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High-efficient acetate production from carbon dioxide using a bioanode microbial electrosynthesis system with bipolar membrane

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Abstract: The aim of this study was to develop an efficient bioanode microbial electrosynthesis system (MES) to convert carbon dioxide into acetate using bioenergy from the wastewater. The bioanode MESs were constructed using proton exchange membrane (PEM) and bipolar membrane (BPM) as separator, respectively, and operated under different voltages (i.e., 0.8, 1.0, 1.2, and 1.4 V). Since BPM could dissociate H_2O into H^+ and OH^- in situ to buffer the pH change in the chambers, the BPM-MES achieved 238% improvement in cathodic acetate production rate, 45% increase in anodic substrate removal efficiency, and more than five times enhancement in current output, as compared to the PEM-MES. The biomass on the surface of anode and cathode, and the relative abundance of *Acetobacterium* in the cathode of BPM-MES was higher than that in PEM-MES. Bioanode MES with BPM should be a useful microbial electrosynthesis strategy for acetate production using bioenergy from wastewater treatment.

Keywords: Microbial electrosynthesis system, bioanode, bipolar membrane, carbon dioxide, acetate production

1. Introduction

The microbial electrosynthesis system (MES) has recently garnered attention in the fields of microbiological and electrochemical engineering, as the system can convert carbon dioxide (CO_2) to multi-carbon products using electrical energy with bacteria as catalyst (Schroder et al., 2015; Wang & Ren, 2013). A typical double-chamber MES comprises an anode chamber and a cathode chamber separated by proton exchange membrane (PEM). The CO_2 conversion in the MES is basically occurred on the cathode side because a set of acetogens can use cathode as the only electron donor to convert CO_2 into acetate (Zhang et al., 2013). Theoretically, various renewable energy such as solar energy, wind energy, and bioenergy, can be used in the MES as the energy source (Gong et al., 2013; Rabaey et al., 2011; Tremblay & Zhang, 2015). However, abiotic electrical current has been tested as the sole energy in most previous studies (Jourdin et al., 2016; Jourdin et al., 2015; Marshall et al., 2013). The protons and electrons generated by water electrolysis in the anode transfer to the cathode and participate the microbial reduction of carbon dioxide. Such a pure electrochemical reaction in anode may use intensive energy (Rabaey et al., 2011) and the produced molecular oxygen can be detrimental to the anaerobic reduction of CO_2 (Mohanakrishna et al., 2015). As an alternative to water-electrolysis anode, bioanode MES has been proposed using pure culture. Gong et al. reported that hydrogen sulfide can be used as an electron source for MES using *Desulfobulbus propionicus* as biocatalyst in anode (Gong et al., 2013). Use of mixed culture

bioanode is attractive as they are readily obtained in large biomass and are more tolerant to environmental fluctuation. Despite various wastewater that containing high potential energy had been tested as the energy source in other bioelectrochemical systems (BESs), there is hardly any work reported for MES. Studies that use the mixed culture bioanode are still needed to fully utilize wastewater as the energy source.

Minimizing the pH imbalance is necessary for the construction of bioanode MES, because the pH imbalance in BES produces a 0.059 V/ pH potential loss (Fornero et al., 2010). Similar with other bioelectrochemical systems, the reactions in anode chamber of MES generate the protons and electrons concurrently. The electrons will be collected by the anode and transfer through the external circuit to the cathode. Although the protons should transfer from the anode to the cathode chamber through the membrane, accumulation of protons and lower the pH have been commonly observed in the anolyte due to the limiting factors, such as membrane permeability, competition from other cations, etc., resulting in inhibition to the start-up and performance of bioanode (Fornero et al., 2010; Rabaey et al., 2011; Rozendal et al., 2006). In the MES, hydrogen ions can be consumed by the bio-cathodic reduction, which, results in the pH rise in cathode chamber according to the following equations:

$$4H_2 + 2CO_2 \rightarrow CH_2COOH + 2H_2O \quad (1)$$

$$8H^+ + 8e^- + 2CO_2 \rightarrow CH_3COOH + 2H_2O \quad (2)$$

The pH value of catholyte should influence the reduction rate and biofilm activity of biocathode significantly. Previous studies reported that the pH range of many

