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**Anaerobic digestion of sludge filtrate using anaerobic baffled reactor assisted by
symbionts of short chain fatty acid-oxidation syntrophs and exoelectrogens:
Pilot-scale verification (Clean)**

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Abstract: The growing amount of sewage sludge from wastewater treatment plant is an emerging challenge in China. The efficient anaerobic digestion of sludge filtrate generated from hydrothermally pretreated sewage sludge can promote the disposal of sewage sludge. Herein, a pilot-scale anaerobic baffled reactor (ABR) assisted by symbionts of short chain fatty acid-oxidation syntrophs (SFAS) and exoelectrogens was developed to improve its stability and efficiency for filtrate treatment. The results demonstrated that the symbionts of exoelectrogens and SFAS, which were enriched by introduction of electrodes in the ABR system, promoted the degradation of butyric, propionic and acetic acids. Therefore, the COD removal efficiency increased from 74.1% to 86.6% and the methane content increased from 81.5% to 92.2% with methane production rising from 241 to 282 mL/g COD_{removed}. Furthermore, the economic evaluation indicated that the energy consumption of electrodes was 0.600 kWh/m³ of sludge filtrate, the net energy profited from increased methane was 2.344 kWh/m³ of sludge filtrate. These results confirmed that the ABR system assisted by symbionts of SFAS and exoelectrogens was feasible for treatment of sludge filtrate in terms of both technical and economic level through pilot-scale verification.

Key words: sludge filtrate; anaerobic digestion; pilot scale; SFAS; exoelectrogens.

1. Introduction

Sludge filtrate is a kind of special organic wastewater generated from mechanical squeezing of hydrothermally pretreated sewage sludge. Sewage sludge, mainly aerobic sludge, largely comes from residual active sludge of wastewater treatment plants (WWTP) in China with production of 34 million tons (80% moisture) and disposal rate of 33% in 2015, and it will reach 60-90 million tons in 2020 (Yang et al., 2015). The main factor limiting treatment of sewage sludge is its hard dewatering due to its hydrophilic nature and structural stability (Li Y. et al., 2015). In view of different techniques for dewatering sewage sludge, hydrothermal pretreatment presents significant advantages for its high efficiency (Neyens et al., 2004), which has also been proved from lab-scale study to pilot-scale verification in our previous studies (Li et al., 2017, 2018). In this process, the raw sludge (85% moisture) was pretreated at 180 °C for 30 min and then dehydrated to moisture content of 35%. Nevertheless, the disposal of generated filtrate with abundant organics is becoming a new challenge. At present, the safe treatment rate of sewage sludge is low in China, which is generally less than 30% (Chen et al., 2018). As a result, the flow of sludge filtrate is small. Therefore, filtrate tends to be mixed with raw wastewater with little or no impact observed in the WWTP. Nonetheless, the increasing disposal rate of sludge inevitably increases the output of filtrate that needs to be treated separately and efficiently in sludge treatment process (Wirth et al., 2015). Given the pollutant migration route in sewage treatment process, pollutants are transferred from sewage to sludge and finally concentrated in the

61 sludge filtrate. Therefore, the efficient treatment of sludge filtrate is important for
62 terminal treatment of pollutants and it also facilitates the sludge treatment with high
63 application value.

64 The anaerobic digestion (AD) technique was used to treat this special wastewater
65 with attached product of biogas. Wirth et al. (2015) studied the influence of hydraulic
66 retention time (HRT) and temperature (37 and 55 °C) and found that the fermentation
67 temperature had little effect. When the HRT and organic load rate (OLR) were 5 days
68 and 5 kg COD m⁻³d⁻¹, respectively, the COD removal rate was 68-75% with methane
69 yield of 180 mL/gCOD_{removed}. Li et al. (2018) treated similar sludge filtrate by a
70 pilot-scale up-flow anaerobic sludge bed (UASB) at 37 °C, and the COD removal rate
71 reached 75% with influent COD of 38400 mg·L⁻¹ at HRT of 12 days. These previous
72 results imply that the challenges for filtrate treatment are low COD removal efficiency
73 and poor methane production. In a traditional AD system, the theoretical rates of
74 hydrolysis and acidification are much higher than the subsequent methanogenesis rate.
75 This imbalance is easy to cause accumulation of volatile fatty acids (VFAs) especially
76 in disposal of high concentrated organic wastewater (Liu et al., 2013). Indeed,
77 accumulation of VFAs was also observed in the AD process of sludge filtrate (Li et al.,
78 2018). A large amount of accumulated VFAs not only severely inhibited the subsequent
79 methanogenesis process, but also prevented the hydrolysis and acidification, which
80 directly resulted in the low efficiency of organic treatment (Palatsi et al., 2010).

