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Enhanced methane production by alleviating sulfide inhibition with a microbial electrolysis coupled anaerobic digestion reactor

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ABSTRACT

Anaerobic digestion (AD) of organics is a challenging task under high-strength sulfate (SO_4^{2-}) conditions. The generation of toxic sulfides by SO_4^{2-}-reducing bacteria (SRB) causes low methane (CH_4) production. This study investigated the feasibility of alleviating sulfide inhibition and enhancing CH_4 production by using an anaerobic reactor with built-in microbial electrolysis cell (MEC), namely ME-AD reactor. Compared to AD reactor, unionized H_2S in the ME-AD reactor was sufficiently converted into ionized HS^- due to the weak alkaline condition created via cathodic H_2 production, which relieved the toxicity of unionized H_2S to methanogenesis. Correspondingly, the CH_4 production in the ME-AD system was 1.56 times higher than that in the AD reactor with alkaline-pH control and 3.03 times higher than that in the AD reactors (no external voltage and no electrodes) without alkaline-pH control. MEC increased the amount of substrates available for CH_4-producing bacteria (MPB) to generate more CH_4. Microbial community analysis indicated that hydrogentrophic MPB (e.g. *Methanosphaera*) and acetotrophic MPB (e.g. *Methanosaeta*) participated in the two major pathways of CH_4 formation were successfully enriched in the cathode biofilm and suspended sludge of the ME-AD system. Economic revenue from increased CH_4 production totally covered the cost of input electricity. Integration of MEC with AD could be an attractive technology to alleviate sulfide inhibition and enhance CH_4 production from AD of organics under SO_4^{2-}-rich condition.

1. Introduction

Anaerobic digestion (AD) is widely applied for decades in treating organic wastewaters due to its low cost and high efficiency (Cai et al., 2016; Kiyuna et al., 2017; Dereli et al., 2019). Methane (CH_4) recovery from organic wastewaters via AD is the most feasible and sustainable route to a bioenergy product (Muyzer and Stams, 2008; Dai et al., 2017). Unfortunately, organic effluents from many industries (such as paper, food, and pharmacy) usually contain high-strength sulfate (SO_4^{2-}) which can cause a serious inhibiting effect on AD of organic matter (Yuan et al., 2014; Chen et al., 2019). To date, enhancing CH_4 production in an anaerobic reactor under SO_4^{2-}-rich condition remains to be a critical challenge (Cetecioglu et al., 2019).

SO_4^{2-} reduction is inevitably involved in the anaerobic treatment process of organic wastewater containing SO_4^{2-} (Liu et al., 2015; Zhen et al., 2019). This is due to the fact that both SO_4^{2-}-reducing bacteria (SRB) and CH_4-producing bacteria (MPB) utilize the same substrates including acetate and hydrogen (H_2) as electron donors (Hu et al., 2015; Reyes-Alvarado, 2018). SO_4^{2-} can stimulate the growth of SRB, which can compete with MPB via thermodynamically favorable processes. For example, the utilization of acetate by SRB is thermodynamically more favorable than that by MPB in terms of the standard Gibbs free energy (SO_4^{2-} reduction $\Delta G^{0\prime}_{Acetate} = -47.6$ kJ/Reaction, methanogenesis $\Delta G^{0\prime}_{Acetate} = -31.0$ kJ/Reaction) (Muyzer and Stams, 2008). SRB have a higher affinity for substrates and grow faster than MPB, resulting in generating large amounts of sulfides (Zhang et al., 2011). The produced sulfides are mainly present as three species including unionized H_2S, ionized HS^- and S^{2-}, depending on the pH of the aqueous environment (Lu et al., 2018; Yuan et al., 2019). Among these species, unionized H_2S is the most toxic specie which penetrates the cell membrane of microorganisms and further inhibits bacterial metabolic activity (Wang et al., 2017; Wu et al., 2018). MPB are generally more sensitive to the toxicity of unionized H_2S than SRB (Li et al., 2015; Hu et al., 2019). Although several methods, such as precipitation

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with metal ion, pH control with alkali, SRB inhibition with molybdate, can be used to decrease unionized H_2S, the operational costs are too high for a long-term operation (Sabumon, 2008; Zhang et al., 2011). Elevating pH by adding alkali is commonly employed sulfide inhibition control method. The unionized H_2S can be converted into the ionized form under alkaline conditions (Wu et al., 2018). At pH of 6, most of the sulfides are in the form of unionized H_2S, while at pH of 8 most is present in the ionized HS^- (McCartney and Oleszkiewicz, 1991). MPB are capable to grow well in weak alkaline conditions with pH ranging from 7.5 to 8.5 (Yuan et al., 2014). Therefore, one underlying principle for enhancing CH_4 production is via the pH regulation to alleviate sulfide inhibition in the anaerobic reactor.

Recent studies have suggested that microbial electrolysis cell (MEC) could simultaneously generate H_2 and alkali (OH^-) (Wang et al., 2017; Cai et al., 2018; Blázquez et al., 2019; Miller et al., 2019). In MECs, the anode oxidizes organic matter to electrons and transfers electrons to the cathode, where they reduce the protons (H^+) to H_2 (Bond et al., 2002; Lu et al., 2012; Park et al., 2019). At the anode, almost all kinds of organic matters, such as volatile fatty acids (VFAs), carbohydrates and alcohols, can be used directly by exoelectrogens for electrohydrogenesis (Cheng and Logan, 2007; Lu et al., 2012; Zou et al., 2017). At the cathode, H_2 production consumes H^+ constantly and further increases the pH (Coma et al., 2013; Luo et al., 2014; Blázquez et al., 2016). Meanwhile, H_2O itself will become the source of H^+ with the pH exceeding 5.0, resulting in OH^- generation as the conjugate-base product after electron transfer (Conway and Tilak, 2002). Therefore, MEC may be a promising alternative to alkali addition for alleviating sulfide inhibition in the AD process. Recently, integrated reactors coupling MEC with AD were employed to efficiently enhance CH_4 production (Zhao et al., 2015; Liu et al., 2016; Cai et al., 2016, 2018). The H_2 produced at the cathode is the preferred substrate for CH_4 production in the AD process. To date, the integration of MEC in AD for alleviating sulfide inhibition and enhancing CH_4 production has not been studied.

Hence, we propose this novel process for alleviating sulfide inhibition and enhancing CH_4 production using a sleeve-type anaerobic reactor with built-in MEC, namely ME-AD system. The objectives of this research are (i) to demonstrate the integration of MEC in AD is capable of efficiently alleviating sulfide inhibition and enhancing CH_4 production, (ii) to understand the differences in microbial communities of the reactors with and without MEC, and (iii) to investigate substrates balance and energy input in terms of electricity in the ME-AD system.