61 homoacetogenic bacteria was acidic-like (Mohammadi et al., 2011). A higher
62 acetate production rate has achieved at lower pH (below 6.0) of catholyte than at
63 higher pH by improving substrate availability and enhancing microbial activity
64 (Batlle-Vilanova et al., 2016; Jourdin et al., 2016). While in most BES studies,
65 strong phosphate or carbon buffers (50 - 200 mM) have been utilized to maintain pH
66 neutrality in the chambers, such chemical modifications cannot be used in large scale
67 applications due to economic and environmental concerns. Since membrane is a
68 necessary component in the structure of MES, the bipolar membrane (BPM) could be
69 an alternative membrane because it dissociated H_2O into H^+ and OH^- in situ to support
70 anodic and cathodic reactions, respectively, minimizing the pH imbalance in the
71 chambers.
72 Therefore, the objective of this study was to develop a MES with bibioelectrodes
73 using artificial wastewater as the energy source. As shown in Fig. 1, in the
74 two-chamber MES, feeding artificial wastewater to the anode bacteria produce
75 electrons, which are further used by the cathode bacteria to convert CO_2 into acetate.
76 BPM was adopted as the separator between the anode and cathode chambers and its
77 effects were compared with that using PEM. Different applied voltages (0.8, 1.0, 1.2,
78 and 1.4 V) were investigated as an auxiliary energy source. Performance of the
79 BPM-MES was analyzed in terms of acetate production, electrode reaction rate,
80 biofilm structure, biomass, and community diversity.

81 **2. Materials and methods**

82 **2.1 Source of microorganisms and medium**

Planktonic cells from a mature bioelectrochemical system described by Liu et al.(Liu et al., 2015) were collected, centrifuged, suspended in fresh anolyte and used as anode inocula for the MESs. The cathode inocula were enriched using granular sludge from the Zhujiang beer brewery (Guangzhou, China), and domesticated under hydrogen-containing syngas mixture (H_2/CO_2(80/20,v/v)) with half of year and already had the ability to produce organics (with an acetate production rate of 5.16 mM/d).

The mineral synthetic medium as catholyte was prepared based on DSMZ-recommended growth medium (DSMZ 311) (Nevin et al., 2010), with deionized water containing per litre: 0.5 g NH_4Cl, 1.0 g NaCl, 0.1 g KCl, 0.4 g $MgCl_2 \cdot 6H_2O$, 0.05 g $CaCl_2$, 2 mg $FeSO_4 \cdot 7H_2O$, 0.23 g KH_2PO_4, 0.35 g K_2HPO_4 and 4 g $NaHCO_3$, 10 ml vitamin solution, 10 ml mineral solution, and adjusted pH to 7. To inhibit methanogen, 10 mM sodium 2-bromoethanesulfonate (Marshall et al., 2013) was added during the whole experimental period. The artificial wastewater was used as anodic medium, with deionized water containing per litre: 4 g CH_3COONa, 4.09 g Na_2HPO_4, 2.54 g NaH_2PO_4, 0.31 g NH_4Cl, 0.13 g KCl (Liu et al., 2015), 10 ml vitamin solution and 10 ml mineral solution (pH about 6.9).