81 Given the low efficiency of conventional AD systems for filtrate treatment, the

bio-electrochemical technology could be an effective method to improve the degradation of VFAs and enhance methane production. Previous study has proved that acetic acid is a favorable substrate for exoelectrogens with wider ecological niches and shorter generation time than the acetoclastic methanogens (Lovley, 2004). Nevertheless, propionic and butyric acids cannot be used efficiently by exoelectrogens (Luo et al., 2017). To simultaneously strengthen the degradation of propionic, butyric and acetic acids in AD process of sludge filtrate, the enrichment of short chain fatty acid-oxidation syntrophs (SFAS) and exoelectrogens could be a feasible approach via introduction of electrodes in an AD system, since the propionic and butyric acids can be degraded to acetic acid by SFAS and then further utilized by exoelectrogens (Stams et al., 2012).

Although the introduction of electrodes in an AD system has already been studied to enhance methanogenesis by changing methanogens community and methanogenic pathway (Amelialea et al., 2014), there is little focus on the degradation of VFAs. Nevertheless, VFAs are the major source of electron donor for methanogens to produce methane (Zhang Z. et al., 2018). Moreover, few reports have studied the degradation of VFAs by symbionts of SFAS and exoelectrogens. On the other hand, most of current studies on bio-electrochemical technology were in lab scale (Zhi et al., 2019). There are few studies concerning energy consumption and benefit of a pilot-scale system. Therefore, the present study aims to verify the efficiency of expanded electrodes on enrichment of SFAS and exoelectrogens in a pilot-scale AD system, so as to achieve effective degradation of sludge filtrate for methane production and further evaluate its

feasibility of application via energy consumption and benefit analysis.

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. The raw sewage sludge, mainly aerobic sludge, was hydrothermally pretreated under 180 °C for 30 min and then cooled to 60 °C for subsequent filter pressing in the dewatering system. More detailed information of typical procedure was described in our previous study (Li et al., 2017). The COD concentration of sludge filtrate was $12.5 \pm 0.4 \text{ g}\cdot\text{L}^{-1}$ when the pH value and alkalinity were 7.21 ± 0.30 and $2620 \pm 46 \text{ mg CaCO}_3/\text{L}$, respectively, with $\text{NH}_4^+\text{-N}$ concentration of $1544 \pm 27 \text{ mg}\cdot\text{L}^{-1}$. The ratio of COD : N : P was about 45 : 6 : 1, the BOD/COD rate of sludge filtrate was 0.54 ± 0.02 .

The inoculated sludge was anaerobic sludge that mixed granular sludge with flocculent sludge containing symbionts of SFAS and exoelectrogens. The granular sludge was collected from an AD system for starch wastewater treatment. The flocculent sludge containing symbionts of SFAS and exoelectrogens was collected from an AD system with propionate and butyrate as carbon source. More detailed information of typical procedure for enriching symbionts of SFAS and exoelectrogens in static trial was described in our previous study (Wang et al., 2018). The granular sludge and the flocculent sludge were mixed at a volume ratio of 9:1 according to our previous study (Wang et al., 2019) and inoculated into the five compartments of anaerobic baffled

reactor (ABR) with same amount of 20.50 ± 0.14 g VSS/L. The inoculation of flocculent sludge aims to accelerate the enrichment of exoelectrogens and SFAS after introduction of electrodes in the ABR system.

Fig. 1 The field installation photos of pilot-scale ABR (a), electrode (b) and the schematic diagram of pilot-scale system (c), ABR represents anaerobic baffled reactor.

