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**Anaerobic digestion of sludge filtrate assisted by symbionts of short chain fatty acid-oxidation
syntrophs and exoelectrogens: process performance, methane yield and microbial community**

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17 **Abstract:** Sludge filtrate is a kind of special organic wastewater generated from
18 hydrothermally pretreated sewage sludge. The efficient treatment of sludge filtrate can promote the
19 development of sludge recycling technology. Herein, the anaerobic baffled reactor (ABR) assisted
20 by symbionts of short chain fatty acid-oxidation syntrophs (SFAS) and exoelectrogens was applied
21 to treat the sludge filtrate. The influence of fermentation temperature and promotion of
22 methanogenesis via symbionts were focused. The results showed that the COD removal efficiency
23 and methane yield of the ABR system assisted by symbionts at 35 °C (R3) were 11.7% and 11.0%
24 higher than the one at 55 °C (R2), respectively. And the COD removal efficiency and methane yield
25 of the R2 system were 9.1% and 12.9% higher than the traditional ABR system at 55 °C (R1),
26 respectively. Large abundances of exoelectrogens such as *Thermincola* and *Geobacter* were found
27 in the R2 and R3 systems, respectively. Moreover, ample *Syntrophobacter*, *Syntrophomonas* and
28 *Methanobacterium* were detected in both R2 and R3 systems. The present research revealed the
29 importance of SFAS, exoelectrogens and hydrogenotrophic methanogens for the improvement of
30 methanogenesis. Besides, the mesophilic condition is conducive to enhancing the methanogenesis
31 rate of sludge filtrate.

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33 **Key words:** Sludge filtrate; Anaerobic digestion; SFAS; Exoelectrogens; Methanogenesis

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1. Introduction

With development of economics and improvement of living standard, large amount of sewage sludge is produced as byproduct from treatment of wastewater. The yield of sewage sludge reached 34 million tons (80% moisture content) in China in 2015 and more than 80% was disposed by improper dumping [1-3]. The main factor limiting treatment of sewage sludge is its hard dewatering, resulted from special properties such as hydrophilic nature and structural stability [4]. In terms of different technologies for dewaterability enhancement of sewage sludge, hydrothermal pretreatment presents obvious advantages due to its high efficiency for subsequent dehydration [5]. It was also proved and applied from lab-scale study to pilot-scale verification in our previous study, in which the moisture content of sewage sludge clearly reduced from 85 to 35% after hydrothermal pretreatment [6]. Nevertheless, the treatment of generated filtrate with high concentration of organic matters is becoming a new problem. The traditional anaerobic technology was applied to dispose of this special wastewater with attached product of biogas. Li et al. [7] treated similar sludge filtrate by a pilot-scale UASB, and the COD removal rate reached 75% when inlet COD was 38400 mg·L⁻¹ with HRT of 12 days. In the study of Wirth [8], the COD removal efficiency was 68-75% and methane production rate reached 180 mL/g COD_{removed} with HRT and OLR of 5 days and 5 kg COD m⁻³d⁻¹, respectively. Therefore, the COD removal efficiency and methane yield need to be further improved for deeper treatment and utilization of sludge filtrate. So, assigned technology should be considered for higher energy recovery from anaerobic digestion of sludge filtrate. In addition, higher energy recovery can offset the energy consumption in hydrothermal pretreatment.

In view of these challenges in traditional anaerobic digestion (AD) of sludge filtrate, which are

generally caused by accumulation of volatile fatty acids (VFAs) such as propionic, butyric and acetic acids [7-9], the bioelectrochemical technology could be one of the effective ways to solve it and thereby improve methane production since: (1) acetic acid, one of the easy accumulated VFAs, is the favorable substrate for exoelectrogens with wider ecological niches and shorter generation time than the acetoclastic methanogens [10,11]; (2) the physical electrodes can provide durable support for the attachment of functional microbes; (3) microbial electrolysis cells (MEC), one of typical bioelectrochemical technology, is in favor of methane production [12]. Nevertheless, propionic and butyric acids cannot be used efficiently by exoelectrogens [13]. Furthermore, 20-43% of methane produced in an anaerobic system is gradually converted from propionic and butyric acids [14]. The oxidation of propionic and butyric acids depends on the metabolism of SFAS [15]. As the metabolic intermediate of propionic and butyric acids is acetic acid [16], it is a feasible way to remove propionic and butyric acids by decomposing them into acetic acid and degrading acetic acid simultaneously. It can be achieved by enrichment of SFAS and exoelectrogens in the AD system via introduction of MEC, since the propionic and butyric acids can be degraded to acetic acid by SFAS and further utilized by exoelectrogens. Although a large amount of effort has been done for exoelectrogens and the MEC system [17-18], there is little focus on the metabolism of VFAs by the symbionts of SFAS and exoelectrogens. Moreover, rare work has been done on treatment of sludge filtrate by the AD system assisted by symbionts of SFAS and exoelectrogens.

