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Submission to Bioresource Technology

The impact of anode acclimation strategy on microbial electrolysis cell treating hydrogen fermentation effluent

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Abstract:

The impact of different anode acclimation methods for enhancing hydrogen production in microbial electrolysis cell (MEC) was investigated in this study. The anodes were first acclimated in microbial fuel cells using acetate, butyrate and corn stalk fermentation effluent (CSFE) as substrate before moving into MECs, respectively. Subsequently, CSFE was used as feedstock in all the three MECs. The maximum hydrogen yield with the anode pre-acclimated with butyrate (5.21 ± 0.24 L H₂/L CSFE) was higher than that pre-acclimated with acetate (4.22 ± 0.19 L H₂/L CSFE) and CSFE (4.55 ± 0.14 L H₂/L CSFE). The current density (480 ± 11 A/m²) and hydrogen production rate (4.52 ± 0.13 m³/m³/d) with the anode pre-acclimated with butyrate were also higher than another two reactors. These results demonstrated that the anode biofilm pre-acclimated with butyrate has significant advantages in CSFE treatment and could improve the performance of hydrogen production in MEC.

Keywords: Microbial electrolysis cell (MEC); Corn stalk fermentation effluent; Pre-acclimation; Hydrogen; Butyrate; Acetate
Introduction

Hydrogen as one promising alternative clean energy source has attracted international attention in recent years (Datar et al., 2007; Guo et al., 2010; Kumar et al., 2016; Li & Fang, 2007). Among all of the available biological routes for H₂ production, biohydrogen production through dark-fermentation can utilize various crop castoffs as feedstock was considered to be a feasible method due to its low energy consumption and ease of operation (Ghimire et al., 2015). However, during the hydrogen fermentation processes, hydrogen production is accompanied with production of volatile fatty acids (VFAs) and alcohols as by-products in which acetate and butyrate were the main component of fermentation effluent (Pan et al., 2010; Xing et al., 2011). The low conversion efficiency of feedstock and residue organics in fermentation effluent are two main bottleneck problems (Marone et al., 2016). Therefore, biohydrogen production is likely to be industrially viable if fermentation processes could be integrated into a combination of processes that are able of utilizing metabolic end products (Ghimire et al., 2015).

Recently, Microbial electrolysis cell (MEC) as one emerging technology for producing hydrogen from fermentation end products, such as acetate and ethanol, has gained increasing attention (Kadier et al., 2014). Compared with the dark-fermentation the MEC has a higher hydrogen recovery and a wider substrate diversity (Escapa et al., 2016). Most of the MEC studies have relied on the use of pure chemical compounds (primarily acetate) and acidogenic wastewater (fermentation effluent) as the substrate (Kadier et al., 2014). The integration of dark fermentation with MECs has been recognized as a promising method to convert biomass to hydrogen. However, when fermentation effluents were used as substrate, the hydrogen production rate was low and there was substantial methane production. For example, the hydrogen production rate could reach to 5.56 m³/m³/d at applied voltage of 0.8 V in single-chamber MEC using sodium acetate as substrate (Liang et al., 2011), while the highest hydrogen production rate only was 1.76 m³/m³/d feeding with hydrogen fermentation effluent.
It is of great importance to improve the hydrogen production rate from fermentation effluent in MEC.

The performance of MECs is directly related to the substrates. VFAs and alcohols as the main end products in dark fermentation, among which acetate and ethanol were easily degradable while butyrate and propionate could not be oxidized efficiently (Lu et al., 2009; Yang et al., 2015). In a previous study (Li et al., 2014), about 90 ± 2% of acetate was removed while the butyrate removal was only 4 ± 2% in MEC. It is important to improve the degradation of butyrate by exoelectrogenic bacteria in MECs, as many effluents of hydrogen fermentation not only contain high concentration of acetate but also contain high concentration of butyrate.

Recently, Ullery and Logan (Ullery & Logan, 2014) examined the impact of anode acclimation strategy (using different substrate: acetate or domestic wastewater) on the treatment efficiency of cellulose fermentation effluent. It was found that the pre-acclimation strategy of using domestic wastewater or acetate in MECs had no significant difference in COD treatment, current generation, and coulombic efficiency. Popov et al (Popov et al., 2016) also investigated the influence of pre-acclimation with acetate and butyrate on biofilm structure for enhancing electricity and hydrogen production. It was found that the anode biofilm acclimated to butyrate had a significant advantage in hydrogen production when using butyrate or acetate and butyrate mixture as substrate. However, the effect of pre-acclimation strategy on the MECs treating corn stalk fermentation effluent has never been reported.