2. Material and methods

2.1. Reactor, inoculum, and medium

The lab-scale ME-AD reactor was constructed as a sleeve-type AD reactor with built-in MEC, which was separated by cation exchange membrane (CEM, Ultrex CMI-7000, Membranes International, USA) into anode chamber and cathode chamber (Fig. 1). The inner wall cylinder was full of circular holes with a diameter of 5 mm. The anode was placed in the outer cylinder and the cathode was in the inner cylinder to achieve functional division. The total volume of the reactor was 1.3 L, including 0.7 L of inner and 0.6 L of outer. The anode and cathode were made of carbon felt (Jilin Carbon Plant, China) and placed close to cylinder wall. The cathode was covered with a Pt catalyst layer (0.5 mg-Pt/cm^2) on one side. The inner cylinder as the AD zone was used to hold anaerobic sludge and cathode for treating organic wastewater containing SO_4^{2-}. The anode in the outer cylinder used organic solution to generate electrons and H^+, while, with input of electrical energy, the cathode in the AD cylinder generated H_2 and OH^-. The produced sulfide was converted into ionized HS^- with the generation of OH^-, resulting in unionized H_2S below the threshold of methanogenesis inhibition. The produced H_2 could increase the amount of substrates available for the AD zone.

The ME-ADO reactor was conducted synchronously with an open

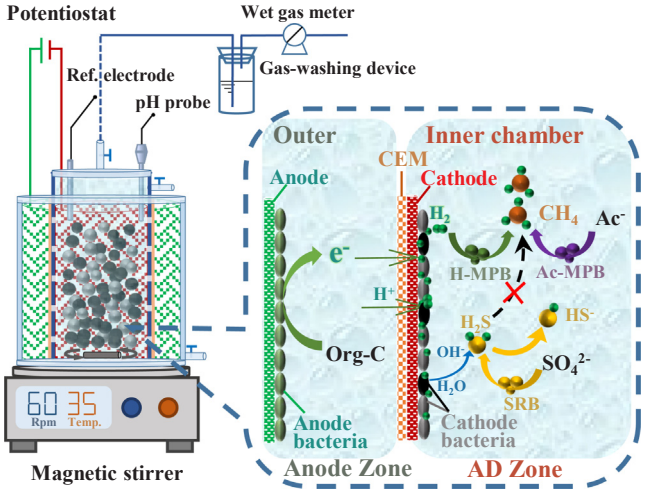

Fig. 1. Schematic diagram of the lab-scale ME-AD reactor.

circuit. For the AD reactor, the anode and cathode were replaced with the same volume of a rubber sheet. The anode, cathode and the reference electrode were connected to a data acquisition (Keithley 2700, USA) with external resistance of 10 Ω. An inserted Ag/AgCl reference electrode was placed in the reactor near to the cathode for potential measurement. The potential control was performed using a CHI-660D potentiostat (CH Instruments, Austin, Texas, U.S.). All potentials were reported with respect to standard hydrogen electrode (SHE). A gas-washing device collected the H_2S gas generated at the top of the AD chamber. The gas production rate was measured in a wet gas meter (Shinagawa WS-1A, Japan). A magnetic stirrer (Jingzao KMS-171E, China) was used to provide gentle mixing (40 rpm) and constant temperature (35 ± 1 °C) for each reactor.

The anaerobic sludge as inoculum was collected from an anaerobic tank of ChengDong Wastewater Treatment Plant, Yancheng, China. The inner cylinder of each reactor was inoculated with 0.3 L of inoculum with volatile suspended solids (VSS) of 6.6 g/L (VSS/total suspended solids (TSS) = 0.86). The inner and outer cylinders were filled with 0.3 L of AD medium and 0.6 L of anodic medium, respectively. Acetate was used as the model substrate in both cylinders of the reactors. The sodium acetate and sodium sulfate were added into oxygen-free deionized water to prepare desired chemical oxygen demand (COD) and SO_4^{2-} concentrations of the AD medium. After mixing well with the inoculum, the COD of the AD medium was fixed at 3000 mg/L and SO_4^{2-} was set to 1500 mg/L, resulting in a COD/SO_4^{2-} ratio of 2:1. Many previous studies suggested that methanogenesis could be inhibited by high sulfide concentration at COD/SO_4^{2-} < 10 (Sabumon, 2008; Zhang et al., 2011; Li et al., 2015). The COD/SO_4^{2-} ratio of 2:1 could seriously cause methanogenesis inhibition due to the toxicity of sulfides and the substrates competition of sulfidogenesis (Sabumon, 2008; Lu et al., 2016; Zeng et al., 2019). The trace element solution was added into the AD medium described by Chen et al. (2009). The anodic medium was prepared by supplementing sodium acetate to obtain a COD concentration of 1000 mg/L. The initial pH of AD and anodic medium was adjusted to 7.0. The AD and anodic medium were added with a 100 mM buffer solution (9.16 g/L Na_2HPO_4, 4.9 g/L $NaH_2PO_4{\cdot}H_2O$, 0.31 g/L NH_4Cl, 0.13 g/L KCl). The AD and anodic medium were purged with nitrogen gas for 10 min to remove dissolved oxygen before being respectively introduced into the inner and outer cylinders.

2.2. Reactors operation

Four reactors were operated in the conditions as shown in Table 1. One reactor with built-in MEC (ME-AD) was operated with the cathode potential controlling at −0.8 V. One reactor (ME-ADO) was operated

Table 1
Operating conditions of each reactor.

Reactor	ME-AD	ME-ADO	AD	ADC
pH control	/	/	/	8.0
Electrode materials	a	a	b	b
Cathode potential (V)	− 0.8	0	/	/

[a]Reactor was equipped with carbon belt.
[b]Reactor was equipped with rubber sheet.

with electrodes and an open circuit. Two reactors (AD and ADC) were operated without electrodes. The pH of ME-AD, ME-ADO and AD reactors were measured but not controlled, while the ADC reactor was automatically controlled at pH of 8.0.

To start-up the bioanode for the ME-AD reactor, the anode was set at 0 V to cultivate electroactive bacteria oxidizing acetate. After operating for 30 days, the bioanode potential reached around −0.35 V under circuit-opening conditions, and the cultivation of the bioanode was regarded to be completed (Wang et al., 2009b). After that, the cathode potential of the ME-AD reactor was controlled at −0.80 V. Each batch test was repeated three times.