Compared to traditional single-phase anaerobic reactor such as continuous stirred tank reactors (CSTR) and up-flow anaerobic sludge bed (UASB), the anaerobic baffled reactor (ABR) presents better performance on methane production by separating acid-producing and

methane-producing phases to facilitate the best niches of different microorganisms [19]. Moreover, it provides a favorable structural support for coupling with microbial electrolysis in an integral reactor due to its baffled compartment [20]. Therefore, the ABR assisted by symbionts of SFAS and exoelectrogens was used to treat the sludge filtrate. Through this study, the enhanced treatment efficiency due to symbionts of SFAS and exoelectrogens and also the optimal control parameters of system can be achieved. The effect of MEC for enriching SFAS and exoelectrogens in the AD of sludge filtrate and the influence of fermentation temperature on the microbial community can be clear. These results are expected to guide the operation of subsequent pilot-scale system. This study aims to obtain a feasible strategy for effective utilization of VFAs in the AD of sludge filtrate for effective methanogenesis.

2. Materials and methods

2.1. Substrate and inoculum

The fresh sludge filtrate was obtained from a pilot-scale dewatering system in Xiamen, China, and stored at 4 °C. In this dewatering system, the raw sewage sludge was hydrothermally pretreated under 180 °C for 30 min, and then cooled to 60 °C for subsequent filter pressing. After that, the filter cake and filtrate were used for biochar and biogas generation via pyrolysis and anaerobic digestion, respectively. More detailed information of typical procedure was described in our previous study [6]. The COD concentration of sludge filtrate in the experiment was $12.5 \pm 0.4 \text{ g}\cdot\text{L}^{-1}$ with pH value and alkalinity of 7.21 ± 0.30 and $2620 \pm 46 \text{ mg CaCO}_3/\text{L}$, respectively. And the $\text{NH}_4^+\text{-N}$ concentration was $1544 \pm 27 \text{ mg}\cdot\text{L}^{-1}$. The BOD/COD rate of sludge filtrate was 0.54 ± 0.02 .

100 The inoculum in the five compartments of ABR systems (R1, R2, R3, Fig.1) owned the same
101 suspended solid (SS) and volatile suspended solids (VSS) with $23.15 \pm 0.12 \text{ g}\cdot\text{L}^{-1}$ and 12.50 ± 0.12
102 $\text{g}\cdot\text{L}^{-1}$, respectively. The inoculum of R1 and R2 systems were collected from an anaerobic digester
103 for sludge filtrate treatment at 55°C , with addition of 5% (V:V) mixed sludge containing
104 thermophilic SFAS and exoelectrogens. The inoculum of R3 was obtained from an anaerobic
105 digester at 35°C for treatment of sludge filtrate, with addition of 5% (V:V) mixed sludge containing
106 mesophilic SFAS and exoelectrogens. The thermophilic sludge used to inoculate R1 and R2 systems
107 was originally derived from the same mesophilic anaerobic digester used to inoculate R3 system,
108 and then domesticated for a long time on thermophilic condition. Using the thermophilic inoculum
109 for R1 and R2 systems at 55°C can shorten the start-up time and achieve stable operation as soon
110 as possible. On the premise of same biomass of inoculum in the three systems, advance acclimation
111 of thermophilic inoculum for the R1 and R2 systems does not affect the decisive influence of
112 temperature on the composition of microorganisms [21]. More detail information of typical
113 procedure for enriching SFAS and exoelectrogens was described in our previous study [22]. The
114 addition of mixed sludge containing SFAS and exoelectrogens aims to accelerate the enrichment of
115 their symbionts after coupling MEC with the ABR system.

116 **Fig.1** Schematic diagram of the adopted experimental apparatus for anaerobic digestion

117 **2.2. Experimental setup and operation**

118 The ABR was made of plexiglass and its working volume was 2.5 L. The anodes and cathodes
119 were fixed in the up-flow region of Compartment 2nd-4th (C2-C4) to construct MECs part in the
120 ABR system (Fig.1). The electrodes in the R1 system was open circuit while the electrodes in the

121 R2 and R3 [systems](#) were close circuit by connecting with external voltage (0.9V). The coupling of
122 MECs in the R2 and R3 systems aimed to enrich the symbionts of SFAS and exoelectrogens to
123 accelerate the degradation of organics and methane production from sludge filtrate.