2.2. Experimental setup and operation

The ABR was applied in the present study due to its advantages for separation of acid-producing and methane-producing phases (Zhu et al., 2008). The pilot-scale ABR is made of plexiglass and its working volume is 360 L with dimension of $1.2 \times 0.3 \times 1.0$ m (Fig. 1a). The volume ratio of downstream region and upstream region is 1:3, and the electrodes are placed in upstream region of the middle three compartments (C2-C4). The operational temperature was controlled at 35 ± 1 °C by a water jacket with a heater and a built-in mercury thermometer. The biogas yield was measured by wet flowmeters (LML-1, Changchun Automobile Filter, China). The substrate was stored at a continuous stirred tank reactor (CSTR) and the inlet flow was controlled by a feed pump (BT100-2i, Longer Pump, Britain). The AD process was divided into four stages (stages I, II, III and IV) based on influent COD and the open/closed circuit status of electrodes during the long time running (Table 1). There were two preliminary stages to mature the system, stage I and II, in which the sludge filtrate was diluted to start up the AD process under HRT of 3 days. After a successful start-up, the HRT was extended to 6 days in stages III and IV to improve the COD removal rates. The stage III with open

circuit and stage IV with closed circuit were performed and compared.

Table 1 The operation of the pilot-scale system

Design of electrodes: both anodes and cathodes were made of carbon cloth. Polyvinyl chloride (PVC) plate was used as framework of electrode module. Each electrode module contained three pieces of carbon clothes in parallel with size of 4 cm × 60 cm (Fig.1b). The design of parallel carbon cloth was conducive to ensuring the voltage stability of electrodes. Waterproof rubber was used to glue the edge of carbon cloth to prevent carbon wire falling off. Two pairs of electrode modules were located in one compartment and the center spacing between anode and cathode was 4 cm. The lower end of module was 20 cm away from the bottom of reactor. The PVC plate was equipped with multiple holes to minimize its impact on flow route. The top end of carbon cloth was 10 cm above the highest water level. The carbon cloth was connected with cooper wire by cooper screw, the cooper screw was 8 cm above the highest water level and coated with waterproof glue. Six pairs of electrode modules in the C2-C4 were connected in parallel by copper wire with external power supply. Before the experiment, the voltage of each electrode module was tested by a voltammeter after they were properly connected with the external power supply, so as to ensure that the terminal voltage was maintained at 0.90 ± 0.02 V.

2.3. Sampling and analytical methods

Substrate and effluent samples were taken from sampling ports and their chemical analyses such as ORP, pH, COD, alkalinity, VSS, SS and $\text{NH}_4^+\text{-N}$ were followed by

standard methods ([APHA, 2012](#)). The gas volume was measured by the gas meters when gas composition was tested via a gas chromatograph (GC9790II, Fuli, China) with nitrogen as the carrier gas and hydrogen for burning of flame. A thermal conductivity detector equipped with a stainless column (TDX-01, 2 m × 3 mm) was used for monitor of biogas, and the temperatures of injector, detector and column were 120 °C, 150 °C and 120 °C, respectively. A flame ionization detector with a capillary column (ATFFAP, 30 m × 0.32 mm × 0.50 μm) was applied to measure the content of VFAs. The temperatures of detector, column and injector were 220 °C, 150 °C and 210 °C, respectively. The samples for VFAs detection were centrifuged at 12000 r·min⁻¹ for 4 min. After that, the supernatants were acidized to pH below 2 via 3 mol·L⁻¹ HCl and then filtered through a 0.22 μm membrane. The conductivity of sludge filtrate was tested by a portable conductivity meter (DDBJ-350, Rex, China).

The sludge samples from each compartment and electrodes were collected for DNA extraction to reveal the microbial composition. Through the analysis of microbial composition, the feasibility of enrichment of SFAS and exoeletrogens via expanded electrogens could be verified, further explaining the performance of system. The DNA of each sample was extracted three times by FastDNATM SPIN Kit for Soil (MP Biomedicals, LLC) and then mixed to conduct the 16S rRNA pyrosequencing. High throughput sequencing technology was used to analyze the community change of microorganisms. The DNA was amplified by PCR using the primers 515FmodF (5'-GTGYCAGCMGCCGCGGTAA-3') and 806RmodR (5'-GGACTACNVGGGTWT-

CTAAT-3') for V4 region of the 16S rRNA gene. Sequencing was performed on an Illumina Hiseq2500 PE250 platform. More information about extraction of DNA, PCR and sequencing can be referred in the previous studies (Zhao et al., 2019; Li X. et al., 2015).

