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Published in:
Environmental Technology (United Kingdom)

Link to article, DOI:
[10.1080/09593330.2018.1540664](https://doi.org/10.1080/09593330.2018.1540664)

Publication date:
2020

Document Version
Peer reviewed version

[Link back to DTU Orbit](#)

Citation (APA):
Wang, T., Lv, N., Pan, X., Li, C., & Zhu, G. (2020). Process performance and microbial community functional structure in a thermophilic anaerobic baffled reactor coupled with biocatalysed electrolysis. *Environmental Technology (United Kingdom)*, 41(12), 1535-1545. <https://doi.org/10.1080/09593330.2018.1540664>

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**Process performance and microbial community functional
structure in a thermophilic anaerobic baffled reactor coupled with
biocatalyzed electrolysis**

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Abstract: In this study, the performances of a conventional anaerobic baffled reactor (ABR) and an ABR combined with microbial electrolysis cells (MECs) for enhancing degradation of volatile fatty acids (VFAs) were evaluated in 55 °C. The ABR-MECs system achieved a total chemical oxygen demand (COD) removal rate of 97.2% and a methane yield of 236 ± 5 mL/g COD removed at organic loading rate (OLR) of 6.9 kg COD $\text{m}^{-3} \text{d}^{-1}$, which were higher than those of the ABR with 77.6% and 207 ± 5 mL/g COD removed, respectively, at OLR of 5.1 kg COD $\text{m}^{-3} \text{d}^{-1}$. The pyrosequencing analysis confirmed that the introduction of MECs into ABR was conducive to establishing stable functional communities of syntrophic fatty acids oxidizing bacteria (SFOB), exoelectrogens and hydrogenotrophic methanogens, such as *Syntrophobacter* (5.4%), *Thermodesulfovibrio* (2.0%), *Methanobacterium* (43.8%), *Methanolinea* (20.4%). The content of unclassified bacteria increased from 12.4% in the ABR system to 52.3% in the ABR-MECs system. In contrast, the proportion of aceticlastic methanogens decreased from 50.1% in the ABR to 24.5% in the ABR-MECs system. The improved performance of thermophilic ABR-MECs system was resulted from phase separation, wide ecological niche and intensification of methanogenesis process via functional microbes, which significantly enhanced the degradation of propionic acid and acetic acid.

Key words: Anaerobic digestion; Microbial electrolysis cell; Methanogenesis; Thermophiles; Microbial community

1. Introduction

Anaerobic digestion (AD) has been widely applied in treating organic wastewater due to its advantage in energy recovery [1,2]. Nevertheless, some challenges such as low processing capacity and instability limited its efficiency for further application [3]. Correspondingly, different methods were adopted to improve efficiency of the AD process. For instance, researchers tried to optimize the conditions via engineering control and online monitoring in the early days. Boe et al. put forward several indicators for monitoring the AD process [4]. The VFAs, especially propionic acid, were important indicators for stable operation of the AD process [5].

In recent years, bioaugmentation technology has been widely investigated and applied in the AD process. Generally, the metabolic rate between acidogens and methanogens is imbalanced, which resulted in instability and accumulation of VFAs [6]. In response to this problem, Li et al. proposed the application of bioaugmentation to prevent failure from ammonia and propionate inhibition [7]. Town and Dumonceaux made use of a small and stable consortium capable of catabolizing acetate for stimulating methanogenesis [8]. These previous studies indicated the bioaugmentation via SFOB (e.g. propionic / butyric oxidizing bacteria) and acid-resistant methanogens was a meaningful attempt to avoid excessive acidification in the AD process. Indeed, bioaugmentation technology can reconstruct microbial community, improve abundance of functional bacteria and make up the imbalance between acidogens and methanogens to certain extent. However, the traditional bioaugmentation technology was hard to

make up the limitations of methanogens (e.g., long generation cycles and narrow ecological niches) with acclimation of SFOB and acid-resistant methanogens [9]. Besides, how to keep stable existence of functional microbes is a key issue for traditional bioaugmentation.