124 Carbon cloth (WOS1009, CeTech, China) was used as electrodes with size of 54.00 cm². The
125 three systems (R1, R2, R3) shared the same substrate tank via different outlets. Each system
126 contained a temperature control device, a gas measuring unit (LML-1, Changchun Automobile
127 Filter, China), inlets and outlets. The temperature of reactors was assured by water jacket
128 connecting to heaters. Each system was equipped with a submerged thermometer to indicate the
129 temperature. The substrate tank was kept at 4 °C by connecting to a cooler. The filtrate was fed by
130 peristaltic pumps (BT100-2i, Longer Pump, China) and the three systems were operated
131 simultaneously. [The R1 and R2 systems were operated at 55°C while the R3 system was run at](#)
132 [35°C](#). A start-up stage was operated in the three systems to make the microorganism adaptable. The
133 start-up stage lasted for 60 days with COD concentration of 2-5 g·L⁻¹. After a successful start-up,
134 the influent COD increased to 12.5 ± 0.4 g·L⁻¹ at HRT of 6 days for another 120 days. The control
135 parameters and performances of [the](#) three systems [during](#) last 60 days are shown in [Table 1](#).

136 **Table 1** Control parameters and process performances of the R1, R2 and R3 systems

137 ***2.3. Sampling and analytical methods***

138 Substrate and effluent samples were taken from the sampling ports and their chemical
139 analyses such as pH, [oxidation reduction potential \(ORP\)](#), COD, alkalinity, VSS, SS and NH₄⁺-N
140 were followed by standard methods [\[23\]](#). The gas volume was measured with the gas meters and

141 gas composition was tested via a gas chromatograph (GC9790II, Fuli, China) with nitrogen as the
142 carrier gas and hydrogen for burning of the flame. A thermal conductivity detector equipped with a
143 stainless column (TDX-01 2 m × 3 mm) was used for monitor of biogas and the temperatures of
144 injector, detector and column were 120 °C, 150 °C and 120 °C, respectively. A flame ionization
145 detector with a capillary column (ATFFAP 30 m × 0.32 mm × 0.50 μm) was applied to measure the
146 content of VFAs and the temperatures of detector, column and injector were 220 °C, 150 °C and
147 210 °C, respectively. The samples for VFAs detection were centrifuged at 12000 r·min⁻¹ for 4 min,
148 [after that](#), the supernatant was acidized to pH < 2 using 3 mol·L⁻¹ HCl and then filtered through a
149 0.22 μm membrane. Data in the steady state (the last 60 days) were presented in [Table 1](#) and
150 analyzed in [Table 2](#).

151 The sludge samples in each compartment of the three systems were collected three times from
152 different altitudes and then they were mixed for DNA extraction. The DNA of each sample was
153 extracted three times and then mixed to conduct the high-throughput 16S rRNA pyrosequencing.
154 The DNA was extracted by FastDNA™ SPIN Kit for Soil (MP Biomedicals, LLC). The V4 region
155 of 16S rRNA gene was amplified by PCR via the primers of 515FmodF
156 (5'-GTGYCAGCMGCCGCGGTAA-3') and 806RmodR (5'-GGACTACNVGGGTWTCTAAT-3').
157 Sequencing was performed on an Illumina Hiseq2500 PE250 platform. More information about the
158 extraction of DNA, PCR and sequencing can be referred in the previous study [\[24\]](#).

159 **2.4. Calculations**

160 The mass balance of COD was calculated to evaluate the methanogenesis rate as Eq. [\(1\)](#).

$$\text{COD mass balance (\%)} = (\text{COD}_{\text{CH}_4} + \text{T-COD}_{\text{eff}}) / \text{T-COD}_{\text{inf}} \times 100 \quad (1)$$

Where COD_{CH_4} , COD_{eff} and COD_{inf} represent CH_4 weight converted to COD (g), COD in the effluent and COD in the influent, respectively.

The acidogenesis and methanogenesis rates were calculated from the COD balance. The COD conversion rates of acidogenesis and methanogenesis were analyzed [based on](#) the Eq. (2,3,4,5). [25]

$$R_{\text{VFAs}} = \frac{(\text{COD}_{\text{VFAs}} + \text{COD}_{\text{CH}_4})_{\text{eff}} - (\text{COD}_{\text{VFAs}})_{\text{inf}}}{V \times \text{HRT}} \quad (2)$$

$$R_M = \frac{\text{COD}_{\text{CH}_4}}{V \times \text{HRT}} \quad (3)$$

$$\text{SR}_{\text{VFAs}} = \frac{(\text{COD}_{\text{VFAs}} + \text{COD}_{\text{CH}_4})_{\text{eff}} - (\text{COD}_{\text{VFA}})_{\text{inf}}}{\text{VSS} \times \text{HRT}} \quad (4)$$

$$\text{SR}_M = \frac{\text{COD}_{\text{CH}_4}}{\text{VSS} \times \text{HRT}} \quad (5)$$

Where COD_{CH_4} represents CH_4 weight converted to COD (g); COD_{VFAs} represents VFAs weight converted to COD (g); “eff” represents effluent; “inf” represents influent; R_{VFAs} represents acidogenesis rate (gCOD/L/d); R_M represents methanogenesis rate (gCOD/L/d); SR_{VFAs} represents specific acidogenesis rate (gCOD/gVSS/d); SR_M represents specific methanogenesis rate (gCOD/gVSS/d).