2.2 Reactor Construction and Operation.

The H type MES was constructed using two identical custom glass chambers that clamped together separating with a 6.15 cm^2 BPM (Fuma-sep-FBM, FuMA-Tech GmbH, Germany) or PEM (Nafion117, DuPont, USA) (Fig. S1). Five pieces of plain graphite plates (2 cm × 5 cm × 0.2 cm) were used as anodes and cathodes,

105 respectively. A saturated calomel electrode (+0.241 V *vs* standard hydrogen
106 electrode (SHE)) was used as a reference electrode to the cathode. All electrode
107 potentials were reported versus SHE.
108 After being assembled and sterilized (115°C for 20 min), the enriched cultures were
109 incubated in the anode and cathode chambers, each of which was with a volume of
110 150 mL containing 130 mL medium. The abiotic MESs were feeding with sterile
111 medium without the addition of inocula and operated under the voltage of 1.4 V. To
112 remove headspace air and dissolved oxygen after inoculation, the cathode and anode
113 chambers were bubbled with 100% CO_2 and 100% N_2, respectively, then were sealed
114 with rubber stoppers. For the MESs reactors, an external resistor (10 Ω) was
115 connected with the negative electrode of the power supply and the cathode, while the
116 positive electrode was connected to the anode. Four fixed voltages (0.8, 1.0, 1.2 and
117 1.4 V) were applied to the above reactors circuit using a power supply (Itech, IT6720,
118 China). Current generation was monitored with a data logger (Keithley 2700,
119 module 7702). The MESs were operated at 30°C and the catholyte was bubbled
120 with 100% CO_2 every 2 d (10 min per time) to buffer the pH (Marshall et al., 2013).
121 According to the current change, 12 d was defined as a cycle, and the MESs were
122 operated at least four cycles.

123 **2.3 Chemical and Physiological Analysis Methods.**

124 The medium pH values were monitored with a pH meter (PHS-3C, Leici, China)
125 every 2 d before and after sparging gas. Organic acids were quantified using a high
126 performance liquid chromatography (HPLC, Agilent Technology 1100 series,

AgilentInc., USA) equipped with an organic acid analysis column (Zorbax SB-Aq (4.6×150 mm，5 μm), Agilent Inc., USA), a UV detector was set at 210 nm. The mobile phase was 0.09 mol/L K_2HPO_4 solution and with a flow rate of 0.8 mL/min. Protein content was quantified to evaluate the biofilm followed Yang and Xiang (Yang et al., 2014). To analyze the biofilm structure and cellular activity, two of carbon plates (0.5 cm ×1 cm) were cut from the electrode and analyzed with confocal laser scanning microscopy (CLSM) (Yang et al., 2015). Biofilm on anode and cathode was sampled and rinsed in sterilized PBS to remove the loosely attached planktonic cells. The samples were then stained with an LIVE/DEAD BacLight staining kit (Invitrogen) and subsequently observed under CLSM (LSM 700, Zeiss). At least 12 random view-fields (650×650 μm for each field) were selected and analyzed for each biofilm sample. To obtain three-dimensional (3D) structure information, the biofilm sample was observed using "Stack" model of the Zen software (Zeiss). Specific viability of each biofilm layer was analyzed and presented by the ratio of viable/total biofilm cells based on per area (obj./total) counting in software (Image-Pro Plus 6.0).

2.4 Calculation

The acetate production rates (P_v) was calculated by

$$P_v = \frac{P_t - P_{t0}}{t - t0} \quad (3)$$

where P_{to} and P_t are the initial and final concentrations of the product (acetate) in the cathode chamber, respectively, and t_0 and t are the initial and final time of the measurements.

Electron recovery (ER) is the efficiency of capturing the electron from the electric

currents to the product (also called coulombic efficiency in cathodic processes (Patil et al., 2015b)). In this study, only the acetate production was considered for electron efficiency calculations according to

$$ER = 100\% \times \frac{C_p}{C_t} \quad (4)$$

Here the total coulombs consumed (C_T) is calculated by integrating the current over the measurement time period, and C_P is coulombs consumed and calculated by $C_P=b\cdot n\cdot F$, where b is the number of electrons (8 electron equivalent per mol for acetate), n is the number of moles of acetate product, and F is Faraday's constant (96,485 C/mol).