Bio-electrochemical system (BES) has been a promising energy-friendly system dealing with the imbalance between acidogens and methanogens via the function of exoelectrogens [10,11]. In a MEC, exoelectrogens transfer electrons to the anode by oxidizing organic matters, and the electrons pass through circuit to the cathode to produce H_2 . Thereafter, methane can be produced in the biocathode via hydrogenotrophic methanogens [12]. The previous study indicated that exoelectrogens compete with methanogens for electrons, realizing the oxidation of organic matter in the anode. In the cathode, exoelectrogens obtain electrons from cathode and conduct the synthesis of methane via H^+ , CO_2 and electrons. BES dominated by exoelectrogens has lots of advantages in utilization of organic matter and biogas production. Clauwaert et al. investigated one mole acetate could be converted into 0.41 mole methane at the temperature ranges of 22 - 28 °C [13]. In addition, Hao et al. observed the exoelectrogens had a better adaptability than methanogens [14]. Therefore, it is quite possible to take advantage of exoelectrogens to enhance the oxidation of acetic acids and methane generation, making up the imbalance of metabolic rates between acidogens and methanogens. Actually, MECs have been already applied in the AD system. Overall, the previous studies on the AD-MECs mainly focused on: (1) Efficiency enhancement,

e.g. the improvement of methane production and energy recovery rate (Gajaraj et al., 2017, Hassanein et al., 2017). (2) Microbial community. The hydrogenotrophic methanogens enriched in the cathode played important role in enhancing methane yield (Gholikandi et al., 2014). Besides, the enrichment of exoelectrogens in the anode improved the removal of organics (Cerrillo et al., 2016, Liu et al., 2017). (3) Electron transfer mechanism. For instance, the zero-valent iron or suitable extra voltage promoted the direct electron transfer of exoelectrogens (Zhang et al., 2013, Choi et al., 2017). Although the enhanced efficiency of the AD system by MECs and microbial composition were mainly focused, synergistic degradation of VFA via functional bacteria has not been paid much attention. Actually, the decomposition of propionic acid and butyric acid by exoelectrogens is much slower than acetic acid (Clauwaert et al., 2008), which limits further application of the AD-MECs system. To accelerate the VFA degradation, the acclimated SFOB was applied to enhance degradation of propionic and butyric acid, further improve the removal of organics and methane production in the AD-MEC system. Through the synergistic effect of SFOB and exoelectrogens in the thermophilic condition, VFA can be fast degraded in this study.

To the best of our knowledge, bioaugmentation technology based on thermophilic SFOB and exoelectrogens for boosting degradation of VFAs has not been applied and evaluated for the AD process. And its performances and responses of microbes have not yet been explored. So, an improved ABR-MECs system was established and evaluated. The ABR-MECs system was compared with a conventional ABR in performances, such

as COD removal, methane production and indicators for stability (i.e., VFAs, pH, alkalinity (ALK) and [Oxidation-Reduction Potential](#) (ORP). Moreover, microbial communities were analyzed to further demonstrate performance of the ABR-MECs system.

2. Materials and methods

2.1. Configuration, start-up and operation of the ABR-MECs system

The volume of the ABR is 2.5 L with circling hot water to keep temperature of 55 ± 1 °C. The ABR is divided into five equal compartments by vertical baffles as shown in Fig.1. [The temperature of the substrate tank was controlled by a water jacket with a cooler \(4 °C\). The temperature of ABR was assured by water jacket and heater, and submerged thermometers were equipped to indicate the temperature in the reactors.](#) Each compartment is divided into down-corner and up-corner regions by hanged baffles of 45°. The synthetic wastewater was fed via a peristaltic pump ([BT100-2i, Longer Pump, Britain](#)). The reactor is sealed and the top of each compartment is connected to a 7 L gas sampling bag for measuring the volume of biogas via a flow meter ([LML-1, Changchun Automobile Filter., China](#)). The anodes and cathodes are fixed in up-flow region of the three middle compartments and connected with power source (0.9 Volt).

[The start-up was performed in a conventional ABR with OLR of 1.4 kg COD m⁻³d⁻¹, and a stable COD removal of \$72 \pm 1\%\$ was achieved in the end of start-up stage \(Period 1, Hydraulic Retention Time \(HRT\)=26 h\).](#) Further COD removal was hard to

be obtained when low influent COD was given as insufficient COD presumably restricted the growth of sludge microorganisms. Correspondingly, OLR in the conventional ABR (Period 2, HRT=21 h) climbed up from 1.7 to 5.1 kg COD m⁻³ d⁻¹. However, when OLR was 5.1 kg COD m⁻³ d⁻¹, the COD removal rate decreased significantly (Fig. 2d) and VFAs were accumulated in the fifth compartment (Fig. 3, 5b). Therefore, MECs were introduced into the traditional ABR by inserting electrodes with applied voltage, which was made as described in Section 2.3 to enhance the degradation of VFAs after 28 days operation of Period 2. Meanwhile, the sludge contained SFOB and exoelectrogens acclimated in the Section 2.3 were inoculated to accelerate the process of the ABR-MECs system. Therefore, the anaerobic process was defined into three periods. The first period was the start-up stage under low OLR in the ABR system. The second period was the operational stage in the ABR system. And the third period was operational stage after the electrodes inserting into the ABR system (ABR-MECs system). The introduction of MECs built a suitable electrochemical environment for stable existence of exoelectrogens. The operation of the ABR-MECs system started with OLR of 5.1 kg COD m⁻³d⁻¹ and finally increased to 6.9 kg COD m⁻³d⁻¹ (Period 3, HRT=21 h). The operation lasted for 72 days as described in Table 1.

