Fatty Acids (VFA) production and profile in anaerobic leach bed reactors digesting a high solids content substrate, Bioresour. Technol. 123 (2012) 263-271. http://doi.org/10.1016/j.biortech.2012.06.060

[130] R. Jariyaboon, S. O-Thong, P. Kongjan, Bio-hydrogen and bio-methane potentials of skim latex serum in batch thermophilic two-stage anaerobic digestion, Bioresour. Technol. 198 (2015) 198-206. http://doi.org/10.1016/j.biortech.2015.09.006

[131] L. Andre, M. Ndiaye, M. Pernier, O. Lespinard, A. Pauss, E. Lamy, T. Ribeiro, Methane production improvement by modulation of solid phase immersion in dry batch anaerobic digestion process: Dynamic of methanogen populations, Bioresour. Technol. 207 (2016) 353-360. http://doi.org/10.1016/j.biortech.2016.02.033

[132] K. Wang, J. Yin, D. Shen, N. Li, Anaerobic digestion of food waste for volatile fatty acids (VFAs) production with different types of inoculum: Effect of pH, Bioresour. Technol. 161 (2014) 395-401. http://doi.org/10.1016/j.biortech.2014.03.088

[133] M. Peces, S. Astals, W.P. Clarke, P.D. Jensen, Semi-aerobic fermentation as a novel pre-treatment to obtain VFA and increase methane yield from primary sludge, Bioresour. Technol. 200 (2016) 631-638. http://doi.org/10.1016/j.biortech.2015.10.085

[134] A. Scoma, F. Varela-Corredor, L. Bertin, C. Gostoli, S. Bandini, Recovery of VFAs from anaerobic digestion of dephenolized Olive Mill Wastewaters by Electrodialysis, Sep. Purif. Technol. 159 (2016) 81-91. http://doi.org/10.1016/j.seppur.2015.12.029
[135] K.C. Wijekoon, C. Visvanathan, A. Abeynayaka, Effect of organic loading rate on VFA production, organic matter removal and microbial activity of a two-stage thermophilic anaerobic membrane bioreactor, Bioresour. Technol. 102 (2011) 5353-5360. http://doi.org/10.1016/j.biortech.2010.12.081
[136] S. Bengtsson, J. Hallquist, A. Werker, T. Welander, Acidogenic fermentation of industrial wastewaters: Effects of chemostat retention time and pH on volatile fatty acids production, Biochem. Eng. J. 40 (2008) 492-499. http://doi.org/10.1016/j.bej.2008.02.004
[137] J. Luo, L. Feng, W. Zhang, X. Li, H. Chen, D. Wang, Y. Chen, Improved production of short-chain fatty acids from waste activated sludge driven by carbohydrate addition in continuous-flow reactors: Influence of SRT and temperature, Appl. Energy 113 (2014) 51-58. http://doi.org/10.1016/j.apenergy.2013.07.006
[138] M.d.P. Anzola-Rojas, M. Zaiat, H. De Wever, Improvement of hydrogen production via ethanol-type fermentation in an anaerobic down-flow structured bed reactor, Bioresour. Technol. 202 (2016) 42-49. http://doi.org/10.1016/j.biortech.2015.11.084
[139] P. Intanoo, P. Rangsanvigit, P. Malakul, S. Chavadej, Optimization of separate hydrogen and methane production from cassava wastewater using two-stage upflow anaerobic sludge blanket reactor (UASB) system under thermophilic operation, Bioresour. Technol. 173 (2014) 256-265. http://doi.org/10.1016/j.biortech.2014.09.039
[140] G. Luo, L. Xie, Z. Zou, W. Wang, Q. Zhou, H. Shim, Anaerobic treatment of cassava stillage for hydrogen and methane production in continuously stirred tank reactor (CSTR) under high organic loading rate (OLR), Int. J. Hydrogen Energy 35 (2010) 11733-11737. http://doi.org/10.1016/j.ijhydene.2010.08.033
[141] C.-M. Liu, S.-Y. Wu, C.-Y. Chu, Y.-P. Chou, Biohydrogen production from rice straw hydrolyzate in a continuously external circulating bioreactor, Int. J. Hydrogen Energy 39 (2014) 19317-19322. http://doi.org/10.1016/j.ijhydene.2014.05.175
[142] O.A. Arikan, W. Mulbry, S. Lansing, Effect of temperature on methane production from field-scale anaerobic digesters treating dairy manure, Waste Manage. 43 (2015) 108-113. http://doi.org/10.1016/j.wasman.2015.06.005
[143] P. Intanoo, P. Chaimongkol, S. Chavadej, Hydrogen and methane production from cassava wastewater using two-stage upflow anaerobic sludge blanket reactors (UASB) with an emphasis on maximum hydrogen production, Int. J. Hydrogen Energy 41 (2016) 6107-6114. http://doi.org/10.1016/j.ijhydene.2015.10.125
[144] J. Zhong, D.K. Stevens, C.L. Hansen, Optimization of of anaerobic hydrogen and methane production from dairy processing waste using a two-stage digestion in