2.4. Calculations

(1) Balance of the COD mass was calculated based on study of Ge et al., (2011):

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

Where COD_{CH_4} , $\text{COD}_{\text{eff.}}$ and $\text{COD}_{\text{inf.}}$ represent the total COD values ($\text{g} \cdot \text{d}^{-1}$) corresponding to CH_4 ($2.86 \text{ g} \cdot \text{COD} \cdot \text{d}^{-1}$), COD in the effluent and COD in the influent, respectively.

(2) Energy consumption analysis

$$P = U^2 / R \quad (2)$$

$$\sigma = 1 / \rho \quad (3)$$

$$\rho = R \cdot S / L \quad (4)$$

$$R = L / (\sigma \cdot S) \quad (5)$$

$$P = U^2 \cdot (\sigma \cdot S) / L \quad (6)$$

Where P is the electricity power (W), U is the voltage (V), S is the surface area of electrode (m^2), L is the electrode distance (m), σ is the conductivity of wastewater (S/m). ρ is the resistivity ($\Omega \cdot \text{m}$) and R is the resistance (Ω).

3. Results and discussion

3.1. COD removal efficiency and VFAs transformation enhanced by symbionts of

SFAS and exoelectrogens

The performances of system in stages III and IV were focused to explore the influence of microbial electrolysis. All analyses were conducted based on the mean values of the last 60 days of stages III and IV, respectively. The Fig. 2 shows that the COD removal rates of C1- C5 in stage III were 52.2%, 61.2%, 68.9%, 72.6% and 74.1%, respectively, while they were 50.3%, 64.1%, 74.6%, 82.5% and 86.6% in stage IV. The total COD removal efficiency was 74.1% and 86.6%, respectively, in stages III and IV. Although the COD removal efficiency of C1 in stage IV was less than that in stage III, the COD removal rate of C4 in stage IV was 9.9% higher than that in stage III. Obviously, the improvement of COD removal efficiency was mainly contributed by C2-C4, which were coupled with electrodes.

According to the four steps theory (Bryant, 1979), the efficient degradation of VFAs is important for improving COD removal efficiency of AD system. It was also showed from the present study that the VFAs concentration of C5 declined from 1013 mg HAc/L in stage III to 245 mg HAc/L in stage IV (Fig. 2). Specially, the concentration of acetic and butyric acids in C4 reduced by 83.9% and 69.9%, respectively (Fig.3). Interestingly, the content of VFAs in C1 rose from 2279 mg HAc/L in stage III to 2793 mg HAc/L in stage IV with an increase of 22.6%. Since the special structure of ABR was beneficial to separation of acid-producing and methane-producing phases (Zhu et al., 2008), the rapid degradation of VFAs in C2-C4 facilitated the acid production in C1 and promoted the total COD removal efficiency.

The efficient degradation of VFAs in stage IV made the pH value and ALKs more reasonable. In stage III, the pH values of C1-C5 were 7.44, 7.38, 7.23, 7.47 and 7.56, respectively, with corresponding ALKs of 5185, 5059, 4963, 5204 and 5202 mgCaCO₃/L (Fig.2). Organics of filtrate were continuously hydrolyzed and acidified, consuming alkalinity and causing decline of pH in C1-C3. This phenomenon was usually observed in an ABR with treating high concentrated organic wastewater (Bodkhe, 2009). In stage IV, the pH value of C1-C5 reached 7.34, 7.52, 7.60, 7.63 and 7.74, respectively, with corresponding ALKs of 4801, 5136, 5216, 5386 and 5426 mgCaCO₃/L (Fig.2). Due to efficient utilization of VFAs, the pH and ALK increased from C1 to C5 resulting in a stronger buffer capacity of VFAs (Martin et al., 2013). It was worth noting that the pH and ALK of C1 decreased from stage III to stage IV, which was consistent with the enhancement of acid production in C1. Thus, the microbial electrolysis strengthened the degradation of VFAs in C2-C4 and facilitated the acidification of organics in C1 of the ABR system. So, the ABR system coupled with expanded electrodes achieved effective treatment of sludge filtrate with strong buffering capacity of VFAs.