Fig.1. Process flow schematic of the ABR-MECs system.

Table 1 Operation of the conventional ABR and ABR-MECs systems

2.2. Characteristics of wastewater and inoculated sludge

The study aimed to verify the feasibility of boosting VFAs degradation by SFOB

and exoelectrogens. So, glucose was applied as single carbon source to reduce interference factors in the experiment. The NH_4Cl and KH_2PO_4 were provided as nitrogen and phosphorus sources. In addition, the NaHCO_3 was given to regulate pH value. The concentration of synthetic wastewater was prepared according to Table 1. Constant element and mineral solution of 1.5 mL^{-1} was also added as nutrients based on our previous study [21]. It was prepared by dissolving 50 mgL^{-1} H_3BO_3 , 30 mgL^{-1} CuCl_2 , 50 mgL^{-1} MnSO_4 , 50 mgL^{-1} $(\text{NH}_4)_6\text{MoO}_{24} \cdot 4\text{H}_2\text{O}$, 50 mgL^{-1} AlCl_3 , 50 mgL^{-1} $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ and 50 mgL^{-1} NiCl_2 in the distilled water. Seed sludge of ABR was obtained from an anaerobic digester treating filtrate from hydrothermally treated sewage sludge [22]. The seed sludge in each compartment of reactor was about half of the working volume (0.5 L per compartment). SS and VSS of Seed sludge were 31.2 gL^{-1} and 12.5 gL^{-1} , respectively.

2.3. Pre-acclimation of exoelectrogens and SFOB in static experiment

Exoelectrogens were pre-acclimated in microbial fuel cells (MFCs). The beaker with 500 mL volume was applied to constitute a single chamber MFC. Resistance of 1000Ω , electrode film and substrate were formed together as a circuit. Sodium acetate was added as carbon sources to provide COD with concentration of 4000 mgL^{-1} while 50 mM phosphate buffer nutrient medium was applied to sustain pH value of 7.0. A mineral solution of 1.5 mL^{-1} was also added as nutrient based on our previous study [21]. The electrode was 54.0 cm^2 with 6.0 cm in width and 9.0 cm in length. The anodes and cathodes were made of carbon cloth (WOS1009, CeTech, Taiwan) and stainless

steel mesh catalyzed by Ni nanoparticles, respectively. Six parallel groups were executed in 55 ± 1 °C. One cycle was finished when COD removal rate was higher than 80% and then substrate was replaced to begin another cycle. Stable voltage higher than 300 mV was generated by MFCs after 5 cycles, indicating that electrodes with enriched exoelectrogens were well prepared. [More detail about the modified method of cathodes and procedure of exoelectrogens acclimation can be found in our previous article \[21\].](#)

The SFOB was acclimated in anaerobic bottles. Sodium propionate and sodium butyrate were applied in equal amount as carbon sources to provide COD with concentration of 4000 mgL⁻¹. The proportion of COD: N: P was 500: 5: 1. A mineral solution of 1.5 mL⁻¹ was also added as nutrients [based on our previous study \[16\]](#). Six parallel groups were executed in 55 ± 1 °C. Substrate was updated every 7 days. After five cycles, the COD removals were more than 93.8%. Besides, butyrate was not detected and the removal rate of propionate was 98.2%, suggesting the acclimated sludge with enriched SFOB was well prepared.

In order to construct an improved ABR-MECs system for enhancing the degradation of VFAs, the acclimated sludge containing 60% exoelectrogens and 40% (V/V) SFOB was inoculated into the middle three compartments [as soon as electrodes inserted into the ABR system after operation of Period 2. The proportion of inoculation was 5% \(V/V\) working volume \(0.5 L\) of per compartment. Besides, in order to keep the sludge of system consistent, equivalent original sludge was removed before inoculation.](#)