induced bed reactors (IBR), Int. J. Hydrogen Energy 40 (2015) 15470-15476. http://doi.org/10.1016/j.ijhydene.2015.09.085
[145] R. Pretel, B.D. Shoener, J. Ferrer, J.S. Guest, Navigating environmental, economic, and technological trade-offs in the design and operation of submerged anaerobic membrane bioreactors (AnMBRs), Water Res. 87 (2015) 531-541. http://doi.org/10.1016/j.watres.2015.07.002
[146] Y. Chen, J.J. Cheng, K.S. Creamer, Inhibition of anaerobic digestion process: A review, Bioresour. Technol. 99 (2008) 4044-4064. http://doi.org/10.1016/j.biortech.2007.01.057
[147] R. Rajagopal, D.I. Masse, G. Singh, A critical review on inhibition of anaerobic digestion process by excess ammonia, Bioresour. Technol. 143 (2013) 632-641. http://doi.org/10.1016/j.biortech.2013.06.030
[148] G. Lettinga, S. Rebac, G. Zeeman, Challenge of psychrophilic anaerobic wastewater treatment, Trends Biotechnol. 19 (2001) 363-370. http://doi.org/10.1016/s0167-7799(01)01701-2
[149] A.G. Brito, A.C. Rodrigues, L.F. Melo, Granulation during the start-up of a UASB reactor used in the treatment of low strength wastewaters, Biotechnol. Lett. 19 (1997) 363-367. http://doi.org/10.1023/a:1018359102949
[150] O. Yenigun, B. Demirel, Ammonia inhibition in anaerobic digestion: A review, Process Biochem. 48 (2013) 901-911. http://doi.org/10.1016/j.procbio.2013.04.012
[151] C.-Y. Lin, W.S. Chai, C.-H. Lay, C.-C. Chen, C.-Y. Lee, P.L. Show, Optimization of Hydrolysis-Acidogenesis Phase of Swine Manure for Biogas Production Using Two-Stage Anaerobic Fermentation, Processes 9 (2021). http://doi.org/10.3390/pr9081324
[152] W. Wang, J.S. Chang, D.J. Lee, Integrating anaerobic digestion with bioelectrochemical system for performance enhancement: A mini review, Bioresour. Technol. 345 (2022). http://doi.org/10.1016/j.biortech.2021.126519
[153] T.H. Pham, K. Rabaey, P. Aelterman, P. Clauwaert, L. De Schamphelaire, N. Boon, W. Verstraete, Microbial fuel cells in relation to conventional anaerobic digestion technology, Eng. Life Sci. 6 (2006) 285-292. http://doi.org/10.1002/elsc.200620121
[154] B. Tartakovsky, P. Mehta, J.S. Bourque, S.R. Guiot, Electrolysis-enhanced anaerobic digestion of wastewater, Bioresour. Technol. 102 (2011) 5685-5691. http://doi.org/10.1016/j.biortech.2011.02.097
[155] R. Zou, G.F. Zhu, J.A. Kumar, C.X. Liu, X. Huang, L. Liu, Hydrogen and methane production in a bio-electrochemical system assisted anaerobic baffled reactor, Int. J. Hydrogen Energy 39 (2014) 13498-13504. http://doi.org/10.1016/j.ijhydene.2014.02.086
[156] C. Zamalloa, J.B.A. Arends, N. Boon, W. Verstraete, Performance of a lab-scale bio-electrochemical assisted septic tank for the anaerobic treatment of black, New Biotechnol. 30 (2013) 573-580. http://doi.org/10.1016/j.nbt.2013.01.009