This study aims to investigate the impact of pre-acclimation of anode biofilm using different substrates in MFC mode on hydrogen production in MEC with corn stalk fermentation effluent (CSFE) as feedstock. The anode biofilm was first enriched using acetate, butyrate and CSFE as substrate, respectively. Subsequently, the potential of using such biofilm for hydrogen production from CSFE in double anodes MEC was investigated. The corresponding operational parameters were optimized in batch tests. In addition, the VFAs and ethanol removal in CSFE...
along with the current and coulombic efficiencies were evaluated.

2. Materials and methods

2.1. Seed microorganism

Cow dung compost as the seed of hydrogen-producing microflora and exoelectrogens was obtained from the dairy in biogas plant. Prior to use, the mixture of water and cow dung compost with liquid/solid ratio of 4:1 (w/w) was sealed in serum bottle, and then treated using microwave irradiated for 1.5 min to suppress the activity of hydrogen-consuming bacteria and methanogens (Song et al., 2012). Thereafter, pre-incubated with basal medium in an anaerobic reactor at 36 °C for about 9 h as the inoculum of corn stalk fermentation. The basal medium contains: sucrose, 10 g/L; NH₄HCO₃, 1 g/L; KH₂PO₄, 0.2 g/L and 10 mL mineral salt solution (comprising 2 g/L NH₄HCO₃, 1 g/L KH₂PO₄, 0.1 g/L MgSO₄·7H₂O, 0.01 g/L NaCl, 0.01 g/L Na₂MoO₄·2H₂O, 0.01 g/L CaCl₂·2H₂O, 0.015 g/L MnSO₄·7H₂O and 0.0278 g/L FeCl₂).

2.2. Characteristics of effluent samples as feedstock of MECs

The effluent was taken from a 5 L batch stirred anaerobic bioreactor, where the batch dark fermentation experiment was performed according to the method previously described (Li et al., 2014; Wang et al., 2012). The flowchart of the integrated hydrogen production process was shown in Fig. S1 (Supplementary data). The corn stalk as the substrate of fermentation was smashed by a vegetation disintegrator with 40-mesh screen before using. The milled corn stalk and H₂SO₄ solution (0.5%) with solid to liquid ratio of 1:10 (w/v) was autoclaved 60 min at 121 °C. Thereafter, the pH was adjusted to 7 with 1 M Ca(OH)₂ solution for the dark fermentation. The stirred anaerobic bioreactor was filled with 3 L mixture containing the pre-incubated inoculum and pretreated corn stalk of 20 g/L. The bioreactor was flushed with nitrogen gas for 15 min and then was operated at 37 °C with 120 rpm stirring speed. At the end of fermentation, the pH of fermentation effluent was adjusted to 7.0 using NaOH. The effluent
was then collected by centrifugal separation to remove the fermentation residue and was further
used as feedstock in MECs for H₂ production. The effluent had a COD of 8842 ± 48 mg/L, with
the following constituents identified: acetate, 3101 ± 21 mg/L; butyrate, 2602 ± 24 mg/L;
propionate, 88 ± 12 mg/L; ethanol, 452 ± 22 mg/L.

2.3. MECs construction

The cubic single-chamber membrane-less MECs were constructed with a total volume of 64
mL as shown in Fig.1. The MECs were equipped with the bioanode separately placed on both
sides of cathode. The anode consisted of two pieces of square graphite felts. The cathode was
made of a square carbon cloth coated with 0.5 mg Pt/cm² (20 wt% Pt/C, JM) and a Nafion (5%,
Dupont). The cathode was placed in the middle of the cubic chamber with 15mm average
spacing to the anodes. Titanium wire was used to connect the electrodes to the circuit. The
electrodes were connected to a battery test system (Neware Battery Testing System TC53,
Shenzhen, China), which was used as a power supply (PS) to control the applied voltage and
record the current generated from MECs.