2.3. Analytical methods

The SO_4^{2-} in liquor samples following 0.45 μm filtration were measured by an ion chromatography (Dionex ICS-3000, USA) equipped with an Ion-Pac AS4A-SC column. The dissolved sulfide in the aqueous phase including unionized H_2S, HS^- and S^{2-} were determined using the methylene blue method (APHA, 2012). Unionized H_2S was calculated based on the following equation: total sulfide/$(1+(K1/10^{-pH}))$ (Speece, 1996), where $K1$ is the first ionization constant of H_2S, which is equal to $10^{-6.83}$ (35 °C). The dissolved sulfide can interfere with the COD measurement of liquor samples. Before COD measurements, liquor samples were adjusted to pH of 2.0 with H_2SO_4 and then were purged with N_2 for 10 min to removal H_2S (Sahinkaya et al., 2018; Yuan et al., 2019). The TSS, VSS, and COD were analyzed according to standard methods (APHA, 2012). Acetate concentration was measured by a gas chromatograph (Shimadzu GC-2010 Plus, Japan) equipped with an Agilent GS-GASPRO column. The pH was analyzed by a pH meter (Mettler-Toledo FE20, China). The compositions of gas (CH_4 and H_2) were measured using a gas chromatography (Agilent 6890, USA) equipped with an Agilent 19091P-MS4 column. For COD mass balance, the CH_4 could be normalized to a COD amount by 0.35 L CH_4 = 1 g COD (Gianico et al., 2013). Voltage and current were recorded using a multimeter (Keithley 2700, USA). Volumetric current density was calculated by the average current in each hour. Coulombic efficiency was calculated by using the equation described by Feng et al. (2015). The bioelectroactivity of the cathode in the ME-AD was examined by polarization curves. A cathode was as a control in the same ME-AD reactor with anodic and cathodic medium but without inoculum. The cathode potential was set from −0.7 V to −1.0 V with steps of 0.05 V. The current density was recorded per minute at each potential and was plotted in the polarization curve.

2.4. Microbial community analysis

Microbial community analysis was performed using eight biomass samples, namely AD sludge, ME-ADO sludge, ME-AD sludge, ADC sludge, cathode biofilm in ME-ADO and ME-AD, anode biofilm in ME-ADO and ME-AD. All these biomass samples were collected at the end of the experiment. Sampling was repeated three times. The details for the DNA extraction, PCR amplification, Illumina MiSeq sequencing, and data analysis are available in the Supplemental Material (Part 1).

3. Results and discussion

3.1. The role of MEC in AD for alleviating sulfide inhibition and enhancing CH_4 production

3.1.1. SO_4^{2-} reduction, unionized H_2S, and pH variation

The SO_4^{2-} reduction, unionized H_2S, and pH variation were first investigated in three reactors (ME-AD, ME-ADO, AD) without pH-control. SO_4^{2-} removal efficiency in ME-AD (92 ± 2.4%) was close to that in ME-ADO (91 ± 1.9%) and that in AD (91.5 ± 1.7%), which showed that there were no significant differences in SO_4^{2-} reduction among the three reactors (Fig. 2a). The total sulfide in all reactors reached up to around 450 mg-S/L. However, unionized H_2S and pH variation showed notable differences between reactors with and without the supplied electrical energy. A conventional anaerobic reactor at under SO_4^{2-}-rich condition usually generates more unionized H_2S (Zhang et al., 2012). However, unionized H_2S in ME-AD (24 ± 3.7 mg-S/L) was much lower than that in ME-ADO (156 ± 4.2 mg-S/L) and that in AD (159 ± 5.3 mg-S/L) (Fig. 2b). The lower amount of unionized H_2S in ME-AD might be attributed to the differences in pH values. The pH in ME-AD had increased to 8.1 ± 0.2 during the three cycles, while the pH in ME-ADO and AD was below 7.4 ± 0.2 (the initial pH of three reactors was 7.0 ± 0.1) (Fig. 2c). The pH variation showed that weak alkaline conditions occurred in ME-AD, which was attributed to protons consumption and OH^- generation at the cathode (Liang et al., 2013; Luo et al., 2014; Blázquez et al., 2016). The performance of ME-AD was also compared to an additional anaerobic reactor (ADC) without electrodes but with controlling pH at 8.0 ± 0.1. The SO_4^{2-} reduction and unionized H_2S variation in ME-AD were similar to those in ADC, indicating that MEC could replace alkaline-pH control in an anaerobic reactor for reducing unionized H_2S.

The toxicity of unionized H_2S could inhibit the methanogenesis process (Chen et al., 2019). In our study, the unionized H_2S was as high as 150 mg-S/L in ME-ADO and AD might have a significant inhibiting effect on methanogenesis, but had little influence on sulfidogenesis. This result was consistent with the pervious findings (Jing et al., 2013). CH_4 formation from a conventional anaerobic reactor was inhibited by 50% with the unionized H_2S exceeding 50 mg-S/L (Stucki et al., 1993; Li et al., 2015). Paula and Foresti (2009) found that the ionized HS^- of 500 mg-S/L showed no inhibiting effect on methanogenesis processes. When the pH was about 8, virtually all sulfides were in the ionized HS^- form (Sabumon, 2008; Luo et al., 2014; Yuan et al., 2019). Methanogenesis could remain steady in AD process at high pH and total sulfide (mainly ionized HS^-) concentration, but decrease at low pH as the total sulfide (mainly unionized H_2S) concentration increased (McCartney and Oleszkiewicz, 1991). It was found that the CH_4 production was not influenced by total sulfide concentration of 450 mg-S/L at alkaline pH of 8.5 (Yuan et al., 2014, 2019). These results indicated that the MEC could create weak alkaline conditions for alleviating unionized H_2S inhibition in the anaerobic reactor.

3.1.2. CH_4 production and acetate removal

The CH_4 production and acetate removal were investigated in three reactors (ME-AD, ME-ADO and AD) without pH-control and one reactor (ADC) with pH-control (Fig. 2d–f). The acetate removal including $acetate_{inner}$ in inner cylinder and $acetate_{outer}$ in outer cylinder was examined in these reactors. The CH_4 production and $acetate_{inner}$ removal in these reactors increased with time during the three cycles. However, both CH_4 production and $acetate_{inner}$ removal were obviously suppressed in ME-ADO and AD compared to ADC (Fig. 2 d and e), possibly due to the inhibitory effect of high unionized H_2S on microbial activity of MPB (Lu et al., 2016; Cetecioglu et al., 2019). Apart from high sulfide, there were no other inhibitors (e.g. high ammonia, accumulated VFAs, and deleterious metal ions) affecting on the AD process in these reactors. The maximum cumulative CH_4 production was 0.91 ± 0.13 m^3-$CH_4/m^3_{reactor}$ in ME-AD during the cycle II, which was