[157] N. Belay, R. Sparling, L. Daniels, Relationship of formate to growth and methanogenesis by Methanococcus thermolithotrophicus, Appl. Environ. Microbiol. 52 (1986) 1080-1085. http://doi.org/10.1128/aem.52.5.1080-1085.1986
[158] M. Tabatabaei, M. Aghbashlo, E. Valijanian, H.K.S. Panahi, A.-S. Nizami, H. Ghanavati, A. Sulaiman, S. Mirmohamadsadeghi, K. Karimi, A comprehensive review on recent biological innovations to improve biogas production, Part 1: Upstream strategies, Renewable Energy 146 (2020) 1204-1220. http://doi.org/10.1016/j.renene.2019.07.037
[159] H. Wang, M. Vuorela, A.L. Keranen, T.M. Lehtinen, A. Lensu, A. Lehtomaki, J. Rintala, Development of microbial populations in the anaerobic hydrolysis of grass silage for methane production, FEMS Microbiol. Ecol. 72 (2010) 496-506. http://doi.org/10.1111/j.1574-6941.2010.00850.x
[160] X. Duan, Y. Chen, L. Feng, Q. Zhou, Metagenomic analysis reveals nonylphenol-shaped acidification and methanogenesis during sludge anaerobic digestion, Water Res. 196 (2021). http://doi.org/10.1016/j.watres.2021.117004
[161] C.M. Huban, R.D. Plowman, Bioaugmentation: Put microbes to work, Chem. Eng. 104 (1997).
[162] S.V. Mohan, C. Falkentoft, Y.V. Nancharaiah, B.S.M. Sturm, P. Wattiau, P.A. Wilderer, S. Wuertz, M. Hausner, Bioaugmentation of microbial communities in laboratory and pilot scale sequencing batch biofilm reactors using the TOL plasmid, Bioresour. Technol. 100 (2009) 1746-1753. http://doi.org/10.1016/j.biortech.2008.09.048
[163] F. Maqbool, R. Kamal, Z.A. Bhatti, S. Pervez, M. Sajid, K. Haleem, F. Faridullah, Effects of hydrocarbon degrading inoculum for carwash effluent treatment in a UASB reactor, Desalin. Water Treat. 164 (2019) 31-38. http://doi.org/10.5004/dwt.2019.24396
[164] W.C. Fuqua, S.C. Winans, E.P. Greenberg, Quorum Sensing in Bacteria – the Luxr-Luxi Family of Cell Density-Responsive Transcriptional Regulators, J. Bacteriol. 176 (1994) 269-275. http://doi.org/10.1128/jb.176.2.269-275.1994
[165] M. Schuster, D.J. Sexton, S.P. Diggle, E.P. Greenberg, Acyl-Homoserine Lactone Quorum Sensing: From Evolution to Application, in: S. Gottesman (Ed.) Annu. Rev. Microbiol.2013, pp. 43-63.
[166] M.B. Miller, B.L. Bassler, Quorum sensing in bacteria, Annu. Rev. Microbiol. 55 (2001) 165-199. http://doi.org/10.1146/annurev.micro.55.1.165
[167] B.L. Bassler, M. Wright, M.R. Silverman, Multiple Signaling Systems Controlling Expression of Luminescence in Vibrio-Harveyi-Sequence and Function of Genes Encoding a 2nd sensory Pathway, Mol. Microbiol. 13 (1994) 273-286. http://doi.org/10.1111/j.1365-2958.1994.tb00422.x
[168] K.L. Visick, J. Foster, J. Doino, M. McFall-Ngai, E.G. Ruby, Vibrio fischeri lux genes play an important role in colonization and development of the host light organ, J. Bacteriol. 182 (2000) 4578-4586. http://doi.org/10.1128/jb.182.16.4578-4586.2000

[169] A.M.L. Barnard, S.D. Bowden, T. Burr, S.J. Coulthurst, R.E. Monson, G.P.C. Salmond, Quorum sensing, virulence and secondary metabolite production in plant soft-rotting bacteria, Philos. T. R. Soc. B. 362 (2007) 1165-1183. http://doi.org/10.1098/rstb.2007.2042
[170] L. Yarmolinsky, M. Bronstein, J. Gorelick, Review: Inhibition of bacterial quorum sensing by plant extracts, Isr. J. Plant Sci. 62 (2015) 294-297. http://doi.org/10.1080/07929978.2015.1067076
[171] D.G. Davies, M.R. Parsek, J.P. Pearson, B.H. Iglewski, J.W. Costerton, E.P. Greenberg, The involvement of cell-to-cell signals in the development of a bacterial biofilm, Science 280 (1998) 295-298. http://doi.org/10.1126/science.280.5361.295
[172] T.R. de Kievit, B.H. Iglewski, Bacterial quorum sensing in pathogenic relationships, Infect. Immun. 68 (2000) 4839-4849. http://doi.org/10.1128/iai.68.9.4839-4849.2000
[173] Z.-C. Zhao, G.-J. Xie, B.-F. Liu, D.-F. Xing, J. Ding, H.-J. Han, N.-Q. Ren, A review of quorum sensing improving partial nitritation-anammox process: Functions, mechanisms and prospects, Sci. Total Environ. 765 (2021) 142703-142703. http://doi.org/10.1016/j.scitotenv.2020.142703
[174] B.L. Hanzelka, E.P. Greenberg, Quorum sensing in Vibrio fischeri: Evidence that S-adenosylmethionine is the amino acid substrate for autoinducer synthesis, J. Bacteriol. 178 (1996) 5291-5294. http://doi.org/10.1128/jb.178.17.5291-5294.1996
[175] W.T. Watson, T.D. Minogue, D.L. Val, S.B. von Bodman, M.E.A. Churchill, Structural basis and specificity of acyl-homoserine lactone signal production in bacterial quorum sensing, Mol. Cell 9 (2002) 685-694. http://doi.org/10.1016/s1097-2765(02)00480-x
[176] W.R.J.D. Galloway, J.T. Hodgkinson, S.D. Bowden, M. Welch, D.R. Spring, Quorum Sensing in Gram-Negative Bacteria: Small-Molecule Modulation of AHL and AI-2 Quorum Sensing Pathways, Chem. Rev. 111 (2011) 28-67. http://doi.org/10.1021/cr100109t
[177] S. Swift, J.A. Downie, N.A. Whitehead, A.M.L. Barnard, G.P.C. Salmond, P. Williams, Quorum sensing as a population-density-dependent determinant of bacterial physiology, in: R.K. Poole (Ed.) Adv. Microb. Physiol.2001, pp. 199-270.
[178] A. Fekete, M. Frommberger, M. Rothballer, X. Li, M. Englmann, J. Fekete, A. Hartmann, L. Eberl, P. Schmitt-Kopplin, Identification of bacterial N-acylhomoserine lactones (AHLs) with a combination of ultra-performance liquid chromatography (UPLC), ultra-high-resolution mass spectrometry, and in-situ biosensors, Anal. Bioanal. Chem. 387 (2007) 455-467. http://doi.org/10.1007/s00216-006-0970-8
[179] C.S. Pereira, J.A. Thompson, K.B. Xavier, AI-2-mediated signalling in bacteria, FEMS Microbiol. Rev. 37 (2013) 156-181. http://doi.org/10.1111/j.1574-6976.2012.00345.x