2.4. MEC Start-up and operations

All anodes were enriched in MFCs inoculated with a 1:1(v/v) mixture of hydrogen
fermentation effluent from the 5 L batch stirred anaerobic bioreactor and nutrient buffer solution
(NBS). The NBS contained: NH₄Cl 0.31 g/L, KCl 0.13 g/L, NaH₂PO₄·2H₂O 2.27 g/L,
Na₂HPO₄·12H₂O 11.54 g/L, trace mineral 12.5 mL/L and vitamin 12.5 mL/L. When the
exoelectrogens colonized on the anode surface indicated by a reproducible maximum voltage,
the inoculum was omitted. The MFCs was then fed with 1000 mg/L acetate (HAc-MFC), 1000
mg/L butyrate (HBu-MFC) and CSFE (CSFE-MFC) as substrate, separately. A resistor of 1000
Ω was used as external load during MFCs operation. After one month, the anodes were
transferred into MECs. Then an applied voltage ranging from 0.5 V to 0.8 V was applied to start
up the MECs. The MECs were operated in batch mode and feed with 45 mL CSFE together with trace mineral 12.5 mL/L, vitamin 12.5 mL/L, \( \text{NH}_4\text{Cl} \) (0.31 g/L), KCl (0.13 g/L), \( \text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O} \) (2.27 g/L) and \( \text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O} \) (11.54 g/L). When the MECs obtained a stable current generation, each reactor was operated independently under different voltages ranging from 0.5 V to 0.8 V. The MECs were purged with nitrogen gas for 10 min to keep an anaerobic environment. All the MECs were operated in duplicate in incubator at 36 ± 1°C.

2.5. Analytical methods

The volume of gas production was measured using water replacement method. Hydrogen concentration was determined by a Gas Chromatograph (GC, Agilent 4890D) equipped with a thermal conductivity detector (TCD) and a 6 feet stainless column packed with Porapak Q (80/100 mesh). The volatile fatty acids (VFAs) and alcohols were measured at the end of a test by analysis of the sample using another GC with a flame ionization detector (FID) and an 8-ft stainless column packed with 10% PEG-20 M and 2% \( \text{H}_3\text{PO}_4 \) (80/100 mesh). \( \text{H}_2 \) yield was calculated by measurements of the gas composition in the headspace plus the total volume of gas production at each time interval using the equation:

\[
V = V_{11} x_{11} + V_{12} x_{12}
\]

Where, \( V \) is the cumulative \( \text{H}_2 \) gas volume at current \( (i) \); \( V_{11} \) is the volume of headspace of the MEC reactor and \( x_{11} \) is the fraction of \( \text{H}_2 \) gas of headspace of the MEC reactor at the time \( (i) \); \( V_{12} \) is the biogas volume discharged from the MEC reactor and \( x_{12} \) is the fraction of \( \text{H}_2 \) gas discharged from the MEC reactor at the time interval \( (i) \).

The hydrogen production rate was calculated using the equation:

\[
\text{HPR} = V / (t \times V_0)
\]

Where \( t \) is the residence time of each batch, \( V_0 \) is the volume of the fresh medium supplemented.

The coulombic efficiency (CE) based on total consumption of the substrate and the energy
efficiency (ηE) were calculated as described previously (Call & Logan, 2008). The current density was calculated based on the net reactor volume. The COD concentration were measured following the standard methods (A.W.W.A, 1998). The conductivity was measured using a conductivity meter (DDS-307, LEICI, China). One-way ANOVA analysis was employed to analyze the signification of the difference (P-values<0.05).

2.6. Cyclic Voltammetry (CV)

Bio-electrochemical behavior of microflora under poised potential conditions with respect to electron discharge was studied by employing cyclic voltammeter (CV) using an electrochemical workstation (CHI630B, China). All electrochemical assays were performed in situ by considering anode and cathode as working and counter electrodes, a saturated calomel electrode (SCE) served as the reference electrode in the electrolyte. CV was performed by applying a potential ramp to the working electrode (anode) at a scan rate of 10 mV/s over the range of -0.65 to +0.2 V.

3. Results and discussion

3.1. The impact of acetate, butyrate or CSFE on the voltage generation in MFC

After the first inoculation of the MFC (approximately 6 days, see Fig. S2) with the hydrogen fermentation effluent, stable electricity can be generated in MFCs enriched with acetate (1000 mg/L), butyrate (1000 mg/L) and CSFE, respectively. An example of voltage output in MFC with the substrate was shown in Fig. 2. The stable voltage of acetate or CSFE acclimated MFC reached 531 ± 5 and 551 ± 4 mV, respectively. However, the voltage output of butyrate acclimated MFC only was 423 ± 8 mV, which is less than that of acetate and CSFE acclimated MFCs. Concomitant with electrical energy generation, the VFA removal of 98 ± 1% was achieved in all the MFCs. The phenomenon was similar with previous studies (Zhang et al., 2011).
Furthermore, low concentrations of acetate (90-120 mg/L) were detected at the stable period in the butyrate acclimated MFCs, butyrate and acetate removal were nearly complete when the voltage was less than 50 mV. This behavior indicated some butyrate was first degraded into acetate, and then the acetate was removed as well. In addition, there was no lag phase of energy output appeared in the butyrate-enriched MFCs, demonstrating that electricity generation from butyrate was primarily due to direct electron transfer by the exoelectrogens attached to the anode surface and did not require accumulation of mediators in the fresh solution. These results were similar to previous study (Liu et al., 2010; Zhang et al., 2011), in which butyrate was first converted into acetate by butyrate degrading acetogenic bacteria.