2.4. Analytical methods

Effluent samples from each reactor and substrate were taken from the sampling ports based on HRT. All chemical analyses of effluent such as pH, alkalinity, COD were followed Standard Methods [23]. The VFAs and biogas were measured via a gas chromatograph (GC9790II, Fuli, China) with He as the carrier gas and hydrogen for burning of the flame. The VFAs were measured with a flame ionization detector using a capillary column (ATFFAP 30 m $\times$ 0.32 mm $\times$ 0.50 μ m), and the temperature of column, injector, and detector were 150 °C, 210 °C, and 220 °C, respectively. The biogas was monitored with a thermal conductivity detector equipped with a stainless steel column (TDX-01 2 m $\times$ 3 mm), and the temperature of column, injector, and detector were 120 °C, 120 °C and 150 °C, respectively. Gas sample was taken from each gas pipeline by a 1mL syringe equipped with metal hub needles to measure the gas composition. A volume of 0.5 mL of gas sample was injected into the gas chromatograph. Microorganisms in sludge and electrodes were also observed by scanning electron microscope (S-4800, Hitachi, Japan). The samples were prepared according to following method [21]: first, samples were fixed by 2.5% solution of paraformaldehyde for 0.5 h, and then dehydrated by ethanol series of 50%, 70%, 80%, 90% and 100% (v/v) for 0.5 h each time, and subsequently dried by a critical point dryer (HCP-2, Hitachi, Japan). Data in the steady state were collected at least three times. The average values of these measurements are presented in Table 2.

The sludge samples of days 45 (mature sludge in the middle three compartments of

conventional ABR) and days 70 (only biofilm of both anodes and cathodes in the ABR-MECs system) were collected to analyze microbial communities via high-throughput 16S rRNA pyrosequencing. The samples collected from anodes were mixed to extract DNA for three times and then the DNA samples were mixed to conduct the high-throughput 16S rRNA pyrosequencing. The samples collected from cathodes and the ABR system were handled as those of anodes. DNA was extracted by FastDNA TM SPIN Kit for Soil (MP Biomedicals, USA). The V3-V4 region of 16S rRNA gene was amplified by PCR using the primers 341 F (CCTACGGGNGGCWGCAG), 785 R (GACTACHVGGGTATCTAATCC) for bacteria and the primers 787 (ATTAGATACCCSBGTAGTCC), 1059 R (GCCATGCACCCWCCTCT) for archaea. Additional information about DNA extraction, PCR and sequencing can be found elsewhere [24]. The raw sequence data were deposited in the NCBI Short Reads Archive database under the accession number [SRP150392](#).

3. Results

3.1. The COD removal efficiency

The COD removal efficiency in different stages is showed in Fig.2 (d). The growing influent COD in the conventional ABR (Period 2) led to a rapid increase of total COD removal rate of 95.3% when influent COD was 3500 mgL⁻¹. Therefore, enough nutrients with moderate alkalinity were beneficial to the operation of ABR. Nevertheless, the COD removal rate decreased significantly as influent COD climbed

up to 4500 mgL⁻¹ with OLR of 5.1 kg COD m⁻³d⁻¹. And 77.6% of COD removal rate was detected in the conventional ABR in the end of Period 2 regardless of adequate ALK. Accordingly, the concentration of VFAs (Fig.3 1b-5b) indicated that propionic acid and acetic acid were dominant in the conventional ABR, causing fast growing of effluent COD. The phenomenon indicated the ABR system was in a critical stable state accompanied with the occurrence of propionic accumulation in Day 45. It was benefited to the investigation on propionic degradation when electrodes were inserted at this time. Therefore, the operation of conventional ABR was stopped and further modified by inserting electrodes and inoculating enriched SFOB and exoelectrogens to establish an improved ABR-MECs system. After modification, the total COD removal rate of the ABR-MECs system increased to 97.2% when the OLR was up to 6.9 kg COD m⁻³d⁻¹, 19.6% higher than that of conventional ABR with OLR of 5.1 kg COD m⁻³d⁻¹. Besides, the COD removal rates in five compartments were 54.3%, 36.7%, 2.2%, 2.3% and 1.8%, respectively, while those in the ABR were 29.1%, 18.4%, 13.9%, 9.2% and 7.1%, correspondingly. Clearly, the COD removal efficiencies were promoted in front parts of ABR-MECs system. The pollutant removal capacity was significantly enhanced in the ABR-MECs system. The improved performance was due to further degradation of VFAs (Fig.3, 1-5a). Specifically, both acetate and propionate were reduced significantly in the ABR-MECs system.

Fig.2. Process changes of COD, ORP, ALK and pH in C1, C2, C3, C4 and C5. C1 to C5 mean the first to fifth compartment of reactor in flow direction.

Fig.3. Contrast of VFAs in (a) the ABR-MECs, (b) conventional ABR.