[180] C.M. Waters, B.L. Bassler, Quorum sensing: Cell-to-cell communication in bacteria, Annu. Rev. Cell Dev. Biol.2005, pp. 319-346.
[181] H. Hu, F. Luo, Y. Liu, X. Zeng, Function of quorum sensing and cell signaling in wastewater treatment systems, Water Sci. Technol. 83 (2021) 515-531. http://doi.org/10.2166/wst.2020.601
[182] L. Chernin, N. Toklikishvili, M. Ovadis, S. Kim, J. Ben-Ari, I. Khmel, A. Vainstein, Quorum-sensing quenching by rhizobacterial volatiles, Environ. Microbiol. Rep. 3 (2011) 698-704. http://doi.org/10.1111/j.1758-2229.2011.00284.x
[183] S. Subramoni, V. Venturi, LuxR-family 'solos': bachelor sensors/regulators of signalling molecules, Microbiology (Reading) 155 (2009) 1377-1385. http://doi.org/10.1099/mic.0.026849-0
[184] Y.-C. Yong, J.-J. Zhong, Regulation of aromatics biodegradation by rhl quorum sensing system through induction of catechol meta-cleavage pathway, Bioresour. Technol. 136 (2013) 761-765. http://doi.org/10.1016/j.biortech.2013.03.134
[185] C.H. Tan, K.S. Koh, C. Xie, M. Tay, Y. Zhou, R. Williams, W.J. Ng, S.A. Rice, S. Kjelleberg, The role of quorum sensing signalling in EPS production and the assembly of a sludge community into aerobic granules, ISME J. 8 (2014) 1186-1197. http://doi.org/10.1038/ismej.2013.240
[186] G. Chong, O. Kimyon, S.A. Rice, S. Kjelleberg, M. Manefield, The presence and role of bacterial quorum sensing in activated sludge, Microb. Biotechnol. 5 (2012) 621-633. http://doi.org/10.1111/j.1751-7915.2012.00348.x
[187] Y.-C. Yong, J.-J. Zhong, N-Acylated homoserine lactone production and involvement in the biodegradation of aromatics by an environmental isolate of Pseudomonas aeruginosa, Process Biochem. 45 (2010) 1944-1948. http://doi.org/10.1016/j.procbio.2010.05.006
[188] H. Feng, Y. Ding, M. Wang, G. Zhou, X. Zheng, H. He, X. Zhang, D. Shen, J. Shentu, Where are signal molecules likely to be located in anaerobic granular sludge?, Water Res. 50 (2014) 1-9. http://doi.org/10.1016/j.watres.2013.11.021
[189] Y.P. Zhang, J.Z. Li, F.Q. Liu, H. Yan, J.L. Li, X. Zhang, A.K. Jha, Specific quorum sensing signal molecules inducing the social behaviors of microbial populations in anaerobic digestion, Bioresour. Technol. 273 (2019) 185-195. http://doi.org/10.1016/j.biortech.2018.11.022
[190] L.Y. Li, M.Y. Zheng, H.L. Ma, S.F. Gong, G.M. Ai, X.L. Liu, J. Li, K.J. Wang, X.Z. Dong, Significant performance enhancement of a UASB reactor by using acyl homoserine lactones to facilitate the long filaments of Methanosaeta harundinacea 6Ac, Appl. Microbiol. Biotechnol. 99 (2015) 6471-6480. http://doi.org/10.1007/s00253-015-6478-4
[191] L. Lv, W. Li, Z. Zheng, D. Li, N. Zhang, Exogenous acyl-homoserine lactones adjust community structures of bacteria and methanogens to ameliorate the performance of anaerobic granular sludge, J. Hazard. Mater. 354 (2018) 72-80. http://doi.org/10.1016/j.jhazmat.2018.04.075
[192] Q. Du, Q.H. Mu, G.X. Wu, Metagenomic and bioanalytical insights into quorum

sensing of methanogens in anaerobic digestion systems with or without the addition of conductive filter, Sci. Total Environ. 763 (2021). http://doi.org/10.1016/j.scitotenv.2020.144509