**3.2. MECs Start-up**

Fig. 3 illustrates the effect of anode acclimation method on the current of MECs. During the start-up period, HBu-MEC always showed a higher current than HAc-MEC and CSFE-MEC. Owing to the higher current density, the hydrogen production rate of HBu-MEC was greater than that of HAc-MEC and CSFE-MEC. Results also show that, after 9 cycles, HAc-MEC and CSFE-MEC could obtain stable current density of 346 ± 11 and 391 ± 9 A/m³, respectively. The hydrogen production rate of HAc-MEC and CSFE-MEC were 3.56 ± 0.22 and 3.87 ± 0.23 m³/m³/d. However, it took longer time, about 15 cycles, for HBu-MEC to achieve the stable performance under 0.8 V external applied voltage. Compared with HAc-MEC and CSFE-MEC, the current density and hydrogen production rate of HBu-MEC have great improvement, reached 480 ± 11 A/m³ and 4.52 ± 0.13 m³/m³/d, respectively. Since higher current density represents faster substrate degradation and more efficient on wastewater treatment in MECs (Lu et al., 2016), this great improvement of current density and hydrogen production rate indicated that the anodes acclimated using butyrate as substrate could degrade the VFAs more fast. When comparing to other MEC systems fed with hydrogen fermentation effluent, the HBu-MEC obtained higher hydrogen production rate. Wu et al. used single-chamber microbial electrolysis
cell fed with effluent from anaerobic baffled reactor and the hydrogen production rate was only 1.31±0.04 m³/m³/d at 0.6 V (Wu et al., 2013). Nam et al. reported H₂ production rate of only 0.49 ± 0.05 m³/m³/d at 0.9 V from cellulose fermentation wastewaters in MEC (Nam et al., 2014).

3.3. The H₂ recovery and treatment efficiency in differently MECs

As shown in Fig. 4a, the H₂ production capability of MECs increased with the applied voltages in a certain range (0.5–0.8 V). The maximum cumulative H₂ volume reached to 234.5 ± 11.2 mL (5.21 ± 0.24 L H₂/L fermentation effluent) in HBu-MEC at 0.8 V. While the maximum cumulative H₂ volume of HAc-MEC and CSFE-MEC were only 189.8 ± 8.7 mL (4.22 ± 0.19 L H₂/L fermentation effluent) and 204.8 ± 6.5 mL (4.55 ± 0.14 L H₂/L fermentation effluent) at 0.8 V, respectively. The removal of COD in MECs with different applied voltage is shown in Fig. 4b. The COD removals obtained using HBu-MEC (71 ± 2%) was higher than that of HAc-MEC (45 ± 3%) and CSFE-MEC (51 ± 2%). The COD removals observed in this study was lower than that of previously reported for fermentation effluent (79-86%) (Ullery & Logan, 2014). That could be due the incomplete removal of butyrate from the fermentation effluent. In addition, there were also other components beside VFAs and alcohols in the effluent from corn stalk dark fermentation, such as cell biomass, proteins, furfural and pigment from corn stalk hydrolysis, which could not be consumed by the exoelectrogens at the anode (Kadier et al., 2014).

Fig. 4c and 4d depict the variation of CE and ηₑ in different MECs at different applied voltages. Seen from Fig. 4c, the CE were not significantly different based on the acclimation methods, the CE ranged from 72 ± 3% to 76 ± 2%. The energy efficiency was significantly higher in the HBu-MEC than the HAc-MEC and CSFE-MEC under same applied voltage. However, the energy efficiency decreased with the increase of applied voltage in the same MEC. For example, the maximum energy efficiency were obtained at 0.5 V, which were 178 ± 9%, 215 ± 11% and 185 ± 14% in HAc-MEC, HBu-MEC and CSFE-MEC, respectively. However, the
energy efficiency respectively decreased to 145 ± 11%, 165 ± 10% and 147 ± 8% with the applied voltage increasing to 0.8V.