3. Results and discussion

3.1. The promotion of methanogenesis via symbionts of SFAS and exoelectrogens

The performances of R1 and R2 systems were compared to elucidate the influence of SFAS and

exoelectrogens enriched in the C2-C4 parts (Fig.2 and Fig.3). As shown in Table 1, the pH values in the four compartments of R1 and R2 systems were located in the range of 7.0 - 8.0 and the ORP levels were below -400 mV. Both pH values and ORP were suitable for anaerobic digestion [26, 27]. Nonetheless, the methane yield and total COD removal efficiency of the R2 system were 246.3 mL/gCOD_{removed} and 71.5%, respectively, 12.9% and 9.1% higher than those of the R1 system.

3.1.1 Effective VFAs transformation by symbionts of SFAS and exoelectrogens

The performance of C1 and C4 in the R1 and R2 systems were compared to figure out the influence of SFAS and exoelectrogens enriched in the C2-C4. In the C1, the COD removal rate of R2 system was only 2.5% higher than that of R1 system. Nevertheless, the COD removal efficiency in the C4 of R2 system was 9.1% higher than that of R1 system. Apparently, the C2-C4 parts made a big contribution on the enhanced COD removal efficiency. The high COD removal rate in the R2 system can be explained by effective degradation of VFAs. The VFAs concentration in the C4 of R2 system was 673 mg·HAc·L⁻¹, 23.5% less than that of the R1 system (Table 1).

The less content of VFAs and COD in the R2 system could be due to its microbial composition. In the MEC parts, the abundances of phyla Firmicutes and Synergistetes in the C4 were 54.7% and 45.8%, respectively, higher than those of the R1 system. It was an encouraging phenomenon since most thermophilic exoelectrogens and SFAS belonged to these two phyla [28-29]. More direct evidences were provided in Fig.6 that the relative abundances of genus *Thermincola* (11.03%), *Syntrophobacter* (3.09%), *Syntrophomonas* (2.57%) in the C4 of R2 system were 165.5%, 194.5% and 97.6% higher than those of the R1 system. The genus *Thermincola* was one of the potential thermophilic exoelectrogens [30], and genus *Syntrophobacter* and *Syntrophomonas* were the SFAS

203 accounted for the utilization of propionic and butyric acids. [31,32]

204 The enrichment of *Thermincola*, *Syntrophobacter* and *Syntrophomonas* resulted in less
205 propionic, butyric and acetic acids in the R2 system, which were 31.7%, 22.8% and 24.4%,
206 respectively, lower than those of the R1 system (Fig. 4a). The propionic and butyric acids were
207 effectively used by *Syntrophobacter* and *Syntrophomonas* to produce acetic acid that further
208 exploited by *Thermincola*. Furthermore, the consumption of VFAs in the C2-C4 parts promoted the
209 hydrolysis fermentation in the C1 [33]. As an evidence, the VFAs produced in the C1 of R2 system
210 was 39.8% higher than the R1 system and more hydrolytic bacteria were found (e.g. phyla
211 Bacteroidetes). Thus, the promotion of hydrolysis and degradation of VFAs by the symbionts of
212 SFAS and exoelectrogen accounted for the high COD removal efficiency in the R2 system.

213 **Fig. 2** Comparison of the COD, VFAs, pH, methane yield and methane content in the C1 of R1, R2 and R3
214 systems.

215 **Fig. 3** Comparison of the COD, VFAs, pH in the C4 and methane yield and methane content in the C2-C4 of R1,
216 R2 and R3 systems

217 **3.1.2 Improved methane production of the ABR system assisted by symbionts of SFAS and** 218 **exoelectrogens**

219 The previous study proved that the electrons obtained from oxidizing reaction at the anode of
220 MEC were transferred to the cathodes for methane generation via hydrogenotrophic methanogens
221 [34]. Theoretically, the fast degradation of VFAs by symbionts of SFAS and exoelectrogens at the
222 anode provides more electrons donors for hydrogenotrophic methanogens at the cathode, thus

223 contributing to the formation of methane. [Nevertheless](#), whether the methane yield could be
224 enhanced in an elevated-temperature MEC system remained to be further demonstrated. Notably,
225 the total methane yield of the R2 system was 12.9% higher than that of the R1 system, and most
226 contributed from the MEC parts [\(C2-C4\)](#). [As an evidence](#), the methane yield in the C2-C4 of R2
227 system was 45.4% higher than that of the R1 system, while the methane yields [in the C1 of both](#)
228 [systems](#) were similar. Therefore, the C2-C4 parts made a significant contribution to the
229 improvement of methane production in the R2 system.