[193] M. Lim, D. Patureau, M. Heran, G. Lesage, J. Kim, Removal of organic micropollutants in anaerobic membrane bioreactors in wastewater treatment: critical review, Environ. Sci. Water Res. Technol. 6 (2020) 1230-1243. http://doi.org/10.1039/c9ew01058k

Table 1 The reactions for substrates degradation and conversion to methane in anaerobic digestion

Substrates	Reactions	$\Delta G^{0'}$ (kJ/mol)
Propionate	$CH_3CH_2COOH + H_2O \rightarrow 2CH_3COOH + 3H_2 + CO_2$	+76.1
Butyrate	$CH_3CH_2CH_2COOH + 2H_2O \rightarrow 2CH_3COOH + 2H_2$	+48.1
Lactate	$CH_3CH_2OH + H_2O \rightarrow CH_3COOH + 2H_2$	-4.2
Ethanol	$CH_3CH_2OH + H_2O \rightarrow CH_3COOH + 2H_2$	+9.6
Formate	$4HCOOH \rightarrow CH_4 + 3CO_2 + 2H_2O$	-130
Acetate	$CH_3COOH \rightarrow CH_4 + CO_2$	-33
Hydrogen	$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$	-135

1443 **Table 2** Summary of properties of the main methanogens

Methanogens	**Methanogenic substrates**	**Temperatures**	**Typical habits**
Methanobacterium	H_2, (formate)	37~45℃	Anaerobic digestors, freshwater sediments, marshy soils, rumen
Methanobrevibacter	H_2, formate	37~40℃	Animal gastrointestinal tracts, anaerobic digestors, rice paddies, decaying woody tissues
Methanosphaera	H_2 + methanol	37℃	Animal gastrointestinal tracts
Methanothermobacter	H_2 or formate	55~65℃	Anaerobic digestors
Methanothermus	H_2	80~88℃	Hot springs
Methanococcus	H_2, formate	35~40℃	Marine sediments
Methanothermococcus	H_2, formate	60~65℃	Marine geothermal sediments
Methanocaldococcus	H_2	80~85℃	Marine geothermal sediments
Methanotorris	H_2	88℃	Marine geothermal sediments
Methanomicrobium	H_2, formate	40℃	Anaerobic digestors, groundwater, rumen
Methanoculleus	H_2, formate	20~55℃	Anaerobic digestors, marine sediments, freshwater sediments, rice paddies, oil fields, hot springs
Methanofollis	H_2, formate	37~40℃	Anaerobic digestors
Methanogenium	H_2, formate	15~57℃	Marine sediments, freshwater sediments, rice paddies, animal gastrointestinal tracts
Methanolacinia	H_2	40℃	Marine sediments
Methanoplanus	H_2, formate	32~40℃	Oil fields
Methanospirillum	H_2, formate	30~37℃	Anaerobic digestors, marine sediments
Methanocorpusculum	H_2, formate	30~40℃	Anaerobic digestors, freshwater sediments
Methanocalculus	H_2, formate	30~40℃	Oil fields
Methanosarcina	(H_2), $MeNH_2$,	35~60℃	Anaerobic digestors,

	Acetate		marine sediments, freshwater sediments, rumen
Methanococcoides	$MeNH_2$	23~35℃	Marine sediments
Methanohalobium	$MeNH_2$	40~55℃	Hypersaline sediments
Methanohalophilus	$MeNH_2$	35~40℃	Hypersaline sediments
Methanolobus	$MeNH_2$	37℃	Hypersaline sediments
Methanomethylovorans	$MeNH_2$	20~50℃	Freshwater sediments, anaerobic digestors
Methanimicrococcus	H_2 + $MeNH_2$	39℃	Animal gastrointestinal tracts
Methanosalsum	$MeNH_2$	35~45℃	Hypersaline sediments
Methanosaeta	Acetate	35~60℃	Anaerobic digestors, freshwater sediments
Methanopyrus	H_2	98℃	Marine geothermal sediments