3.2. The methane content performance

Average contents of methane in the first to fourth compartments were 56.3%, 63.7%, 66.2% and 73.8%, respectively, in the stable stage of the ABR system. In contrast, Average contents of 58.6%, 72.8%, 80.9% and 83.5% were detected correspondingly in the stable stage of the ABR-MECs system. The maximum content of CH₄ (83.5%) in biogas was detected in C4 with a total production rate of 236 ± 5 mL CH₄/g COD removed in the ABR-MECs system. The maximum content of CH₄ in conventional ABR was 73.8% and the total production rate was 207 ± 5 mL CH₄/g COD removed. Besides, contents of CO₂ in the middle three compartments (MECs) were 25.3%, 17.6% and 14.2%, respectively, while those of conventional ABR were 30.4%, 27.3% and 25.7%, correspondingly. More net methane and less carbon dioxide were detected in the ABR-MECs system when compared with the ABR system. They were also higher than the previous study [25], in which methane content was usually from 50% to 75% while the carbon dioxide content was from 25% to 50% in the AD system. The results indicated that energy production was enlarged in the ABR-MECs system.

3.3. The characteristics of ORP, alkalinity and pH values

The ORP, alkalinity and pH values were important indicators of stability in the anaerobic system and their changes were depicted in Fig.2 (a-c). The ORP is one of key factors influencing acid fermentation types and methane production. As can be seen in Fig. 2a, the range of ORP in the ABR-MECs system is between -240 mV and -280 mV.

Notably, it is higher than the ORP with the range of -280 mV to -320 mV in the ABR system. Higher ORP was obtained in the ABR-MECs system to produce methane than other results made by Khanal and Huang [26], who observed that the best ORP for methanogens was -350 mV. Besides, the ORP of the middle three compartments (MECs parts) were higher than the first and last compartment in the ABR-MECs system. It indicated that the introduction of MEC increased the ORP of the AD system. As for alkalinity and pH, the influent alkalinity in the conventional ABR (Period 2) grew fast to keep enough buffer capability. In the ABR-MECs (Period 3), higher effluent alkalinity (2912.9 mgL⁻¹) was detected with lower influent alkalinity (2900 mgL⁻¹) when compared with the corresponding 2769.6 mgL⁻¹ and 3200 mgL⁻¹ in the conventional ABR. The pH values of the middle three compartments grew up from 6.9 - 7.1 to 7.2 - 7.3 when the ABR transformed to ABR-MECs system.

3.4. Microorganisms morphology analysis

The sludge in the conventional ABR and ABR-MECs systems were analyzed by SEM to characterize the structure and morphology, as showed in Fig. 4. The major species of C2-C4 changed from globular bacteria in the ABR to bacillus of anodes in the ABR-MECs system, revealing difference of functional microbes in the methanogenic phase. Notwithstanding, there was no distinct change of microorganism morphology in the C1, suggesting that the main functional microorganism in the acidogenic phase did not change much between the ABR and ABR-MECs system. It was consistent with the results obtained by Liu et al.[27], who found that shot rod-shaped and filamentous

bacteria in initial compartment of the ABR were responsible for hydrolysis and acidification. Clearly, the electrochemical system only changed microbial communities of methanogenic phase in the ABR-MECs system, making it more effective in degradation of product from acidification. Particularly, microbial community would be further analyzed in the pyrosequencing analysis.

Fig.4. Photograph of scanning electron microscope: (a) 01, sample from C1 in the ABR stage, (a) 02, sample from C1 in the ABR-MECs stage, (b) 01, sample from C2-C4 in the ABR stages, (b) 02, sample from anodes in the ABR-MECs stage.

3.5. Pyrosequencing analysis.

Pyrosequencing analysis was performed to find the specific difference of microbial communities between the conventional ABR and ABR-MECs systems. Communities of functional eubacteria at phylum level in the ABR and ABR-MECs systems were described in Fig.6. Although the studies on the microorganism of integrated AD-MEC system were the research hot spot, the achievement of thermophilic condition are relatively deficient. However, some comparisons can still be found. (1) The dominant bacteria in both ABR and ABR-MECs systems were *Firmicutes* and *Proteobacteria* with contents of 14.7% and 9.8% in the ABR-MECs system, and 49.0% and 16.5% in the ABR system. The analysis of microbial was in agreement with the previous studies [28,29] that *Firmicutes* was dominant in the thermophilic electrochemical system and thermophilic anaerobic system. The difference lies in a large increase of *Firmicutes* in the ABR-MECs system. It further proved the significance of *Firmicutes* in the