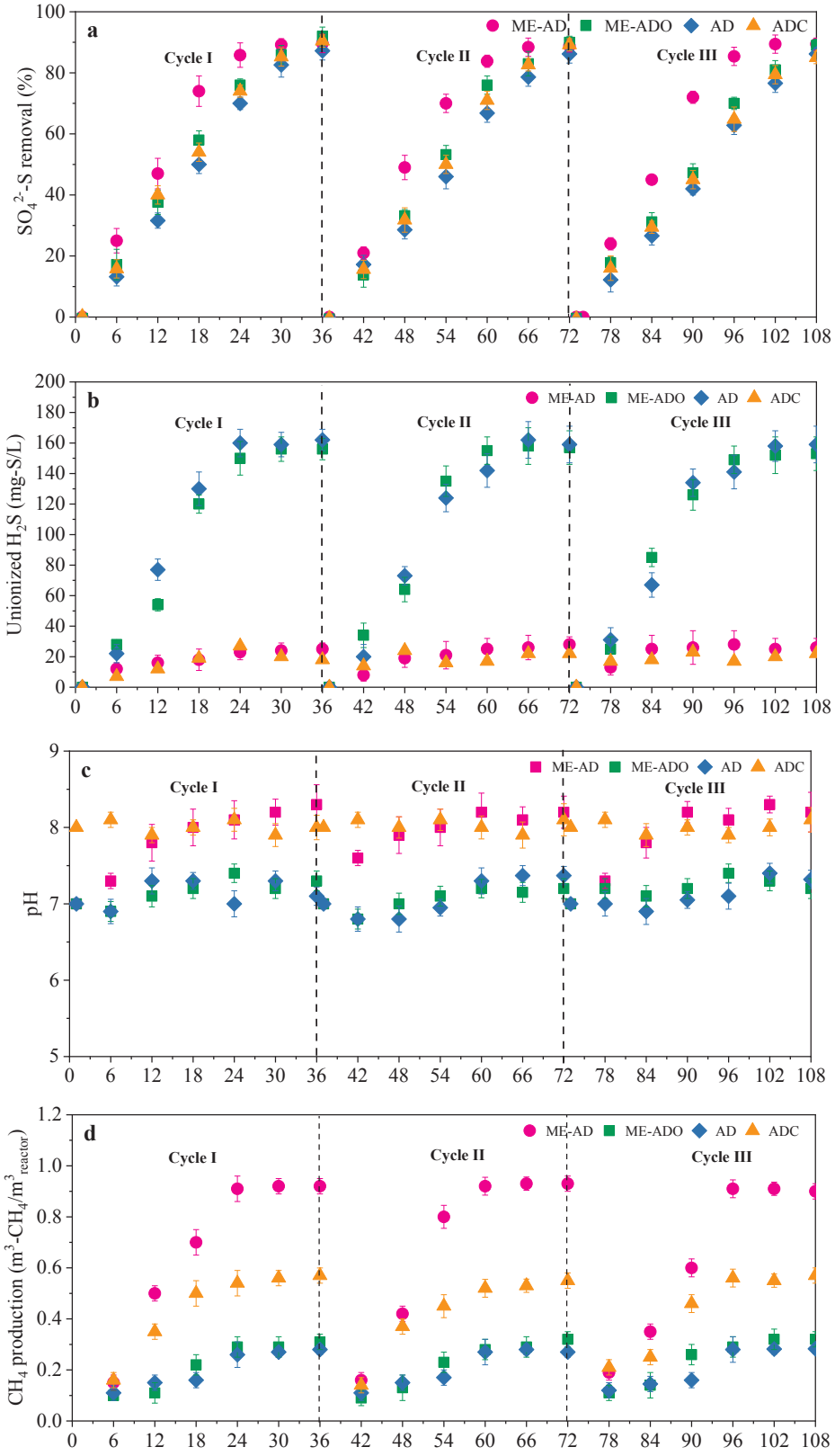

Fig. 2. The SO_4^{2-} reduction, unionized H_2S, pH variation, CH_4 production and acetate removal of different reactors during three consecutive cycles. (a) SO_4^{2-}-S removal efficiency; (b) unionized H_2S concentration; (c) pH variation; (d) cumulative CH_4 production; (e) $acetate_{inner}$ removal; (f) $acetate_{outer}$ removal.

3.03 ± 0.89 times higher than that in ME-ADO and was 3.25 ± 0.32 times higher than that in AD (Fig. 2d).The $acetate_{inner}$ removal in ME-AD (86 ± 3.4%) was 1.47–1.56 times higher than that (57 ± 4.6%) in ME-ADO and that in AD (55 ± 2.6%) (Fig. 2e). These results confirmed that both CH_4 production and $acetate_{inner}$ removal occurred in ME-AD without sulfide inhibition.

The potential of CH_4 production in ME-AD was comparable with that in ADC. The $acetate_{inner}$ removal was greater than 80% in both ME-

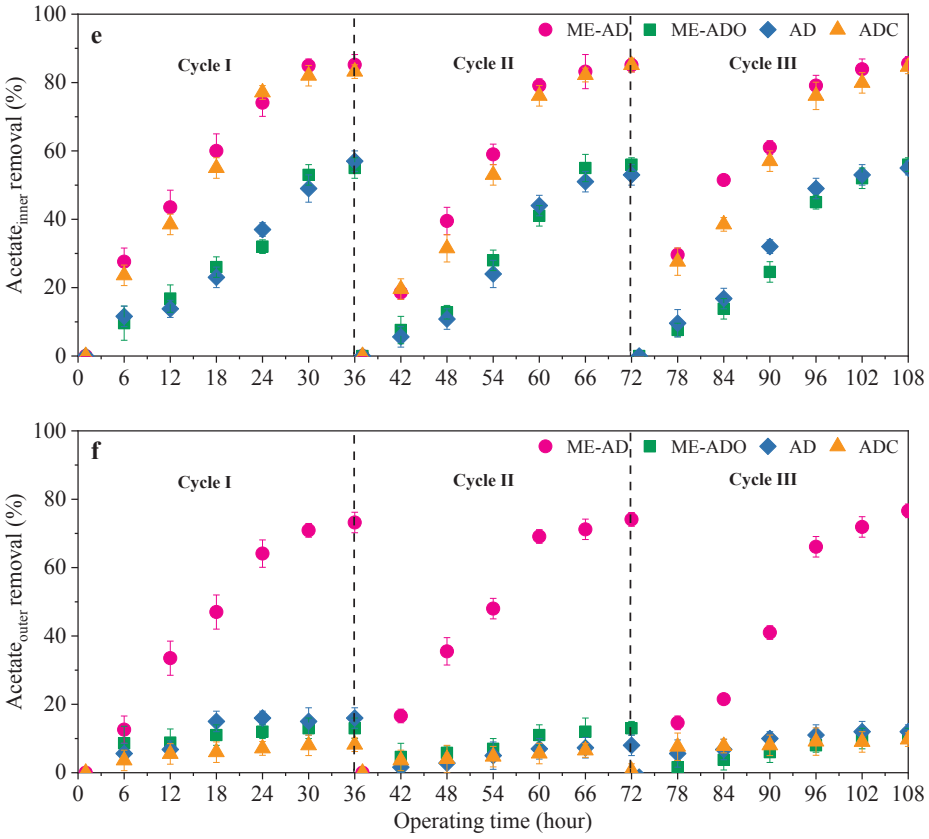

Fig. 2. (*continued*)

AD and ADC, and there were no significant differences in acetate$_{inner}$ removal between reactors (Fig. 2e). However, the maximum cumulative CH_4 production in ME-AD was 1.56 ± 0.33 time higher than that in ADC (Fig. 2d). These results suggested that MEC increased the amount of substrates available for MPB to produce more CH_4. Compared with the open circuit (ME-ADO), more than 3 times higher CH_4 production was observed in ME-AD, which indicated that the supplied electrical energy could enhance CH_4 production.