230 A taxonomic analysis on the microbial community was performed to compare the overall
231 community structure in the R1 and R2 systems ([Fig.6](#)). The dominant archaea in the C4 of R1
232 system were genus *Methanobacterium* (14.0%), *Methanosaeta* (11.0%), *Methanolinea* (1.4%). More
233 hydrogenotrophic methanogens (eg. *Methanobacterium*, 17.9%) and less acetoclastic methanogens
234 (eg. *Methanosaeta*, 6.1%) [were detected](#) in the C4 of R2 system. Similar compositions were
235 observed in the other MEC parts of R2 system. Generally, the methanogenesis in traditional
236 anaerobic system is dominated by acetoclastic methanogens, accounting for about 60% of methane
237 yield [\[35\]](#). In this methanogenesis process, a molecule of CH_3COOH is decomposed into a molecule
238 of CH_4 and a molecule of CO_2 ($\text{CH}_3\text{COOH} \rightarrow \text{CH}_4 + \text{CO}_2$), inevitably resulting in the diversion of
239 carbon to CO_2 and limiting the methane yield. However, in the bio-electrochemical system treating
240 actual wastewater, hydrogenotrophic methanogens usually play a dominant role in the cathode [\[36\]](#).
241 In this process, the synthesis of one molecule of CH_4 and two molecules of H_2O consumes one
242 molecule of CO_2 and four molecules of H_2 ($4\text{H}_2 + \text{CO}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}$), the carbon of CO_2 flows to
243 methane completely. The results [revealed](#) that the CO_2 contents of C2-C4 parts in the R2 and R1

244 systems were 14.7% and 18.8%, respectively, with methane content of 84.6% and 80.3%. Therefore,
245 the improvement of methane yield in the R2 system was due to [effective](#) consumption of CO₂. The
246 increase of hydrogenotrophic methanogens consuming CO₂ and [the](#) decrease of aceticlastic
247 methanogens producing CO₂ were the biological bases for improvement of methane production.

248 Particularly, the *Methanobacterium* could be one kind of methanogens realizing extracellular
249 respiration [37]. Lin et al [38] also confirmed that the methane could be produced by
250 *Methanobacterium* via capture of electrons directly from cathode with the reaction of $\text{CO}_2 + 8\text{H}^+ +$
251 $8\text{e}^- = \text{CH}_4 + 2\text{H}_2\text{O}$. Combining the present and previous results, we inferred that the enrichment of
252 *Methanobacterium* and its direct electron transfer accounted for the improvement of [methane](#)
253 [production](#) in the R2 system.

254 Nevertheless, it should be noted that the methane content of 84.6% in the R2 system was not
255 high enough when compared with [previous results of](#) MEC on mesophilic condition. Choi et al. [39]
256 found that the methane content of MEC at 35 °C can reach more than 90%. Further research found
257 that the efficiency of electron transfer via thermophilic exoelectrogens was lower than the
258 mesophilic exoelectrogens, since the thermophilic exoelectrogens mainly belong to Galanz positive
259 bacteria with thick cell wall and cannot transfer [electrons](#) via flagella [40]. Given the big difference
260 between thermophilic and mesophilic microorganism, it is necessary to make research on the
261 influence of fermentation temperature to achieve the best treatment of sludge filtrate.

262 **Fig.4** The composition of VFAs in the C1 and C4 of R1, R2 and R3 systems (a); COD mass balance in the R1, R2
263 and R3 systems (b).

264 **3.2. Effect of fermentation temperature on the ABR systems assisted by symbionts**

265 **of SFAS and exoelectrogens**

266 The efficient operation of anaerobic system is directly related to the composition of
267 microorganisms and the factors affecting the composition of microorganisms include temperature,
268 pH, substrate, trace elements and so on. Among them, temperature has a significant impact on the
269 microbial community. Firstly, temperature can significantly affect the species of microorganisms
270 and their organic metabolism pathway [21]. Secondly, temperature can [impact](#) the activity of
271 biological enzymes and has a significant influence on the metabolic rate of organisms [41]. In
272 addition, temperature changes the solubility, conversion rate and flow direction of organic
273 compounds, which greatly affects the [performance](#) of anaerobic systems [42]. Therefore, the R2 and
274 R3 systems were compared to investigate the influence of temperature ([Fig. 2 and Fig. 3](#)).

275 **3.2.1 Effect of fermentation temperature on VFAs transformation and COD removal**

276 **efficiency**

277 The pH of C4 in [the](#) R3 system was 7.64 and lower than that of [the](#) R2 system. As neutral pH
278 value was in favor of exoelectrogen and SFAS, [when compared with the R2 systems,](#) the VFAs in
279 the C4 of R3 system was 42.1% lower, the contents of propionic and acetic acids were greatly less,
280 and the COD removal efficiency was also 9.7% higher. These results suggested that the
281 performance of R3 system [at 35 °C](#) was better than the R2 system [at 55 °C](#), which could be
282 elucidated from the microbial community.