thermophilic electrochemical system. In order to find the further difference between these two systems, eubacteria at genus level were described in Fig.5 (b). (2) The potential exoelectrogens, a significant growth of *Thermodesulfovibrio* [30] was found in the ABR-MECs system with proportion of 2.01% when it was not detected in the ABR system. It was different from other thermophilic system [18] that *Desulfuromonadaceae* was the most active family in a thermophilic MEC. Furthermore, *Geobacter*, one kind of main exoelectrogens discovered in the mesophilic MECs system, was completely disappeared in the thermophilic ABR-MECs system. It's worth noting that the proportion of *Unclassified bacteria* in the ABR-MECs system was nearly 5 times (52.6%) of that in the ABR system. All these finding further indicated the complexity of thermophilic electrochemical system, and the physiological characteristics of thermophilic exoelectrogens remained to be further studied. On the other hand, the content of *Syntrophobacter*, one of SFOB that made use of propionate, was promoted 272.4% in the ABR-MECs system, accounting for the effective degradation of propionic acid in the ABR-MECs system. The increased abundance of *Syntrophobacter*, *Thermodesulfovibrio* and *Unclassified bacteria* further confirmed that the introduction of MECs into ABR system was conducive to establishing stable communities of exoelectrogens and SFOB in the anode.

Communities of functional archaea in both ABR and ABR-MECs systems were illustrated in Fig.6. The abundance of hydrogenotrophic methanogens in the cathode was 68.4%, nearly double of that in the ABR. At genus level, as depicted in Fig.5 (a),

Methanobacterium, one kind of hydrogenotrophic methanogens, was detected at 43.8 % of archaea in the ABR-MECs system, which was nearly double of those in the conventional ABR system. The abundance of *Methanolinea* (20.4%) in the ABR-MECs system, one of hydrogenotrophic methanogens, is 10 times of that in the ABR. This finding suggested that both hydrogenotrophic methanogens were well enriched in the cathode of ABR-MEC system. In contrast, the proportion of acetoclastic methanogens decreased from 50.1% in the ABR to 24.5% in the ABR-MECs system. The increase of hydrogenotrophic methanogens and exoelectrogens as well as decrease of acetoclastic methanogens made fewer generation of CO₂, building up the biological basis of purification of methane in site.

Fig.5 Genus of Archaea and Eubacteria in both ABR and ABR-MECs systems: (a) Archaea of middle three compartments in the ABR system and archaea of cathodes in the ABR-MECs system; (b) Eubacteria of middle three compartments in the ABR system and Eubacteria of anodes in the ABR-MECs system.

Fig.6 Relative abundances of functional microbes in both ABR and ABR-MECs systems:

Table 2 Process performance for the thermophilic ABR and ABR-MECs systems in the stable state

4. Discussion

In this study, an effective AD-MECs process was established to accelerate degradation of VFAs and generation of methane. Some main parameters in stable period

were depicted in Table 2. The new system has a better efficacy than traditional ABR system in COD removal and methane production. Besides, the performance was also better than the results of previous study [31], in which COD removal rate was 68 % -72 % with influent COD concentration of 5000 mgL⁻¹ and HRT of 120h at operational temperatures of 37 °C and 55 °C, respectively. The improved performance was resulted from further degradation of VFAs (Fig.3, 1-5a). The total VFAs in five compartments were 4107.3 mgL⁻¹, 3169.2 mgL⁻¹, 1938.8 mgL⁻¹, 1179.2 mgL⁻¹ and 869.4 mgL⁻¹, respectively, in the conventional ABR. In contrast, a sharp decrease was sketched in the ABR-MECs system, about 63.0% drop of VFAs was detected in effluent. Specifically, both acetate and propionate were reduced significantly in the ABR-MECs system. It is consistent with the previous studies that propionic acid can be fast oxidized into acetic acid by propionate oxidation bacteria in the AD process [32] and the concentration of acetic acid had a substantial decrease in the MECs system [33]. The results further indicated that SFOB and exoelectrogens performed well in the ABR-MECs system. As an evidence, the *Syntrophobacter* (5.4%), *Thermodesulfovibrio* (2.0%), *Methanobacterium* (43.8%), *Methanolinea* (20.4%) were well enriched in the ABR-MECs system as showed in Fig.5. Thus, it was an effective way to improve COD removal efficiency by enriching both SFOB and exoelectrogens in the anode, making the new system better adapt to propionic acid fermentation.

As for methane production, more methane and less carbon dioxide were achieved in the ABR-MECs system and the advantages were still obvious when compared with

other AD process. The content of CH₄ was 73.0% - 75.4% in a pilot-scale UASB [34] and 180 mL CH₄/g COD removed was detected by Wirth et al. [31]. The results in Table 2 indicated that more and purer methane (236 mL CH₄/g COD removed) was generated in the ABR-MECs system. One direct reason was that the abundance of hydrogenotrophic methanogens (67.2%) in the ABR-MECs system, which were nearly double of that (37.5%) in the ABR system as depicted in Fig.6. They were also significantly higher than the traditional AD system in which aceticlastic methanogens were dominant in the methanogenic process. The significant increase of hydrogenotrophic methanogens can be due to the enhanced production of H₂ by exoelectrogens [35]. In all, the exoelectrogens boosted the degradation of acetic acid, removed acetic inhibition for SFOB and simultaneously promoted the abundance of hydrogenotrophic methanogens and generation of methane.