The effective degradation of VFAs could be explained from biomass and microbial community. The VSS and VSS/SS are shown in Fig 4a. From day 1 to day 120, the VSS and VSS/SS of each compartment increased slowly. After 120 days, the biomass of each compartment was basically stable. In day 180, the VSS of C1, C3 and C5 were 29.38, 32.33 and 28.92 gVSS/L, respectively, and they were 29.48, 32.31 and 25.94 gVSS/L in

day 330. Apparently, the biomass (VSS) of C1, C3 and C5 did not increased during stage IV, but VSS/SS rate rose up slightly in C3. These results suggested that the electrolysis had no significant effect on biomass but could promote the biological activity, and it could be further explained from microbial community.

The relative abundances of phyla Proteobacteria, Bacteroidetes and Chloroflexi of C1 in stage III were 1.2%, 8.1% and 3.1%, respectively, and they increased to 10.3%, 11.0% and 7.2% in stage IV (Fig.5). The phyla Bacteroidetes and Chloroflexi are related to the acid production and fermentation (Liu et al., 2011). Hence, the increase of phyla Bacteroidetes and Chloroflexi promoted the hydrolysis and acidification in C1. Besides, the abundances of phyla Proteobacteria of C2-C4 in stage IV were 18.4%, 26.0% and 34.3%, respectively, while they were only 1.6%, 3.6% and 2.9% in stage III. Kiely et al. (2010) also found large quantities of Proteobacteria in the anodes of microbial electrolysis cell (MEC). In addition, the abundances of phyla Synergistetes in C2-C4 in stage IV were 15.3%, 17.7% and 3.0%, respectively, while they were only 6.0%, 3.8% and 3.2% in stage III. Apparently, the phyla Proteobacteria and Synergistetes enriched in stage IV were directly related to the micro-electrolysis.

In genus level, the relative abundances of fermentation bacteria, such as *Anaerolineaceae* of C1, increased from 2.0% in stage III to 12.3% in stage IV. As typical exoelectrogens, the abundances of *Geobacter* in C2-C4 were 11.2%, 12.9% and 7.2% in stage IV, respectively, while they were all below 1% in stage III. Since butyric fermentation took place in the system, the relative abundances of *Syntrophomonas* in

C2-C4 reached 7.7%, 5.1% and 4.5% in stage IV, respectively, while they were only 4.0%, 3.8% and 0.8% in stage III. Thus, the expanded electrodes enriched fermentation bacteria, SFAS and exoelectrogens simultaneously, resulting in rapid acidification of organics and efficient degradation of VFAs.

Fig. 2 The time course of pH (a), ALK (b), ORP (c), VFAs (d), COD (e) and methane (f) in C1~C5 of pilot-scale system. C1~C5 represent the first to five compartments in flow direction. ALK represents alkalinity, ORP represents oxidation-reduction potential, VFAs represent volatile fatty acids, COD represents chemical oxygen demand.

Fig. 3 The VFAs constitution in C1~C5 of pilot-scale system in stage III (a) and IV (b). The analysis was conducted based on the data of the last 60 days of stage III and IV, respectively.

Fig. 4 The VSS and VSS/SS (a), the ammonia-nitrogen concentration and COD removal rate (b) of pilot-scale system at different time. VSS represents volatile suspended solid, SS represents suspended solid.

3.2. Methane production improvement in the pilot-scale system assisted by symbionts of SFAS and exoelectrogens

Previous studies have proved that the coupling of MEC can improve the methane production in an AD system (Park et al., 2018). The electrons from organic oxidization in the anode of MEC were transferred to the cathode for methane generation via hydrogenotrophic methanogens (Bo et al., 2014). However, most studies have focused on the composition of methanogens and methanogenic pathways at the cathode, while less attention has been paid to the electron generation at the anode (Lovley, 2017). For

the application of enlarged MEC, the improvement of organic oxidation at the anode is important for preventing decline of its efficiency (Cotterill et al., 2017). Theoretically, the fast degradation of VFAs by symbionts of SFAS and exoelectrogens at the anode could provide more electrons for hydrogenotrophic methanogens at the cathode, thus contributing to the formation of methane. Also, it was studied and proved in the present pilot-scale study.