1448 **Table 3** Overview of the syntrophic community in methanogenic system

Bacteria	**Methanogens**	**Substrates**
S organism	*Methanobacterium bryantii*	Ethanol, rumen fluid
Desulfovibrio vulgaris	*Methanobacterium formicicum*	Lactate, ethanol
Syntrophomonas wolfei	*Methanospirillum hungatei, Methanobacterium arbophilieum*	Butyrate
Desulfovibrio sp.	*Methanosarcina barkeri*	Lactate
Syntrophus buswellii	*Methanospirillum hungatei, Desulfovibrio sp.*	Benzoate
Syntrophospora bryantii	*Methanospirillum hungatei, Desulfovibrio sp.*	Fatty acids with 4-11 carbon atoms
Pelobacter carbinolicus, Pelobacter propionicus	*Methanospirillum hungatei*	Primary alcohols/diols
Acidogenic bacteria (Butyribacterium methylotrophicum, Sporomusa ovata, Acetobacterium woodii)	*Desulfovibrio vulgaris, Methanobrevibacter arboriphilus strain AZ*	Methanol
Sporomusa acidovorans	*Methanospirillum hungatei, Desulfovibrio vulgaris G6, Methanobacterium formicicum*	Methanol
Hyperthemophilic sulfidogens Thermococcus celer, Thermococcus stetteri, Staphylothermus marinus, and Pyrococcus furiosus	*Methanococcus thermolithotrophicus or Methanococcus jannaschii*	Starch, peptone
Pelotomaculum schinkii	*Methanospirillum hungatei*	Propionate
Pelotomaculum propionicicum	*Methanospirillum hungatei, Methanothermobacter thermautotrophicus*	Propionate
Desulfovibrio vulgaris	*Methanococcus maripaludis*	Lactate
Desulfovibrio alaskensis G20	*Methanosprillum hungatei JF-1*	Lactate
Desulfovibrio vulgaris Hidenborough	*Methanococcus maripaludis*	Lactate

Pelobacter carbinolicus	*Geobacter sulfurreducens*	Ethanol
Syntrophomonas wolfei	*Methanospirillum hungatei*	Butyrate
Clostridium sporogene	*Methanobacterium formicicum* *Methanosarcina barkeri*	Isoleucine
Desulfovibrio vulgaris	*Methanobacterium formicicum*	Ethanol, lactate
Desulfovibrio alaskensis G20	*Methanococcus maripaludis*	Lactate
Syntrophomonas wolfei	*Methanobacterium formicicum*	Butyrate
MPOB(Syntrophobacter fumaroxidans)	*Methanospirillum hungatei*, *Methanobacterium formicium*	Propionate
Syntrophospora bryanti	*Methanospirillum hungatei*, *Methanobacterium formicicum*	Butyrate
Themacetogenium phaeum	*Methanothermobacter thermautotrophicus*	Acetate
Syntrophomonas wolfei	*Methanospirillum hungatei*	Butyrate, crotonate
Syntrophus aciditrophocus	*Methanospirillum hungatei*	Benzoate,cyclohexane-1-carboxylate

1449

1450

1451

Figure Captions

Fig. 1 Process of anaerobic digestion

Fig. 2 The mechanisms of interspecies H_2 or formate transfer.

Fig. 3 Syntrophic microorganisms involved in interspecies hydrogen and formate transfers.

Fig. 4 The different mechanisms of three direct interspecies electron transfers.

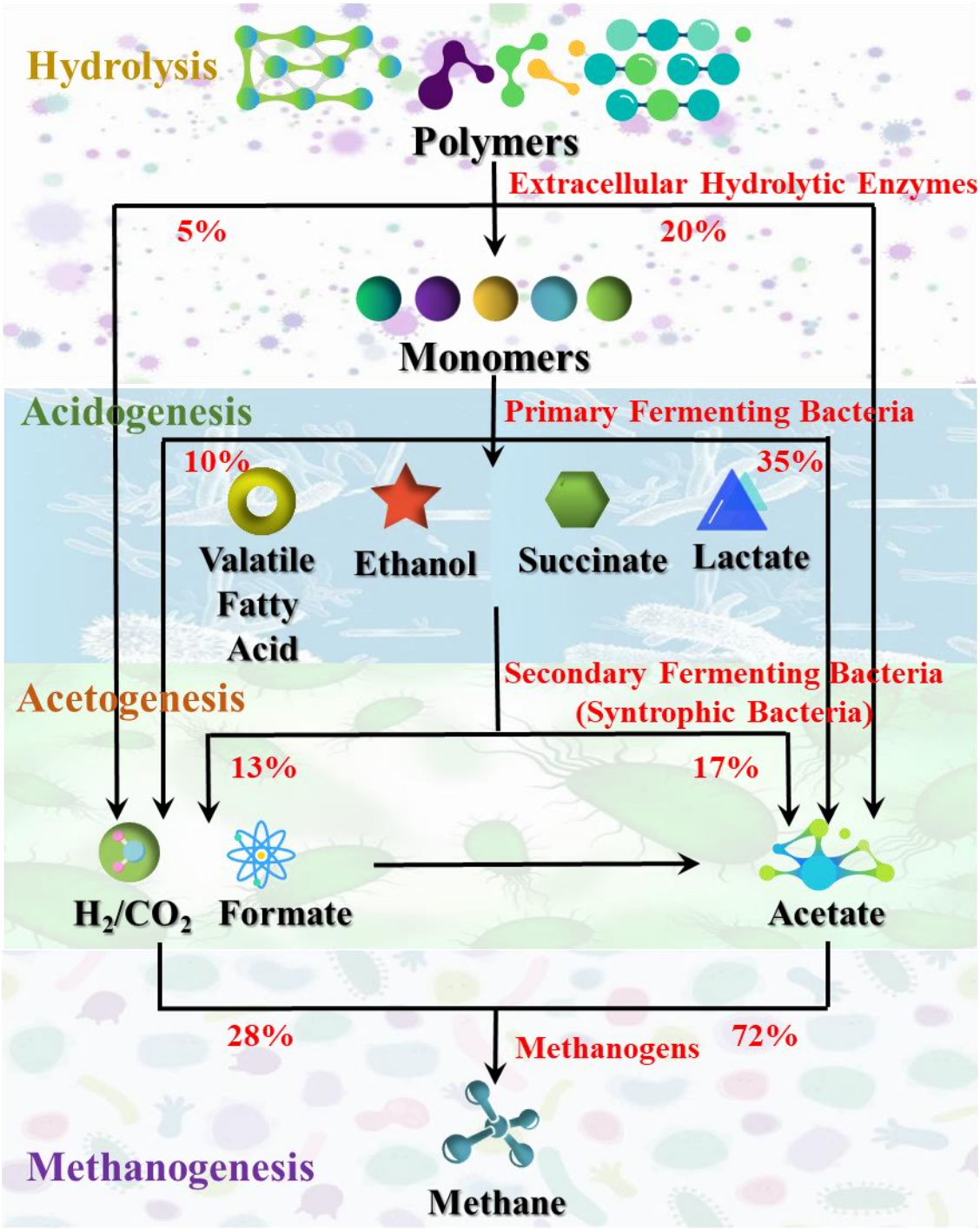
Hydrolysis
Polymers
Extracellular Hydrolytic Enzymes
5%
20%
Monomers
Acidogenesis
Primary Fermenting Bacteria
10%
35%
Valatile Fatty Acid
Ethanol
Succinate
Lactate
Acetogenesis
Secondary Fermenting Bacteria (Syntrophic Bacteria)
13%
17%
H2/CO2
Formate
Acetate
28%
Methanogens
72%
Methanogenesis
Methane