The removal of VFAs in different acclimated MECs at different applied voltage is showed in Fig. 5. The concentration of acetate in the original effluent decreased from 3101 ± 21 mg/L to 79 ± 15 mg/L, while propionate and ethanol concentrations decreased to 8 ± 2 mg/L in all MECs. The removal of butyrate reached to 62 ± 3% in HBu-MEC, which was much higher that of HAc-MEC (8 ± 3%) and CSFE-MEC (15 ± 2%). These results demonstrated that the butyrate acclimated MEC had significantly improvement on butyrate and COD removal compared with the MECs acclimated with acetate and fermentation effluent. Many previous studies have demonstrated the butyrate were difficult to degrade in MECs (Liu et al. 2012; Li et al., 2014; Yang et al., 2015; Popov et al., 2016), and the acetate acclimated anodes performance was limited when degraded butyrate. However, the butyrate acclimated anodes performance was enhanced when degraded butyrate and acetate (Michie et al., 2013; Popov et al., 2016). The predominant microbial composition were different due to the different microbial anode acclimation strategies associated with acetate and butyrate (Zhang et al., 2011; Michie et al., 2013; Popov et al., 2016). These results were further supported in this study in terms of MEC response to fermentation treatment and H₂ production.

Moreover, the average contents of H₂ and CO₂ were detected to be 87 ± 3% and 13 ± 3% respectively in gas phase, and there was no methane observed in all MECs. Results indicated that the single-chamber MEC with double anodes can produce efficiently additional H₂ from the effluent of hydrogen fermentation, and the growth of methanogens in MEC was inhibited. That is due to the pretreatment method of enriching exoelectrogens at anode provided an effective alternative method to inhibit the methanogenesis which is commonly considered to be one of major problems in MECs for H₂ production.

Several previous studies on H₂ production in MECs fed with fermentation effluent were
compared with this study. As shown in Table 1, comparison results showed that several key parameters in the present study were much higher than that of the literatures. Taken together, the highest COD and butyrate removal were achieved in HBu-MEC. In order to get higher hydrogen production rate and cumulative H2 volume, an applied voltage of 0.8 V was considered the most favorable for H2 production. Furthermore, as the microbial hydrogen oxidation often take place at the anode in single chamber MECs, higher hydrogen production rate is contributive to rapidly release of H2 from the MEC reactor for efficient collection (Lee and Rittmann, 2010; Lu et al., 2016). The values for current density and hydrogen gas volumes were also consistent over multiple cycles in MECs.

3.4. Electrochemical analysis of the bioanode

CV tests were conducted to analyze the exoelectrogenic activity of bioanodes acclimated in different procedures (Fig. 5). Voltammograms showed the variation in the electron discharge pattern with the function of external applied potential and different acclimated methods. The abiotic anodes MEC (Control) showed no electrochemical activities, but clear oxidation peak were observed on HAc-MEC, HBu-MEC and CSFE-MEC. e.g. oxidation peaks were observed at -200 mV for the HAc-MEC and -250 mV for the CSFE-MEC. HBu-MEC can generate a higher current peak (26.8 mA) than HAc-MEC (24.0 mA) and CSFE-MEC (25.7 mA). The CV could be performed to characterize the electroactivity of the biofilms (Zhu et al., 2014), and the exoelectrogenic catalytic activity of bioanodes also could be improved by using the MEC with double anodes (Liang et al., 2011). In this study, the bioelectrochemical behavior of anode microorganisms also was affected by the acclimating substrates, which could be because that the type of substrate acclimated bioanode significantly affected the microbial community (Zhang et al., 2011). The anodes acclimated by using butyrate as substrate had higher electroactivity than that acclimated using acetate and fermentation effluent as substrate.

4. Conclusions
Pre-acclimation using different substrates had appreciable impact on the corn stack fermentation effluent treatment for hydrogen production in MECs. The differences in acetate, propionate and ethanol removal were not significant. Differences in butyrate removals and hydrogen yield were relatively appreciable. The butyrate removal in butyrate acclimated MEC could reach to $62 \pm 3\%$ and the maximum hydrogen yield was $5.21 \pm 0.24$ L H$_2$/L fermentation effluent. Moreover, the current generation and hydrogen production rate also were enhanced in the butyrate acclimated MEC. This study provides an efficiency anode acclimation strategy to enhance the fermentation effluent treatment for hydrogen production.