Coulombic efficiency (CE) in anodic side, is the total coulombs to the theoretical consumption coulombs of acetate in anode and was calculated by

$$CE = 100\% \times \frac{C_T}{b(C_t - C_{t0})VF} \quad (5)$$

where C_{t0} and C_t are the initial and final concentrations of acetate in the anode chamber (M), respectively, V is the volume of the cathode solution (L).

The overall energy recovery (η_{E+C}) was the ratio of energy content of energetic production (acetate) produced to the inputs from both electrical energy and substrate (acetate in anode) and was calculated by

$$\eta_{E+C} = 100\% \times \frac{(P_t - P_{t0})\Delta H}{\int_{to}^{t}(IU - I^2R_{ex})dt + (C - C_0)\Delta H} \quad (6)$$

Here ΔH is the combustion heat of acetate (ΔH=870.28 kJ/mol) (Call & Logan, 2008). I is the current (A), U is the applied voltage (V), and R_{ex} is the external resistance (Ω).

The total energy consumption (E) (i.e., the energy consumption for the production of

1 kg acetate, kWh/kg) in the MES included the electricity input from the power supplier and the energy from acetate utilization by exoelectrogens in the anode chamber (Liu et al., 2014)

$$E = \frac{\int_{to}^{t} UIdt + (C_t + C_{t0})\Delta H}{3600(P_t - P_{t0})MV} \quad (7)$$

where M is the acetate molar weight (60.05 g/mol).

2.5 Bacteria Community

Samples were cut from the biofilm of anode and cathode electrodes with sterile scissor at the end of cycle. Total genomic DNA extraction from the samples was conducted with a DNA kit according to the manufacturer's manual (K182001, Invitrogen Bio-Tek, USA). The DNA qualities of the samples were examined with 1% agarose gel electrophoresis. The V4 region of the 16S rRNA gene was amplified for pyrosequencing using bacterial primers 515F (GTGCCAGCMGCCGCGGTAA) and 806R (GGACTACHVGGGTWTCTAAT) were used for PCR amplification. A PCR reaction volume of 50 μL was used with the following procedure: the initial denaturation of DNA for 3 min at 95 ^{o}C, 30 cycles of 30 s at 94 ^{o}C, 1 min at 55 ^{o}C,1 min at 72 ^{o}C, and then a final extension for 10 min at 72 ^{o}C. The final products were purified, quantified and then sequenced on an Illumina Miseq platform by Majorbio (Shanghai, China).

3. Results and discussion

3.1 Enhanced acetate production and substrate utilization in the BPM-MES

As shown in Fig.2 A, in the cathode of the BPM-MES, the average acetate production values were 4.80 ± 0.91, 9.85 ± 0.37, 12.26 ± 0.31, and 16.74 ± 1.07 mM, respectively,

192 with the applied voltages of 0.8, 1.0, 1.2, and 1.4 V. The acetate yield in the
193 BPM-MES was positively correlated to the applied voltage. In the cathode of the
194 MES with PEM as separator (PEM-MES), the average acetate production values
195 ranged in 4.39-5.17 mM with the different applied voltages. The maximum acetate
196 yield in the BPM-MES was 238 ± 6.76% higher than that in the PEM-MES. The
197 maximum acetate production rate in the BPM-MES was 1.39 ± 0.09 mM/d
198 normalized to total catholyte volume, which was much higher than those in other
199 studies (0.42 - 1 mM/d) (Jourdin et al., 2014; Patil et al., 2015a). Besides acetate,
200 formate and propionate were detected but with concentrations lower than 5 mg/L,
201 indicating high product specificity in our system. In addition, acetate production
202 rates in the BPM-MES maintained at a relative constant value during 48 d of
203 operation (with 4 cycles), while, production rates in the PEM-MES decreased
204 significantly after 5 d within each cycle. The results might be attributable to the
205 changes of electron transfer rates that affected by many parameters in the MES as
206 discussed later.
207 In this study, bioanode was successfully applied to use artificial wastewater and its
208 performance under different applied voltages were investigated. As shown in Fig. 2
209 B, the average removal efficiencies of COD in the anode of the BPM-EMS during the
210 4 cycles were 51.5 ± 4.5%, 63.2 ± 5.8%, 71.3 ± 5.5% and 87 ± 4.19%, respectively,
211 with 0.8, 1.0, 1.2, and 1.4 V. An increase of the electron donor removal rate in anode
212 with increasing of the applied voltage from 0 to 2 V has been reported previously by
213 Coma et al (Coma et al., 2013). The result was likely due to that higher applied