Nevertheless, If it was the only way consuming hydrogen via hydrogenotrophic methanogens, hydrogen should be detected in the system, as hydrogenotrophic methanogens cannot completely remove hydrogen if methane production was only caused by converting hydrogen [36]. In fact, hydrogen was not discovered in the ABR-MECs system. There may be other ways to generate methane in the ABR-MECs system. The electrochemical systems offer possibilities for new methanogenic pathways. The exoelectrogens were defined as a kind of microorganisms with extracellular respiration. Specifically, methane could be produced by exoelectrogens through direct electron transfer in bio-cathode with the reaction of $\text{CO}_2 + 8\text{H}^+ + 8\text{e}^- = \text{CH}_4 + 2\text{H}_2\text{O}$ [37].

Indeed, *Methanobacterium*, one kind of methanogens with electrochemical active, was detected at 43.83 % of archaea in the cathodes of the ABR-MECs system, which was nearly double of the ABR as described in Fig.5 (a). Correspondingly, more CO₂ was fixed in site realizing the purification of methane [38]. The significance of *Methanobacterium* discovered in cathodes not only certified such a new way to generate methane but also proved that it can play a leading role in certain conditions. Thus, higher content of methane in the ABR-MECs system was resulted from enrichment of hydrogenotrophic methanogens and methanogens with electrochemical active (e.g. *Methanobacterium*) in the cathodes. It is an effective way to improve generation of methane by coupling biocatalyzed electrolysis reactor with conventional AD process.

The interaction of SFOB, exoelectrogens and hydrogenotrophic methanogens in the ABR-MECs system originated from two aspects: (1) organic matters that hard to utilized (i.e., propionic acid, butyric acid) were fast degraded to acetic acid by SFOB and in turn exploited by exoelectrogens in the anode; (2) Electrons in the cathode obtained by exoelectrogens directly or transformed to H₂ and then made use by hydrogenotrophic methanogens to produce methane. The existent of SFOB reduced the accumulation of propionic acid and provided enough substrate for exoelectrogens. The consumption of acetic acid by exoelectrogens not only removed the acetic inhibition of SFOB, but also promoted the enrichment of the hydrogenotrophic methanogen. Therefore, the SFOB and exoelectrogens were the most important functional microorganisms in the ABR-MECs system to realize fast degradation of VFAs.

It is worth noting that the purer methane was generated stably in the ABR-MECs system when ORP was in the ranges of -250 mV ~ -280 mV. Higher ORP was obtained to produce methane compared with conventional ABR and other results made by Khanal and Huang [26], who observed that the best ORP for methanogens was -350 mV, meaning wider ecological niche in the ABR-MECs system [39]. Wang et al. found that maximum electric current was generated in a modified MFC with ORP of -219 mV. The finding demonstrated the exoelectrogens can adjust themselves well in high ORP [39]. Thus, methane can be produced without such strict anaerobic conditions as conventional ABR did, facilitating the running of anaerobic system. It may be a practical advantage in further application of the AD-MECs system. Besides, high ALK and pH values resulted from efficient degradation of VFAs were considered to other factors which were beneficial to the stability of system [21]. It was reported by Gil et al. that MFC achieved a maximum current density with an optimal pH range of 7.0 - 8.0 [40], while O'Flaherty et al. reported that the growth rates of methanogens could be inhibited when pH was above 7.5 or below 7.0 [41]. Therefore, pH level of 7.2 - 7.3 in the MECs parts of the ABR-MECs system were in favor of methane production. Consequently, a wide ecological niche containing suitable ALK, pH and wide range of ORP was the chemical foundation of the ABR-MECs system to perform well in the COD removal and methane production.

In a traditional AD process, methanogenesis was the speed-limiting step and unbalanced metabolic rate between methanogens and acidogens resulted in poor

treatment and unstable performance. In the ABR-MECs system, middle three compartments were coupled with electrodes to produce methane while the first compartment performed as acidogenic phase, making them more integral and effective. Favorable structure of ABR realized cascade-utilization of organic matter in different units with phase separation. Similar research was conducted by Cusick et al.[42], who coupled several electrodes in [series](#) as an integral MEC in pilot scale and obtained better efficiency. The better performance of the ABR-MECs system fundamentally originated from phase separation, wide ecological niche and synergistic degradation of VFAs via SFOB, exoelectrogens and hydrogenotrophic methanogens.