283 The microbial communities of R2 and R3 systems are depicted in [Fig. 5 \(b\) \(c\) and Fig.6](#). The

284 dominant bacteria in [the](#) C1 of R2 system were phyla Firmicutes (22.4%), Bacteroidetes (10.4%),
285 Synergistetes (7.7%), Chloroflexi (7.1%), Atribacteria (7.4%), Thermotogae (6.7%) and
286 Proteobacteria (6.1%). In contrast, more phyla Bacteroidetes (26.8%), Proteobacteria (19.3%) and
287 less Firmicutes (5.1%), Synergistetes (4.9%), Chloroflexi (5.2%) were detected in the C1 of R3
288 system. The dominant bacteria changed from phyla Firmicutes in [the](#) R2 system to phyla
289 Bacteroidetes in the R3 system. The enrichment of [genus](#) *Bacteroides*, one of hydrolytic
290 fermentation bacteria, made more acetic and butyric acids in the C1 of R3 system ([Fig.4a](#)),
291 suggested that the hydrolytic rate of the R3 system was higher than that of the R2 system.

292 In the C4 of R3 system, the main bacteria were phyla Proteobacteria (22.3%), Bacteroidetes
293 (14.6%), Synergistetes (11.0%), while the main bacteria in the C4 of R2 system were phyla
294 Firmicutes (26.3%), Synergistetes (10.5%) and Proteobacteria (9.4%). Previous studies [have](#) found
295 that many mesophilic exoelectrogens belong to the phyla Proteobacteria [\[43\]](#). This significant
296 difference between the R2 and R3 systems indicated that more Firmicutes were detected in the
297 elevated-temperature MECs, while Proteobacteria were mainly formed in the moderate MECs.

298 The abundance of genus *Geobacter*, one of typical mesophilic exoelectrogens, was 12.06 % in
299 the C4 of R3 system. The abundance of genus *Thermincola*, a thermophilic exoelectrogens, was
300 11.03% in the C4 of R2 system. In terms of the relative abundance of exoelectrogens, the R3 system
301 did not have obvious advantages over the R2 system. However, *Geobacter* was superior to
302 *Thermincola* [on](#) its ability to metabolize acetic acid and deliver electrons directly [\[30\]](#). Therefore,
303 the R3 system with *Geobacter* as the main exoelectrogens was superior to the R2 system. On the
304 other hand, the relative abundance of SFAS (e.g. *Syntrophobacter*) in the R3 system was 36.0%

305 higher than that of the R2 system. Altogether, [these](#) results demonstrated that the moderate
306 fermentation temperature benefited to enrich exoelectrogens with stronger metabolic capability and
307 achieve higher abundance of SFAS.

308 **Fig. 5** Taxonomic composition at phylum level in [the](#) C1 and C4 of R1 system (a); R2 system (b); R3 system (c).

309 3.2.2 Effect of fermentation temperature on methane production

310 Effective degradation of VFAs transferred more carbon into methane in the R3 system with
311 total methane yield of 297.3 mL/g·COD_{removed}, 20.7% higher than that of the R2 system. The
312 methane yield of C1 and C2-C4 in the R2 system were 238.9 and 269.8 mL/g·COD_{removed},
313 respectively, with the highest methane content of 84.6 % detected in C4. In contrast, the methane
314 yield in C1 and C2-C4 of the R3 system reached 272.0 and 305.6 mL/g·COD_{removed}, respectively,
315 with the highest methane content of 91.4% detected in C4. The methane yield in both C1 and
316 C2-C4 parts of the R3 system were higher than those of the R2 system, affiliated with purer
317 methane content. These results indicated that moderate temperature was better than elevated
318 temperature to promote the methane production from anaerobic digestion of sludge filtrate. It could
319 be elucidated from the composition of phyla Euryarchaeota.

320 It was found that the abundance of phyla Euryarchaeota in the C2-C4 parts of R3 system was
321 significantly higher than that of R2 system. It can be inferred that the moderate temperature
322 benefited for the increase of methanogens, resulting in better performance of methane yield. Similar
323 report was made by Yin et al [\[21\]](#) that the mesophilic digester obtained 15% higher methane yield
324 than the thermophilic one. [The dominant methanogen was *Methanosaeta* in the inoculums of both](#)

R2 and R3 systems, with the abundances of 16.4% and 14.7%, respectively. Nevertheless, the dominant methanogen changed to *Methanobacterium* in both R2 (17.9%, C4) and R3 (24.2%, C4) systems after a long time operation. These results revealed that the methanogen community changed fundamentally due to the coupling of MECs in both R2 and R3 systems. Moreover, it was proved that the moderate temperature benefited to enrich exoelectrogens with strong metabolic capability (e.g. *Geobacter*) and achieved high abundance of SFAS. The effective utilization of VFAs in the anodes by symbionts of SFAS and exoelectrogens provided more electron donors in the cathodes and facilitated the enrichment of hydrogenotrophic methanogen (e.g. *Methanobacterium*) in the R3 system. The above analysis theoretically explains that the moderate temperature is more conducive to the enrichment of SFAS, exoelectrogens and hydrogenotrophic methanogens, promoting the methanogenesis in anaerobic digestion of sludge filtrate.