voltage could promote the electron transfer between anode and cathode, and further stimulate the biofilm growth and activity (Gong et al., 2013). However, in the PEM-MES, the average removal efficiencies were similar with the different applied voltages (i.e., 45.2 ± 2.6%, 45.6 ± 0.4%, 47.8 ± 0.8% and 45.1 ± 0.4%, respectively, with 0.8 to 1.4 V). The result might be related to pH increase to offset the positive effect by the electro-potential on anode biofilm. The improved performance of the BPM-MES was most likely due to the improvement of microbial activities on both anode and cathode chambers because little change of the acetate concentration was observed in abiotic MES (Fig. S2A).

3.2 BPM Enhanced the current output and energy efficiency of MES

With refreshment of substrate in the anode and cathode chambers, the current output of the MES increased significantly within 48 h in each cycle (Fig. 2C), suggesting that the biofilm in the MES was mature and had a stable activity. A negligible current was obtained in the abiotic MES throughout the experiments with applied voltage of 1.4 V (Fig. S2B), indicating that the current output was promoted by the catalysis of electrochemical-active biofilm. The average current densities in the BPM-MES were 0.101 ± 0.009, 0.190 ± 0.026, 0.272 ± 0.033, and 0.369 ± 0.041 A/m^2, respectively, with 0.8, 1.0, 1.2, and 1.4 V. In the PEM-MES, the average current densities ranged from 0.072 to 0.078 A/m^2 with the voltages from 0.8 to 1.4 V. In the BPM-MES, a positive correlation was observed between the average current and applied

voltage with R^2=0.999 (Fig. S3). The maximum current of 0.369 ± 0.041 A/m^2 was observed at the voltage of 1.4 V, which was more than five times higher than that in the PEM-MES. The result was consistent with the improved electrode reactions in the BPM-MES. To eliminate the effect of CO_2 shortage, the cathode chamber of MES was bubbled with pure CO_2 gas every 48 h. In the BPM-MES, current fluctuation in a range of 0.1-0.8 mA was observed shortly after the batch supplement of CO_2. The fluctuation range was also positively correlated with the applied voltage. Interestingly, such fluctuation was not observed in the PEM-MES. The results implied that the CO_2 supplement could be a rate-limiting factor in the BPM-MES and a continuous mode could be adopted to achieve higher performance of the MES. To better compare the performance between the MESs, the columbic output, current efficiency, electron recovery efficiency, and energy consumption within a cycle was calculated (Table 1). From 0.8 to 1.4 V, electrons (i.e bioenergy) harvested in the BPM-MES increased gradually from 1033.5 ± 102.0 to 3780.9 ± 424.2 C, while electrons in the PEM-MES ranged from 737.1 ± 18.8 to 800.9 ± 9.4 C. Compared to the PEM-MES, the BPM-MES improved the columbic production from 40% to 405% with the increase of applied voltages. In the BPM-MES, the average current efficiencies (*CE*) were 41.2 ± 2.5%, 62.6 ± 3.8%, 80.0 ± 4.9% and 89.1 ± 6.1%, respectively, with 0.8, 1.0, 1.2. and 1.4 V. The correspondingly *CE* values in the PEM-MES were 33.5 ± 1.4%, 34.4 ± 1.2%, 34.3 ± 0.7%, and 34.1 ± 0.9%, respectively. The improvement of both COD removal rate and *CE* in the BPM-MES