5. Conclusion

Biocatalyzed electrolysis significantly improved the performance of anaerobic system in organic removal efficiency and methane production. More importantly, microbial analysis certified that, it was the synergy effect of exoelectrogens and SFOB in the anode correspondingly with hydrogenotrophic methanogens and methanogens with electrochemical activity in the cathode achieved rapid degradation of organic matter and considerable methane production in the new system. These finding further confirmed that it provided an insight into methanogenic steps from cascade degradation of VFAs by the SFOB, exoelectrogens and hydrogenotrophic methanogens.

Acknowledgement

The authors would like to thank the supports from the National Key Research and Development Program of China (Contract No. 2017YFD0800804-03), the National Natural Science Foundation of China (Contract No. 21477122, 51678553, 21276248), the Project of the Natural Science Foundation of Fujian Province (Contract No. 2016J01273, 2017J05092), IUE CAS Young Talents Frontier Project (Contract No. IUEQN201501) and the Japanese - China Research Cooperative Program (Contract No. 2016YFE0118000).

Conflict of interest

The authors declare that they have no conflict of interest.

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Figures Captions

- 591 **Fig.1.** Process flow schematic of the ABR-MECs system.
- 592 **Fig.2.** Process changes of COD, ORP, ALK and pH in C1, C2, C3, C4 and C5. C1 to C5 mean the
593 first to fifth compartment of reactor in flow direction.
- 594 **Fig.3.** Contrast of VFAs in (a) the ABR-MECs, (b) conventional ABR.
- 595 **Fig.4.** Photograph of scanning electron microscope: (a) 01, sample from C1 in the ABR stage, (a) 02,
596 sample from C1 in the ABR-MECs stage, (b) 01, sample from C2-C4 in the ABR stages, (b) 02,
597 sample from anodes in the ABR-MECs stage.
- 598 **Fig.5** Genus of Archaea and Eubacteria in both ABR and ABR-MECs systems: (a) Archaea of
599 middle three compartments in the ABR system and archaea of cathodes in the ABR-MECs system;
600 (b) Eubacteria of middle three compartments in the ABR system and Eubacteria of anodes in the
601 ABR-MECs system.
- 602 **Fig.6** Relative abundances of functional microbes in both ABR and ABR-MECs systems:

Fig.1

Fig.2

Fig.3

Fig.4

Fig.5

Fig.6

Table 1 Operation of conventional ABR and the ABR-MECs systems

Stage	Days	COD (mgL ⁻¹)	HRT (h)	OLR (kg COD m ⁻³ d ⁻¹)	Alkalinity (mg CaCO ₃ L ⁻¹)	NH ₄ ⁺ -N (mgL ⁻¹)	PO ₄ ³⁻ (mgL ⁻¹)
Period.1	1-20	<u>1500 ± 50</u>	26	<u>1.4 ± 0.1</u>	580-1100	<u>75 ± 5</u>	<u>5 ± 1</u>
Period.2	21-48	1500-4500	21	1.7-5.1	1100-3200	75-225	5-15
Period.3	49-72	4500-6000	21	5.1-6.9	2400-2900	225-300	15-20

Period.1: start-up of conventional ABR, Period.2: conventional ABR system, Period.3: the ABR-MECs system.

Table 2 Process performance for the thermophilic ABR and ABR-MECs systems in the stable state

Items	ABR-MECs	ABR	Items	ABR-MECs	ABR
COD (mg L ⁻¹)	<u>6000 ± 150</u>	<u>4500 ± 100</u>	Effluent VFAs (mgL ⁻¹)	<u>321.4 ± 9.1</u>	<u>869.4 ± 23.2</u>
OLR (kg COD m ⁻³ d ⁻¹)	<u>6.9 ± 0.1</u>	<u>5.2 ± 0.1</u>	ORP	-250 mV to -280 mV	-300 mV to -330 mV
Maximum COD removal rate (%)	<u>97.2 ± 0.5</u>	<u>77.6 ± 0.5</u>	pH	6.5 - 7.4	6.9 - 7.1
Average CH ₄ content (%)	78.6 ± 1.0	65.3 ± 1.0	Influent ALK (mgL ⁻¹)	<u>2900 ± 50</u>	<u>3200 ± 50</u>
Maximum CH ₄ content (%)	<u>83.5 ± 2.0</u>	<u>73.8 ± 2.0</u>	Effluent ALK (mgL ⁻¹)	<u>2912 ± 23</u>	<u>2769 ± 52</u>
Average CH ₄ yield (L/g COD removed)	236 ± 5	207 ± 5			