Fig. 6 Comparison of the R1, R2 and R3 systems on microbial taxonomy at genus level in the C1 and C2 (a); in the C3 and C4 (b)

3.3. Analysis on the carbon conversion efficiency in different systems

In order to analyze the carbon conversion efficiency of R1, R2 and R3 systems, the COD mass balance was calculated according to the average value of last 60 days and depicted in Fig.4b. The error ranges of the total COD balance rates in the three systems were all within 10%, indicating that the relevant data were accurate. The 56.9% of COD mass in the flesh sludge filtrate was converted into methane in the R1 system. In contrast, the COD mass corresponding to methane increased greatly to 67.4% and 78.4% in the R2 and R3 systems, respectively. Therefore, the R3 system was more advantageous in conversion of carbon source. The methanogenesis rates of the three systems

are depicted in Table 2. The volumetric methanogenesis rates of the R1, R2 and R3 systems were 0.639, 0.917 and 1.373 g·COD/L-reactor/d, respectively. In term of specific methanogenesis rates, they were 0.073, 0.092 and 0.114 g·COD/g·VS/d, respectively. In the previous study of Jang et al [42], thermophilic condition was in favor of high volumetric methanogenesis rate. In this experiment, the R3 system had higher volumetric and specific methanogenesis rates since sufficient electron donors for methanogenesis derived from rapid degradation of VFAs.

Interestingly, the comparative results of acidogenesis rates between the R1, R2 and R3 systems were similar with those of methanogenesis rates ($R1 < R2 < R3$). Generally, the high temperature is beneficial to hydrolytic fermentation [41]. Nonetheless, the higher acidogenesis rate of R3 system at 35 °C was due to more hydrolytic fermentation bacteria enriched in the C1. In addition, this result also suggested that not only methanogenesis rate, but also acidogenesis rate was intensified by symbionts of SFAS and exoelectrogens. Thus, the R3 system at mesophilic condition has obvious advantages on treatment of sludge filtrate in term of both acidogenesis and methanogenesis rates.

How to improve the abundance and methanogenic activity of methanogens in an anaerobic system is one of the key challenge to improve its methanogenesis rates. The results demonstrated that coupling MEC with an AD system can enrich SFAS, exoelectrogens and hydrogenotrophic methanogens simultaneously at both thermophilic and mesophilic conditions, providing a technical route for enhancement of methane production. However, these results were obtained from a lab scale experiment. In the amplification of MEC, the electrochemical performance of MEC could be significantly reduced, which is one of the key challenge affecting the practical application of MEC system [44]. The components of small MEC are simple and make it stable in electrochemical

367 performance. But the enlarged MEC is prone to loss voltage due to large internal resistance [45]. So,
368 by increasing the current density of MEC, the hydrogen release rate in the cathode could be
369 theoretically increased, thus directly increasing the methane production and the carbon conversion
370 efficiency via hydrogenotrophic methanogens [46].

371 Therefore, it is necessary to improve the carbon conversion efficiency of an AD-MECs system
372 by means of electrode optimization and optimal operation in the enlarged application. The current
373 density can be effectively increased by increasing the external voltage. In addition, the potential loss
374 of electrodes can be reduced by optimization of electrode materials, electrode combination mode
375 and reactor structure. Considering the differences of reactor structures, it is important to optimize
376 the flow path and microbial adhesion conditions in the [coupling](#) system. In conclusion, although the
377 ABR system assisted by symbionts of SFAS and exoelectrogens achieved effective degradation of
378 VFAs and high methane production from [treatment](#) of sludge filtrate, the coupling mode of
379 electrodes and operational conditions still need to be scientifically optimized before it can be used
380 in the enlarged engineering.

381 **Table 2** Conversion rates of acidogenesis and methanogenesis in [the](#) R1, R2 and R3 systems

382 **4. Conclusion**

383 In this study, the ABR system assisted by symbionts of SFAS and exoelectrogens was applied
384 to treat sludge filtrate. By controlling different operational conditions, conducting control
385 experiments and analyzing the microbial structure, the effects of symbionts and fermentation
386 temperature on methanogenesis were analyzed.

387 The results demonstrated that the COD removal rate, methane content and methane yield of the
388 R2 system reached 71.5%, 84.6%, 246.3 mL/gCOD_{removed}, respectively. Compared with the R1
389 system, they increased by 9.1%, 4.3% and 12.9%, respectively. The R3 system performed best, its
390 COD removal efficiency, methane content and methane yield reached 83.2%, 91.4% and 297.3
391 mL/gCOD_{removed}, respectively.