Fig.1

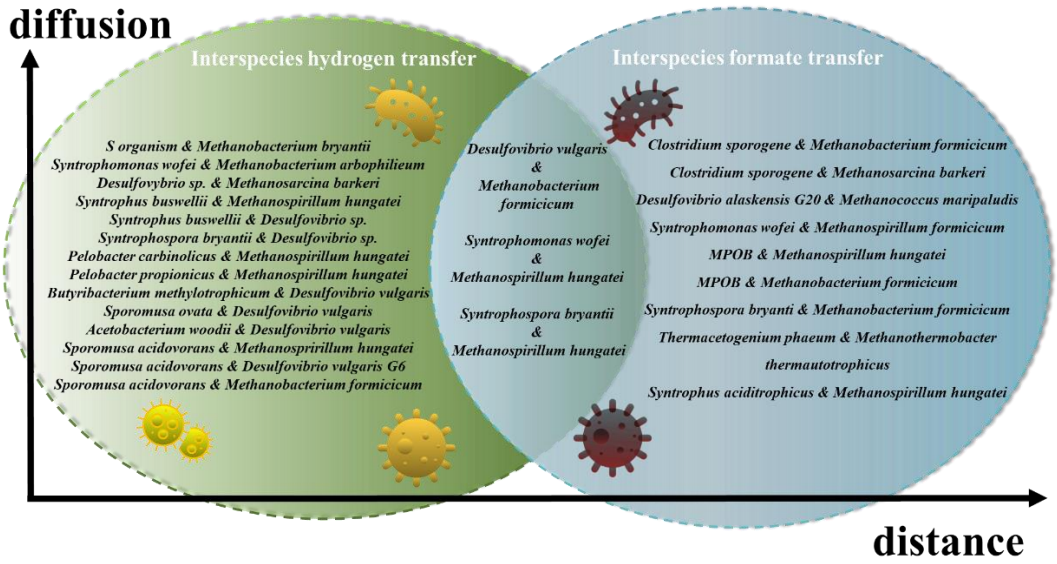
diffusion
Interspecies hydrogen transfer
S organism & Methanobacterium bryantii
Syntrophomonas wofei & Methanobacterium arbophilieum
Desulfovybrio sp. & Methanosarcina barkeri
Syntrophus buswellii & Methanospirillum hungatei
Syntrophus buswellii & Desulfovibrio sp.
Syntrophospora bryantii & Desulfovibrio sp.
Pelobacter carbinolicus & Methanospirillum hungatei
Pelobacter propionicus & Methanospirillum hungatei
Butyribacterium methylotrophicum & Desulfovibrio vulgaris
Sporomusa ovata & Desulfovibrio vulgaris
Acetobacterium woodii & Desulfovibrio vulgaris
Sporomusa acidovorans & Methanospririllum hungatei
Sporomusa acidovorans & Desulfovibrio vulgaris G6
Sporomusa acidovorans & Methanobacterium formicicum
Desulfovibrio vulgaris & Methanobacterium formicicum
Syntrophomonas wofei & Methanospirillum hungatei
Syntrophospora bryantii & Methanospirillum hungatei
Interspecies formate transfer
Clostridium sporogene & Methanobacterium formicicum
Clostridium sporogene & Methanosarcina barkeri
Desulfovibrio alaskensis G20 & Methanococcus maripaludis
Syntrophomonas wofei & Methanospirillum formicicum
MPOB & Methanospirillum hungatei
MPOB & Methanobacterium formicicum
Syntrophospora bryanti & Methanobacterium formicicum
Thermacetogenium phaeum & Methanothermobacter thermautotrophicus
Syntrophus aciditrophicus & Methanospirillum hungatei
distance