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Table 1. Performance of different MECs fed with fermentation effluent.

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>Substrate</th>
<th>$E_{ap}$ (V)</th>
<th>CE (%)</th>
<th>H$_2$ yield mL/g COD</th>
<th>$\eta_E$ (%)</th>
<th>HPR (m$^3$/m$^3$/d)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activated sludge</td>
<td>Sludge fermentation liquid</td>
<td>0.8</td>
<td>92±10</td>
<td>1200</td>
<td>155±5</td>
<td>1.76±0.03</td>
<td>(Liu et al., 2012)</td>
</tr>
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<td>Domestic wastewater</td>
<td>Molasses fermentation effluent</td>
<td>0.8</td>
<td>78</td>
<td>1155</td>
<td>170</td>
<td>1.52</td>
<td>(Lu et al., 2009)</td>
</tr>
<tr>
<td>Cow dung compost</td>
<td>Corn stalk fermentation effluent</td>
<td>0.8</td>
<td>71±2</td>
<td>1000±50</td>
<td>166±10</td>
<td>3.43±0.12</td>
<td>(Li et al., 2014)</td>
</tr>
<tr>
<td>Activated sludge</td>
<td>Sludge fermentation effluent</td>
<td>0.6</td>
<td>-</td>
<td>2780±110</td>
<td>138.6±3.1</td>
<td>1.31±0.04</td>
<td>(Wu et al., 2013)</td>
</tr>
<tr>
<td>Domestic wastewater</td>
<td>Cellulose fermentation effluent</td>
<td>0.9</td>
<td>66±11</td>
<td>1090±240</td>
<td>-</td>
<td>0.49±0.05</td>
<td>(Nam et al., 2014)</td>
</tr>
<tr>
<td>Wastewater</td>
<td>Corn stover fermentation effluent</td>
<td>0.5</td>
<td>-</td>
<td>750±180</td>
<td>230±50</td>
<td>1.0±0.19</td>
<td>(Lalaurette et al., 2009)</td>
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<tr>
<td>Cow dung compost</td>
<td>Corn stalk fermentation effluent</td>
<td>0.5</td>
<td>72±3</td>
<td>780±35</td>
<td>215±11</td>
<td>2.41±0.12</td>
<td>This study</td>
</tr>
<tr>
<td>Cow dung compost</td>
<td>Corn stalk fermentation effluent</td>
<td>0.8</td>
<td>76±2</td>
<td>870±40</td>
<td>155±10</td>
<td>4.52±0.13</td>
<td>This study</td>
</tr>
</tbody>
</table>

17
Figure Captions

Fig. 1. Schematic illustration of MEC with double anodes

Fig. 2. Voltage generation from different substrates in MFC with external load 1000 Ω.

Fig. 3. The performance of Current density during MECs start-up period. (The applied voltages of each cycle were 0.8 V except for the first three cycles. Cycle 1: 0.5 V; Cycle 2: 0.6 V; Cycle 3: 0.7 V.)

Fig. 4. (a) Cumulative hydrogen production, (b) COD removal, (c) Coulombic efficiency (CE) and (d) Energy efficiency in MECs under different applied voltage.

Fig. 5. Removal variation of VFAs and ethanol in different MECs at different applied voltages.

Fig. 6. Cyclic voltammogram comparison of anodes in different MECs. (Control: No microorganisms on the anodes)
Fig. 1.
Figure 2
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![Figure 2](Fig.2.docx)

Fig. 2.
Fig. 3.

Figure 3

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Fig. 4.

Figure 4
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Figure 5
Click here to download Figure: Fig.5.docx

**Acetate Removal (%)**

- **a** Acetate Removal (%) for different applied voltages (V) with varying removal percentages for HAc-MEC, HBu-MEC, and CSFE-MEC.

**Propionate Removal (%)**

- **b** Propionate Removal (%) for different applied voltages (V) with varying removal percentages for HAc-MEC, HBu-MEC, and CSFE-MEC.

**Butyrate Removal (%)**

- **c** Butyrate Removal (%) for different applied voltages (V) with varying removal percentages for HAc-MEC, HBu-MEC, and CSFE-MEC.

**Ethanol Removal (%)**

- **d** Ethanol Removal (%) for different applied voltages (V) with varying removal percentages for HAc-MEC, HBu-MEC, and CSFE-MEC.

*Fig. 5.*
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