Acetate$_{outer}$ removal in outer cylinders of all reactors was also analyzed to evaluate the role of the anode (Fig. 2f). Acetate$_{outer}$ removal in ME-AD was 76 ± 3.2%, while acetate$_{outer}$ removal in ME-ADO, AD and ADC was only 9.5 ± 2.2%, 11.1 ± 1.9% and 8.4 ± 1.5%, respectively. The differences in acetate$_{outer}$ removal among these reactors could be the result of anodic oxidation by exoelectrogens (Zhao et al., 2014; Liu et al., 2016). The high acetate$_{outer}$ removal in ME-AD indicated that elelctrohydrogenesis of bioanode could make full use of acetate. Therefore, we hypothesized that the additional substrate in inner cylinder (AD zone) might be the generated H_2 at the cathode. These results indicated that the role of the MEC was to not only control sulfide inhibition, but also enhance CH_4 production via providing substrate electrons.

3.2. Sulfur balance and substrate electrons consumption

The sulfur balance was shown in Fig. 3(a). The amount of sulfur in the reactors mainly consisted of ionized HS^-, unionized H_2S, gaseous H_2S and residual SO_4^{2-} in the effluent (eff.-SO_4^{2-}). The proportion of SO_4^{2-} reduced to total sulfide (Ionized HS^- + Unionized H_2S + Gaseous H_2S) accounted for around 94% in all the reactors. The proportion of gaseous H_2S was 3.2–4.4% of total sulfide in the reactors, whereas aqueous sulfide (Ionized HS^- + Unionized H_2S) accounted for 87.2–91.6%. However, unionized H_2S as the inhibitor of methanogenesis only accounted for about 4.8 ± 1.2% in ME-AD which was much lower than that in ME-ADO (33.2 ± 3.5%) and that in AD (31.8 ± 4.2%). The sulfur species in ME-AD were similar to those in ADC under weak alkaline condition. These results confirmed that the aqueous sulfides were mainly present in the ionized HS^- form due to the integration of MEC in AD.

The substrate electrons (in terms of COD) consumption in both inner cylinder and outer cylinder of these reactors were analyzed. The CH_4 production in ME-AD was 1.56 ± 0.33 time larger than that in ADC, suggested that ME-AD utilized more substrate electrons than ADC. To prove this, we began with the assumption that acetate was the only substrate electron because no other organic compounds were detected. Then we divided the thermodynamic electrons$_{inner}$ consumption (TEC) for CH_4 production and SO_4^{2-} reduction by the practical electrons$_{inner}$ consumed (PEC). Calculation produced a ratio of 127.2 ± 6.2% (TEC/PEC) in ME-AD, which was over 100% (Fig. 3b), showing that a portion of electrons (at least 27%) was inorganic substrate generated from MEC. After that, we calculated the electrons practically consumed including electrons$_{outer}$. The ratio of TEC to the sum of electrons$_{inner}$ and electrons$_{outer}$ practically consumed (SEC) (TEC/SEC) was 93.5 ± 3.2% (ME-AD) (Fig. 3c), indicating that electrons$_{outer}$ were converted into H_2 via MEC which accounted for a portion of substrates electrons for AD. These results were consistent with the fact the electron transfer pathway from acetate to hydrogen could be introduced by bioelectrolysis (Lu et al., 2012; Villano et al., 2013; Cai et al., 2016; Zou and He, 2018).

3.3. Microbial community structure and pathways for CH_4 formation

The microbial communities were analyzed at the end of the experiment to compare community structure, diversity, and function in the four reactors (AD, ME-ADO, ME-AD, and ADC). The microbial communities of ME-AD sludge had the richest diversity among all the reactors, with the Shannon index of 4.39 (Table. S1), which indicated

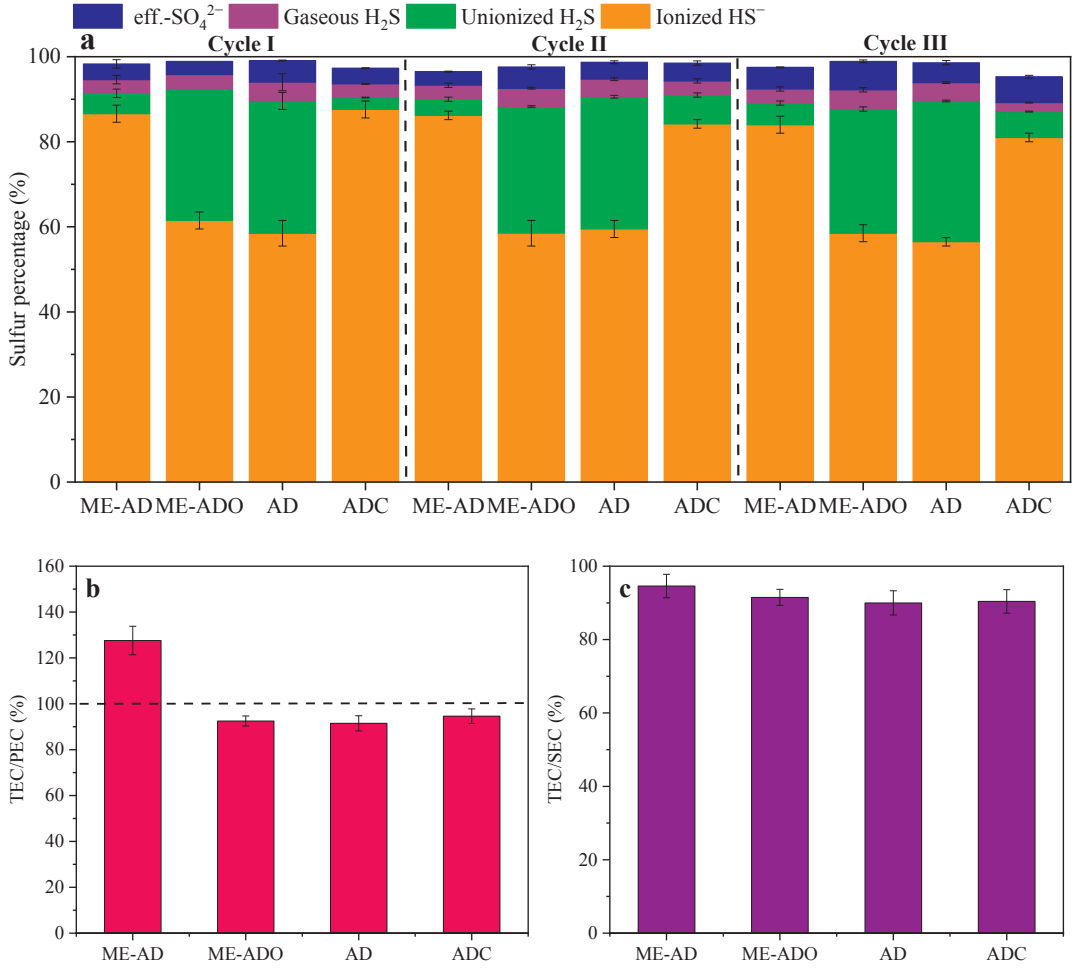

Fig. 3. The sulfur balance and substrate electrons consumption. (a) sulfur balance; (b) TEC/PEC; (c) TEC/SEC.