Fig. 2

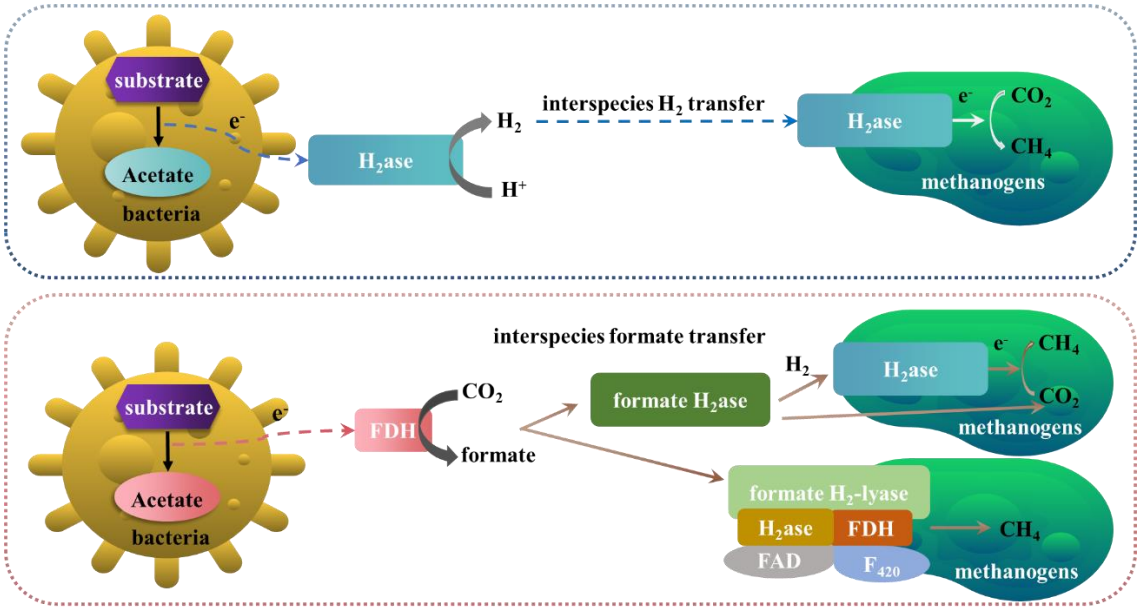
substrate
e⁻
Acetate
bacteria
H₂ase
H₂
H⁺
interspecies H₂ transfer
H₂ase
e⁻
CO₂
CH₄
methanogens
interspecies formate transfer
substrate
e⁻
Acetate
bacteria
FDH
CO₂
formate
formate H₂ase
H₂
H₂ase
e⁻
CH₄
CO₂
methanogens
formate H₂-lyase
H₂ase
FDH
FAD
F₄₂₀
CH₄
methanogens

Fig. 3

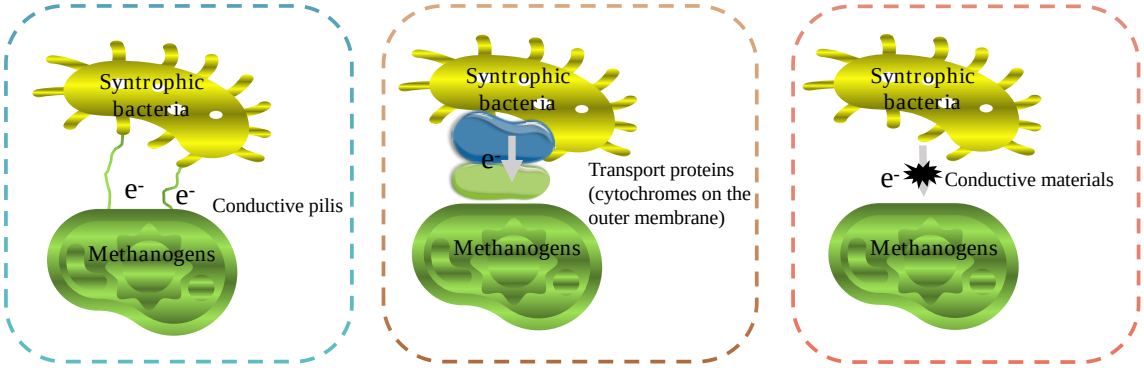
Syntrophic bacteria
e-
e-
Conductive pilis
Methanogens
Syntrophic bacteria
e-
Transport proteins (cytochromes on the outer membrane)
Methanogens
Syntrophic bacteria
e-
Conductive materials
Methanogens

Fig. 4