In the stage III, the methane contents of C1-C4 were 60.1%, 65.2%, 76.5% and 81.5%, respectively, with total methane yield of 241 mL/g COD_{removed} (Fig.2). In the stage IV, the methane content of C1 was maintained at 61.9%, but the methane contents of C2-C4 increased to 82.7%, 89.7% and 92.2%, respectively, with a total methane yield of 282 mL/g COD_{removed}. Compared with stage III, the methane yield was enhanced by 17.0% with purer methane content in stage IV. Therefore, the ABR system assisted by symbionts of SFAS and exoelectrogens owns better methanogenesis than the ABR system. Moreover, the methane content was also higher than that of our previous study on pilot-scale UASB with methane content of 72% - 75% (Li et al., 2018). The enhanced methane yield indicated that the present system was not inhibited with NH₄⁺-N concentration of about 1500 mg·L⁻¹ (Fig. 2). In the previous study, significant ammonia-nitrogen inhibition occurred in the microbial methanogenic consortia with NH₄⁺-N concentration of 1000 mg·L⁻¹ (Zhang C. et al., 2018). The better methanogenesis of the present system with high ammonia-nitrogen concentration could be due to the enrichment of hydrogenotrophic methanogens, since hydrogenotrophic

methanogens have a higher tolerance for ammonia-nitrogen concentration than aceticlastic methanogens (Tian et al., 2019). Its microbial community is analyzed as follows, and the main performance of system is summarized at Table 2, .

Table 2 The main performances of the pilot-scale system

In phyla level, the abundances of Euryarchaeota in the cathodes of C2-C4 were 19.5%, 29.2% and 46.0%, respectively, while those in the anodes were 26.2%, 11.7% and 12.2%. The abundance of Euryarchaeota in the cathode was obviously higher than that of anode and it could be due to the increase of hydrogenotrophic methanogens (Evans et al. 2019).

In genus level, the abundances of *Methanobacterium* in C2-C4 in stage IV were 4.1%, 18.1% and 36.0%, respectively, while they were only 5.5%, 4.7% and 11.9% in stage III. The enrichment of *Methanobacterium* in stage IV resulted in methane content increasing from C2 to C4 (Clauwaert et al., 2008). In addition, the abundances of *Methannosarcina* in C2-C4 in stage IV were 14.3%, 6.2% and 0.3%, respectively, while the corresponding ones in stage III were all below 1.0%. The *Methanobacterium* is a kind of hydrogenotrophic methanogens, while *Methannosarcina* can produce methane through both hydrogen and acetic acid (Gajaraj et al., 2017).

Generally, the methanogenesis in traditional anaerobic system is dominated by aceticlastic methanogens, accounting for about 60% of methane yield (Lee et al., 2017). In this process, a molecule of CH₃COOH is decomposed into a molecule of CH₄ and a molecule of CO₂ (CH₃COOH→CH₄+CO₂), inevitably resulting in the diversion of

carbon to CO₂ and limiting the methane yield. However, in the bio-electrochemical system treating actual wastewater, hydrogenotrophic methanogens usually play a dominant role in the cathode (Sangeetha et al., 2016). In its methanogenesis process, the synthesis of one molecule of CH₄ and two molecules of H₂O consumes one molecule of CO₂ and four molecules of H₂ ($4\text{H}_2 + \text{CO}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}$), the carbon of CO₂ flows to methane completely. Particularly, the *Methanobacterium* is one kind of methanogens realizing extracellular respiration (Yilmazel et al., 2017). Lin et al (2018) also confirmed that methane can be produced by *Methanobacterium* via direct capture of electron from cathode with the reaction of $\text{CO}_2 + 8\text{H}^+ + 8\text{e}^- = \text{CH}_4 + 2\text{H}_2\text{O}$. It provides another way to purify methane. Thus, the promotion of organic degradation by symbionts of SFAS and exoelectrogens at the anodes induced the formation of *Methanobacterium* and *Methannosarcina* at the cathode, resulting in the improvement of methane content and methane production in the pilot-scale system. In conclusion, the substantial enrichment of *Syntrophomonas*, *Geobacter*, *Methanobacterium* and *Methannosarcina* on the expanded electrodes verified its feasibility for efficient treatment of sludge filtrate with methane production.

Fig. 5 The microbial distribution in phylum level in stage III (a) and stage IV (b).

Fig. 6 The microbial distribution in genus level in stage III (a) and stage IV (b).