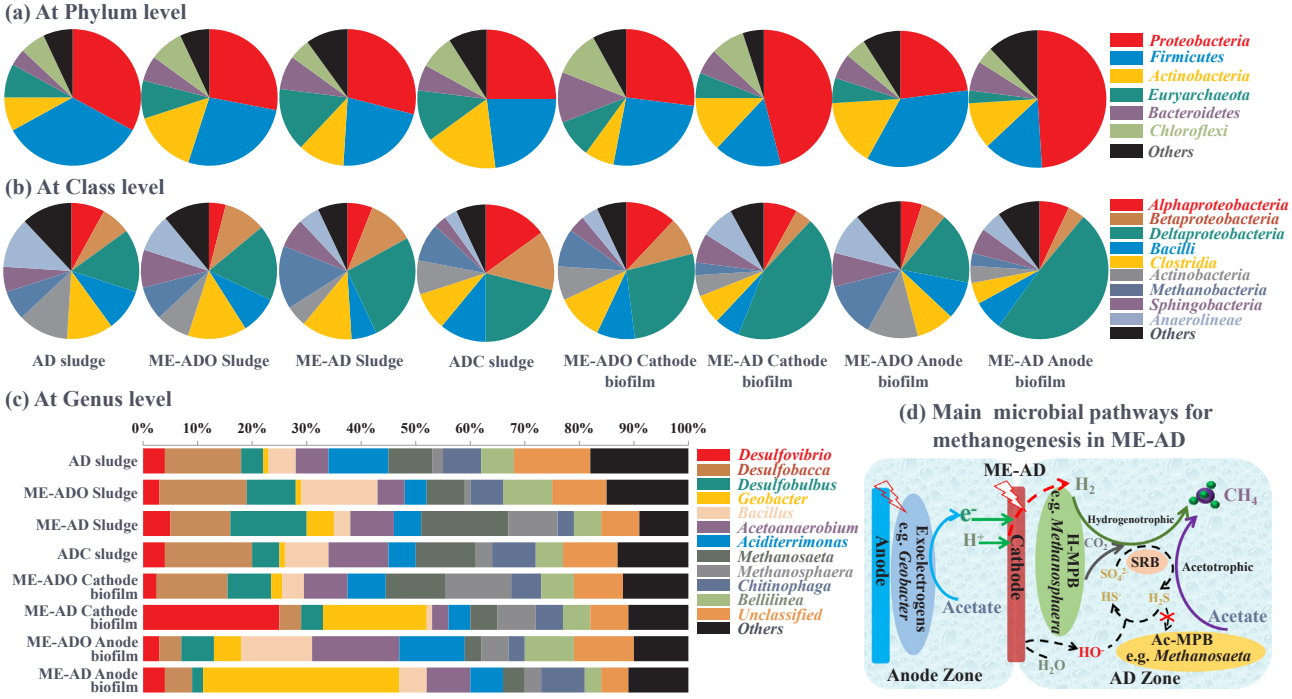

Fig. 4. Microbial communities of the reactors at Phylum (a), Class (b), Genus (c) levels, and (d) Main microbial pathways.

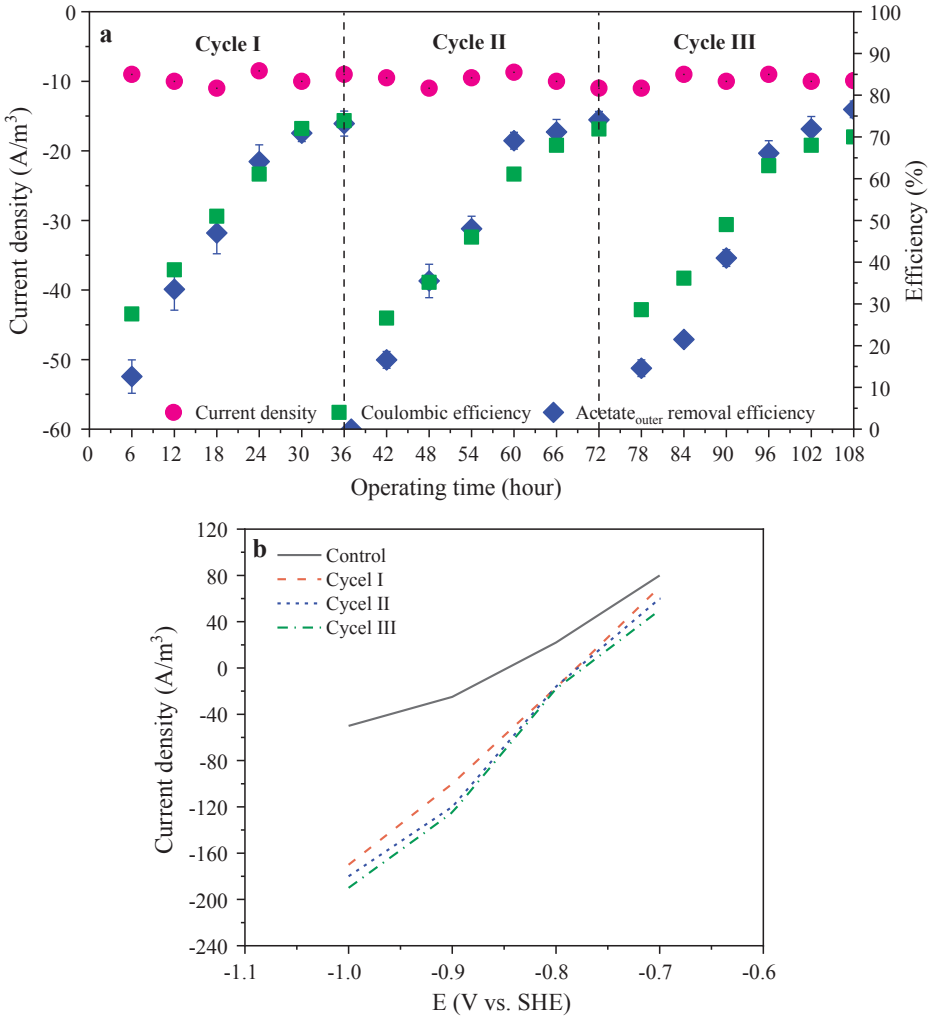

Fig. 5. Current density and coulombic efficiency (a), and polarization curve (b) in ME-AD reactor during the three consecutive cycles.