392 The coupling of MEC with the ABR system enriched symbionts of SFAS and exoelectrogens,
393 [thereby](#) achieved efficient degradation of VFAs and induced dominant hydrogenotrophic
394 methanogens to produce methane. Compared with the thermophilic condition, the mesophilic
395 condition was conducive to forming exoelectrogens with strong metabolic ability and facilitating
396 enrichment of SFAS and hydrogenotrophic methanogens, so as to improve the methanogenesis rates
397 from anaerobic digestion of sludge filtrate.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Figures Captions

Fig. 1 Schematic diagram of the adopted experimental apparatus for anaerobic digestion

Fig. 2 Comparison of the COD, VFAs, pH, methane yield and content in the C1 of the R1, R2 and R3 systems.

Fig. 3 Comparison of the COD, VFAs, pH in the C4 and methane yield and content in the C2-C4 of the R1, R2 and R3 systems

Fig. 4 The composition of VFAs in the C1 and C4 of R1, R2 and R3 systems (a); COD mass balance in the R1, R2 and R3 systems (b). [All values are based on the average value of the last 60 days.](#)

Fig. 5 Taxonomic composition at phylum level in [the](#) C1 and C4 of R1 system (a); R2 system(b); R3 system (c).

Fig. 6 Comparison of the R1, R2 and R3 systems on microbial taxonomy at genus level in the C1 and C2 (a); in the C3 and C4 (b)

1. Substrate tank	4. ABR reactor	7. Water-sealed tank
2. Circulating cooler	5. Circulating heater	8. Gas meter
3. Peristaltic pump	6. Electrodes	9. Effluent tank
- C1~C4 : the first to fourth compartments of reactors
- Anode wire — Cathode wire — Feed pipe — Circulating bath

Fig. 1

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546

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Fig. 2

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Fig. 3

(a)

(b)

Fig. 4

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Fig. 5

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Fig. 6

Table 1 Control parameters and process performances for the R1, R2 and R3 systems

Items	Unit	R1			R2			R3		
		C1	C2-C4	System	C1	C2-C4	System	C1	C2-C4	System
Configuration	Temperature	° C	55	55	55	55	55	35	35	35
parameter	HRT	days	6	6	6	6	6	6	6	6
	T-COD	g/L/d	ND	ND	2.1	ND	ND	2.1	ND	2.1
	pH		7.64±0.02	7.75±0.02	7.75±0.02	7.26±0.04	7.83±0.03	7.83±0.03	7.55±0.02	7.64±0.02
	T-COD	g·L⁻¹	6.9±0.5	4.5±0.3	4.5±0.3	6.4±0.5	3.3±0.2	3.3±0.2	5.2±0.3	2.2±0.2
	S-COD	g·L⁻¹	6.5±0.5	4.3±0.2	4.3±0.2	6.1±0.3	3.2±0.2	3.2±0.2	5.0±0.3	2.1±0.1
	S-COD Red	%	45.7	16.7	62.4	48.2	23.3	71.5	58.3	24.9
	VFAs	mg HAc /L	1810±100	880±25	880±25	2330±160	673±70	673±70	2550±140	390±40
	NH ₄ ⁺ -N	mg·L⁻¹	1573±20	1650±30	1650±30	1545±30	1601±20	1601±20	1504±30	1550±20
	ALK	mg·L⁻¹	4792±110	5650±h13	5650±130	4828±100	6320±150	6320±150	4562±100	5940±150
	VS	g·L⁻¹	25.46±1.2	20.23±0.9	22.35±1.5	28.54±1.4	22.76±1.5	25.38±1.3	33.52±0.5	29.77±1.1
	ORP	mV	-425±5	-425±5	-425±5	-425±5	-425±5	-425±5	-425±5	-425±5
	Biogas									
	CH ₄	%	66.8±1.2	80.3±1.2	71.6	67.7±2.4	84.6±1.3	79.4	75.9±1.5	91.4±1.1
	CO ₂	%	26.6±0.9	18.8±0.3	25.5	24.8±0.8	14.7±0.5	18.1	20.7±1.2	7.9±0.5
Methane	Yield	mL/gCOD _{removed}	230.3	185.2	218.2	238.9	269.8	246.3	272.0	305.6
COD mass balance	COD mass	%	ND	ND	95.6	ND	ND	97.2	ND	ND

**Note:* All values are based on the average measured results of the last 60 days.

^a ‘Red.’ is designated as ‘Reduction’, ‘ND’ is designated as ‘No detect’

Table 2 Conversion rates of acidogenesis and methanogenesis in the R1, R2 and R3 systems

Items		Units	R1	R2	R3
Reaction rates	Acidogenesis	gCOD/L-reactor/d	0.732	0.973	1.379
	Methanogenesis		0.639	0.917	1.373
Specific reaction rates	Acidogenesis	gCOD/gVS/d	0.084	0.098	0.115
	Methanogenesis		0.073	0.092	0.114