In the methanogenesis process, the relationships between SFAS, exoelectrogens and methanogens are shown in Fig. 7. There were five methanogenic pathways (Shen et al., 2016; Lu et al., 2012; Kiely et al., 2011): (1) In the process of interspecies hydrogen

transfer, VFAs were oxidized by SFAS and electrons was transferred to cell membrane of SFAS to form hydrogen via hydrogenase. And then hydrogen was diffused to the surface of hydrogenotrophic methanogens to reduce CO₂ to methane under enzyme catalysis; (2) In the process of interspecies formate transfer, VFAs were oxidized by SFAS and electrons were used to form formate via formate dehydrogenase in the cell membrane of SFAS. And then, formic acid was diffused to generate methane by hydrogenase complex of hydrogenotrophic methanogens. Or hydrogen was formed through formate hydrogenase and combined with CO₂ via hydrogenase to form methane by hydrogenotrophic methanogens; (3) The acetic acid generated by SFAS was utilized by exoelectrogens. The electrons were transferred to the anode via direct interspecific electron transfer (DIET) to produce methane via hydrogen releasing in the cathode; (4) Electrons transferred to the cathode could be directly captured by the electroactive methanogens to produce methane; (5) The electrons obtained by exoelectrogens could also be directly transmitted the electroactive methanogens via DIET to realize methanogenesis. The previous results of Storck et al. (2016) revealed that the electron transfer rate of DIET was 8 times higher than that of interspecies hydrogen transfer. Therefore, it could be concluded that the enrichment of SFAS and exoelectrogens in the electrodes accelerated the degradation of VFAs via DIET, strengthened the methane production through hydrogenotrophic methanogens, induced the methane production via DIET and eventually improved the methane conversion rate of sludge filtrate.

Fig. 7 The electron transfer relationship between SFAS, exoelectrogens and methanogens ((1)-(5))

represent five methanogenic pathways)

3.3. Material balance analysis and economic evaluation

3.3.1 Material balance analysis

The data of stage IV presented in [Table 2](#) are used to calculate the COD balance based on Eq. (1). The results manifested that 72.7% of COD in the fresh sludge filtrate converted to methane, 11.7% of COD remained in the liquid in form of VFAs and other fermentation intermediates, only 3.7% of COD suspended in the liquid as particulate matter. The total balance rate of COD mass was 88.1%, the reasons for the missing COD could be : (1) physical adsorption of organic matter caused by microorganisms ([Feng et al., 2019](#)). Some microorganisms generated sticky extracellular secretions to gather organics around the cell. This part of organics was not converted to methane in time and removed from the liquid, resulting in the loss of detected COD; (2) "sugar storage" caused by microorganisms ([Morin et al., 2017](#)). In the process of actual wastewater treatment, as an emergency response to adverse environment, some substrates were absorbed by microorganisms and stored in cell as reserved glycogen. This part of organic matter was removed from liquid but not converted to methane, leading to the loss of detected COD. Due to a large amount of sludge and abundant microorganisms in the pilot-scale system, the phenomenon of physical adsorption and "sugar storage" was commonly occurred, resulting in the loss of detected COD.

3.3.2 Economic evaluation

Energy consumption is one of important indexes to evaluate the economic

feasibility of engineering application. The main energy consumption of this pilot-scale system comes from feed pump, heating pump and the MEC parts. No exogenous reagents were added in this experiment, so there was no such expenditure. The energy consumption calculation of the whole process is listed in the [supplementary material](#). The results of energy consumption analysis are presented in [Table 3](#).

Table 3 The energy consumption and benefit analysis in stages III and IV of the pilot-scale system

The [Table 3](#) manifested that the maximum energy consumption of the pilot-scale system came from heating pump with 29.167 kWh/m³, accounting for 98.0% of total energy consumption. This part of energy consumption is only related to the specific heat capacity of wastewater and the fermentation temperature. Hence, full utilization of waste heat, heat loss reduction and effective methane production could be the feasible approaches to realize energy balance of anaerobic process.

Compared with traditional ABR system, the difference of the present system is its introduction of electrodes, so their energy consumption and benefits attract much attention. The energy consumption of MECs was 0.600 kWh/m³ of filtrate. The electric charge is \$ 0.140/kWh in Xiamen, China. Thus, the operating cost of MECs was \$ 0.084/m³ of filtrate.