that more biochemical reactions might occur in ME-AD. Shannon index showed the anode and cathode biofilm in ME-AD hold the relatively lower diversity (2.13 and 2.32) than that of ME-ADO, indicating the selective enrichment of some specific bacteria by electrochemical stimulation. The bacterial community abundances of among the eight biomass samples (i.e. sludge in AD, ME-ADO, ME-AD and ADC, cathode biofilm in ME-ADO and ME-AD, anode biofilm in ME-ADO and ME-AD) were identified at the phylum level (Fig. 4a). The main difference among these samples was the different distribution of phylum *Proteobacteria*, *Firmicutes*, *Actinobacteria*, *Euryarchaeota, Bacteroidetes*, and *Chloroflexi*. Anode and cathode biofilm of ME-AD obviously enriched *Proteobacteria* (49.3% and 46.4%) compared with that of ME-ADO (27.6% and 23.8%). *Proteobacteria* include a diversity of electroactive and sulfur-metabolizing microbes (Bond et al., 2002). *Euryarchaeota* enriched in the ME-AD sludge (15.6%) and ADC sludge (11.21%), which accounted for 8.3% and 9.5% in the AD sludge and ME-ADO sludge, respectively. *Euryarchaeota* included methanogens which were usually responsible for CH_4 production in an anaerobic reactor (Antwi et al., 2017). At the class level (Fig. 4b), *Deltaproteobacteria* dominated in anode and cathode biofilm of ME-AD, which accounted for 49.5% and 44.6% of the class. *Deltaproteobacteria* could improve the efficiency of the electron transfer between bacteria and electrode (Wang et al., 2017). In addition, higher abundances of *Methanobacteria* (15.5% and 13.6%) were observed in ME-AD and ADC sludge compared with the ME-ADO and AD sludge.

To further understand the function of microbial communities in different reactors, the bacterial community abundances were classified at genus level (Fig. 4c). The distribution of dominant genus in the cathode biofilm showed a significant difference between the ME-ADO and the ME-AD reactors. *Desulfovibrio* and *Desulfobacca* accounted for 25.3% and 4.3% in cathode biofilm of ME-AD, respectively, whereas 2.5% and 13.1% in cathode biofilm of ME-ADO. *Desulfovibrio* and *Desulfobacca* as SBR belonged to *Deltaproteobacteria* which were capable of reducing SO_4^{2-} to S^{2-} in anaerobic condition (Kaksonen et al., 2004; Muyzer and Stams, 2008; Sun et al., 2018). *Desulfovibrio* as an electroactive and hydrogentrophic SRB for SO_4^{2-} reduction was found in many MEC systems (Zhao et al., 2009; Rago et al., 2015; Blázquez, et al., 2016). The *Desulfovibrio* could grow on the cathode and drive SO_4^{2-} reduction at pH ranging from 4.5 to 10.5 (Liang et al., 2013). *Desulfovibrio* was selectively enriched on the cathode of ME-AD (with voltage), which could utilize H_2 as substrate for SO_4^{2-} reduction, thereby increasing the acetate substrate for MPB. However, *Desulfobacca* as acetotrophic SRB dominated on the cathode of ME-ADO (without voltage). The *Desulfobacca* also dominated in ME-ADO sludge (16.3%), AD sludge (14.6%) and ADC sludge (15.7%) for reducing SO_4^{2-}. Among MPB, *Methanosaeta* and *Methanosphaera* accounted for 8.08% and 2.13% in AD sludge, 7.12% and 1.32% in ME-ADO sludge, 16.54% and 9.65% in ME-AD sludge, 11.32% and 2.52% in ADC sludge, respectively. *Methanosaeta* as acetotrophic MPB was obligate to utilize acetate as substrate for CH_4 production (Demirel and Scherer, 2008; Yang et al., 2015). *Methanosphaera* was classified into the hydrogenotrophic MPB which could produce CH_4 from H_2 and CO_2 (Demirel

and Scherer, 2008). *Methanosaeta* and *Methanosphaera* were obviously higher in the ME-AD sludge than those in AD, ME-ADO and ADC sludge, which was in accordance with the higher CH_4 production in the ME-AD reactor. Other exoelectrogens (e.g. *Geobacter*) were enriched, especially on the anode and cathode of ME-AD, which were responsible for transferring electrons between the electrodes (Cai, et al., 2018).

We proposed two possible microbial pathways which mainly contributed to the CH_4 production in ME-AD reactor (Fig. 4d): (i) The hydrogentrophic MPB (e.g. *Methanosphaera*) enriched in the ME-AD reactor indicated that generated hydrogen could be utilized as an extra substrate for CH_4 formation; (ii) The acetotrophic MPB (e.g. *Methanosaeta*) showed a higher abundance in the ME-AD reactor, suggesting that acetotrophic methanogenesis was also enhanced for CH_4 production.

3.4. Bioelectrochemical analysis of anode and cathode

A potential mechanism for enhancement of CH_4 production in ME-AD was that introducing a MEC system controlled sulfide inhibition and provided additional substrate electrons for generating more CH_4. Organic matter (acetate) was converted into electrons at the anode and the applied voltage was utilized to generate H_2 at the cathode. To further understand the contribution of the MEC in ME-AD, volumetric current density and coulombic efficiency were analyzed (Fig. 5). At a fixed cathode potential of −0.8 V, an average volumetric current density reached −9.5 A/m^3 (Fig. 5a). The current density maintained stable during the entire test. Anode potential was less than −0.34 mV during the test, suggesting that the anode maintained its electroactivity of acetate oxidation. Coulombic efficiency increased from 27.5% to 71.7% with the increase of $acetate_{outer}$ removal from 12.6% to 74.5% (Fig. 5a), indicating that the contribution of the anode converting $acetate_{outer}$ into electrons was enhanced with time. There was no obvious difference in SO_4^{2-} reduction efficiency among these reactors, but the CH_4 production in ME-AD was 1.56–3.03 times higher than that in other reactors. These results illustrated that the inner cylinder (AD zone) of ME-AD used extra substrate electrons, which created via the anode in outer cylinder. In this study, H_2 was detected only during the initial hours. Even though not detected in the later test, H_2 as an intermediate product could be used in-situ by hydrogenotrophic MPB (Blázquez et al., 2016; Luo et al., 2017). The hydrogenotrophic MPB and SRB were enriched substantially in ME-AD, resulting in minor detection of H_2.