In terms of energy recovery, the methane yield in stage IV was 282 mL/g COD_{removed}, the average influent COD was 12500 g·m⁻³ and the average COD removal rate was 86.6%, so the methane production rate was calculated as 3.05 m³ CH₄/ m³ of filtrate. The electricity generation from one cubic meter methane is about 3.59 kWh

(Tabatabaei and Ghanavati, 2018). Therefore, the reclaimed energy of methane in stage IV was 10.95 kWh/m³ of filtrate.

The methane yield in stage III was 241 mL/g COD_{removed}. The methane production rate was calculated as 2.23 m³ CH₄/m³ of filtrate, and the recoverable energy from methane in stage III was 8.006 kWh/m³. Thus, through microbial electrolysis, the extra energy recovered from methane was 2.944 kWh/m³. The power consumption of MECs was 0.600 kWh/m³, so the net energy benefit was 2.344 kWh/m³. In conclusion, the ABR system assisted by symbionts of SFAS and exoelectrogens achieved more efficient exploitation of sludge filtrate than the ABR system, the methane production benefited from MECs was superior to its own energy consumption.

3.3.3 The feasibility analysis for the ABR system assisted by symbionts of SFAS and exoelectrogens for treatment of sludge filtrate

Herein, a pilot-scale ABR system assisted by symbionts of exoelectrogens and SFAS was developed to treat sludge filtrate. The results demonstrated that the symbionts of SFAS and exoelectrogens can reduce accumulation of VFAs, so as to improve COD removal efficiency and methane production. These results further verified the technical feasibility to enhance the degradation of VFAs and improve the methanogenesis via "MECs dominated by symbionts of SFAS and exoelectrogens". However, in terms of engineering application, there are still some key technical and economic challenges in enriching symbionts of SFAS and exoelectrogens in an anaerobic system:

Firstly, the combination mode of electrodes directly affects the distribution of

functional microorganism and the performance of AD system (Callegari et al., 2018). The coupling of electrodes affect the flow path and could result in dead zone of current. It is necessary to optimize the combination mode of electrodes via internal structure and hydraulic analysis.

Secondly, the construction cost of electrochemical system is relatively high, which is mainly reflected in conductive materials and automatic monitoring equipment. Controlling the construction cost is important in practical application.

Thirdly, the introduction of MECs requires additional power input. Although the external voltage is not high, a long-term operation still causes certain power consumption. For example, the energy consumption of MECs in this system was 0.06 kWh/m³. Reducing the energy consumption of an anaerobic system is a long-term pursuit of both scientific research and technical management. In view of this challenge, based on the understanding of energy consumption calculation, the authors suggest that by reasonably controlling the spacing of electrodes, the surface area of electrode, and the number and combination mode of electrodes, the energy consumption can be effectively reduced on the premise of effective methanogenesis.

To sum up, the ABR system assisted by symbionts of exoelectrogens and SFAS is feasible for treatment of sludge filtrate in terms of both technical and economic level, despite more work can be done to improve its efficiency and economy.

4. Conclusion

A pilot-scale ABR system assisted by symbionts of SFAS and exoelectrogens was

developed to treat sludge filtrate. The performance and economic evaluation were studied and the main results were as follows:

(1) The microbial electrolysis improved the treatment efficiency of sludge filtrate. The COD removal efficiency increased from 74.1% to 86.6% and the methane content increased from 81.5% to 92.2% with methane production rising from 241 to 282 mL/g COD_{removed}.

(2) The enrichment of *Syntrophomonas*, *Geobacter*, *Methanobacterium* and *Methannosarcina* on the expanded electrodes verified its feasibility to enhance the methanogenesis rate through enriching SFAS, exoelectrogens and hydrogenotrophic methanogens simultaneously.

(3) The economic analysis demonstrated that the pilot-scale system achieved efficient exploitation of organic energy from sludge filtrate, and the benefit of MECs for methanogenesis was superior to its own energy consumption. The ABR system assisted by symbionts of SFAS and exoelectrogens is feasible for treatment of sludge filtrate in terms of both technical and economic level through the pilot-scale verification.

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Conflict of interest

The authors declare that they have no conflict of interest.

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