Polarization curves of the cathode in ME-AD reactor were analyzed at the end of each cycle (Fig. 5b). A cathode without inoculum served as the reference group. The cathode in ME-AD during the three cycles had similar current densities, which were higher than that of the reference cathode. At a cathode potential of −0.80 V, the current density (−19 A/m^3) of cathode in ME-AD was higher than that (−0.45 A/m^3) of the reference cathode (Fig. 5b), indicating the formation of the cathodic biofilm. The cathodic biofilm could accelerate the electron transfer and modify the cathode surface towards more efficient H_2 evolution (Yu et al., 2011; Pozo et al., 2016; Lin et al., 2019).

3.5. Practical implication

This work proposed a new way for efficient CH_4 production from SO_4^{2-}-rich wastewater by using an anaerobic reactor with built-in ME. The anode chamber could use the organic wastewater to generate electrons, and H_2 was generated in-situ on the cathode in the AD chamber which could be controlled with a selective applied voltage. It was worth evaluating the input energy recovery efficiency as additional cost. The input energy of the ME-AD reactor was calculated in terms of electrical energy invested per volume (m^3) of increased production of CH_4 compared to the AD reactor (Increased CH_4 production (0.63 ± 0.1 m^3-CH_4/$m^3_{reactor}$) = CH_4 production (0.91 ± 0.13 m^3-CH_4/$m^3_{reactor}$) of the ME-AD reactor − CH_4 production (0.28 ± 0.11 m^3-CH_4/$m^3_{reactor}$) of the AD reactor). The input energy of the ME-AD reactor was 0.30 kWh/m^3-CH_4 (U × I × T/Y, U = 0.6 V; I = 9.5 A/m^3; T = 36 h; Y = 0.63 ± 0.1 m^3-CH_4/$m^3_{reactor}$) in one batch, overall electricity cost was 0.02 €/m^3-CH_4 (0.01€/ $m^3_{reactor}$) (the average price of electricity in China = 0.07 €/kWh). In the ME-AD reactor, the increased CH_4 production was 0.63 ± 0.1 m^3-CH_4/$m^3_{reactor}$ in one batch, corresponding to 0.21 €/$m^3_{reactor}$ (the average price of natural gas in China = 0.34 €/m^3-CH_4). The cost of the increased CH_4 production in the ME-AD reactor was almost 20 times higher than that of electrical consumption.

The economic analysis indicated that introduction of MEC in AD totally self-covered the cost caused by electrical energy input. Hence, the benefits of the ME-AD system should not be only focused on CH_4 recovery but also on the efficient treatment of SO_4^{2-}-rich wastewater. The COD removal in ME-AD reactor was significantly higher than that in the AD reactor, which facilitated the reduction of COD loading for the following treatment processes. SO_4^{2-} was efficiently converted into dissolved sulfide for avoiding more gaseous H_2S generation. The dissolved sulfide could be oxidized to elemental sulfur (S^0) (an important sulfur source) in a subsequent sulfide oxidation process (Chen et al., 2016; Huang et al., 2018; Guerrero and Zaiat, 2018). Moreover, the ME-AD process could be more competitive as the applied voltage supplied by renewable energy devices such as a solar cell (Blázquez et al., 2016; Liu et al., 2016). The ME-AD system for treating SO_4^{2-}-rich wastewater had two distinct advantages: (i) Unionized H_2S could be effectively converted into ionized HS^- which relieved toxicity toward methanogenesis; (ii) The CH_4 production could be significantly enhanced with the generation of H_2 at the cathode.

In previous works, bioelectrochemical systems were developed for SO_4^{2-} removal (Luo et al., 2014; Pozo et al., 2016; Blázquez et al., 2016), which could be employed as a pretreatment unit for AD to provide an alternative approach of avoiding the sulfide inhibition. However, keeping the sulfur in the system as in this work may receive additional benefit. As known, ammonium (NH_4^+) usually exists in the AD effluent and is required to be removed in the following processes. The generated HS^- from AD can be used as an inorganic electron donor for denitrification which produces much less sludge compared to the organics as electron donors (Wang et al., 2009a; Wu et al., 2020). Recently, Zhang and Angelidaki (2015) developed a biopolar MEC system, which could simultaneously recover SO_4^{2-} and NH_4^+ from wastewater. In the case of coupling this system with AD, the post nitrogen removal process can be saved. However, the realization of this system strongly relies on a proper downstream industry to condense and purify the recovered chemicals. As a comparison, the ME-AD system just makes moderate modifications for the previous system, which is likely easier to be applied in the future.

Besides sulfide, free ammonia (NH_3) is another inhibition factor for CH_4 production in AD system (Liu et al., 2019). The p*Ka* of NH_3/NH_4^+ is known as 8.95 (35 °C) (Martinelle and Häggström, 1997), indicating the pH in the ME-AD system should be controlled without being too high. However, as the p*Ka* distance between H_2S/HS^- (6.83, 35 °C) and NH_3/NH_4^+ is over 2 units, the pH control would not be that difficult. The alkalinity production rate in MEC system is strongly associated with the current density. The development of the real-time pH control system by adjusting the current density is therefore warranted in the future.

4. Conclusion

Under SO_4^{2-}-rich condition, MEC could create weak alkaline conditions in an anaerobic reactor to significantly decrease the unionized H_2S that was the main factor inhibiting anaerobic digestion. The CH_4 production in the ME-AD system was much higher than that in the controls (no electrodes or no applied voltage) with and without alkaline-pH control. MEC increased the amount of substrates available for anaerobic digestion, thereby enhancing the CH_4 production in the ME-

AD system. Acetotrophic and hydrogenotrophic MPB were enriched in the sludge and cathode biofilm, respectively. Acetotrophic and hydrogenotrophic methanogenesis were the two major pathways for CH_4 formation in the ME-AD system. The additional revenue of the ME-AD system from increased CH_4 production could cover the energy input in the form of electricity. This research suggested that MEC has the potential to be an alternative strategy for controlling sulfide inhibition and enhancing CH_4 production in anaerobic digestion of organic wastewater containing sulfate.

CRediT authorship contribution statement

Ye Yuan: Conceptualization, Writing - original draft, Data curation, Visualization. **Haoyi Cheng:** Methodology, Writing - review & editing. **Fan Chen:** Methodology, Data curation. **Yiqian Zhang:** Supervision. **Xijun Xu:** Supervision. **Cong Huang:** Investigation. **Chuan Chen:** Investigation. **Wenzong Liu:** Writing - review & editing. **Cheng Ding:** Writing - review & editing. **Zhaoxia Li:** Writing - review & editing. **Tianming Chen:** Supervision, Resources, Writing - review & editing. **Aijie Wang:** Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2020.105503.

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