indicated that the activity of anode bacteria especially the electrochemical active bacteria was improved efficiently, therefore, more substrate (acetate) was converted to electricity for acetate production in the cathode chamber (Gong et al., 2013). It's worth to noted that, even though higher acetate was produced in the BPM-MES, the energy consumption normalized to acetate production was lower than that in PEM-MES in most cases. For example, with the applied voltage of 1.0 V, the energy consumption was 19.6 ± 1.59 and 24.3 ± 2.17 kWh/kg (acetate) by the BPM-MES and PEM-MES, respectively.

The electron recovery in acetate ranged from 44.6 ± 6.6% to 51.8 ± 6.6% in the BPM-MES. In the PEM-MES, the higher electron recovery from 60.0 ± 12.3% to 66.4 ± 4 % might be due to that BPM consumed part of the electrons for water electrolysis. The *energy recovery efficiency* in the BPM-MES were comparable with those in other mixed-culture MESs, but lower than those in pure culture MESs. In the mixed-culture MESs, the pathways of electron loss may include the concomitant production of non-identified products (Mohanakrishna et al., 2016; Nevin et al., 2011), imperfect catalysis of mixed cultures on the cathode surface, ohmic loss due to the electrode and the electrical circuit(Rabaey & Rozendal, 2010), and for biomass maintenance and growth. The enhancement (Lu et al., 2016) of *ER* could be achieved by cooperation of operation mode and MES structural optimization, to realize the active acetate extraction from the cathode chamber.

3.3 Enhanced mechanisms for BPM-MES performance

3.3.1 The pH variations

Microbial electrosynthesis from CO_2 to acetate is strongly dependent on pH (Jourdin et al., 2016). Fig. 3 shows the pH changes in anolyte and catholyte with the applied voltages during four cycles. The pH change trends in the BPM-MES and PEM-MES were similar, i.e., increase in catholyte and decrease in anolyte. The pH values of catholyte were affected more by the applied voltages than those of anolyte. In the MES, bio-anodic oxidation reactions release electrons and protons simultaneously, which can lead to accumulation of protons and decrease pH due to the slow and incomplete diffusion of protons from anode to cathode. The bio-cathodic reduction process consumes hydrogen ions and results in pH rise in the cathode chamber (Marshall et al., 2013). This result was different from some previous studies that operated under continuous mode (Batlle-Vilanova et al., 2016; Patil et al., 2015a). In the MES with continuously purged with N_2:CO_2 mixed gases, the pH of catholyte decreased with the production of acetate due to the accumulation of VFAs in the system. The pH decline in the continuous MES also could be attributed to the buffering capacity of CO_2 with the formation of bicarbonate. Moreover, a significant difference in the change range was observed between the PEM-MES and BPM-MES. For the PEM-MES, the pH values increased from 6.5 to 9.0 in the catholyte and decreased from 7.0 to 5.2 in the anolyte. For the BPM-MES, the pH value increased from 6.5 to 7.8 in the catholyte and decreased from 7.0 to 6.3 in the anolyte. It has been reported that pH 5.8 is the optimum value of catholyte achieved a higher acetate production rate than at higher pH by improving substrate availability and enhancing

microbial electrosynthesis (Batlle-Vilanova et al., 2016; Jourdin et al., 2016). Therefore, the BPM could be an important alternative membrane for the MES system because it dissociated H_2O into H^+ and OH^- in situ to support anodic and cathodic reactions, respectively, buffering the pH change in the chambers.

3.3.2 Biomass and Long-term Viability of Biofilm

In the BPM-MES, the biofilm protein values in the anode were 284 ± 8.26, 365 ± 50.0, 422 ± 30.5, and 596 ± 47.9 μg/cm^2, respectively, at 0.8, 1.0, 1.2, and 1.4 V (Fig. 4A). Correspondingly, the biofilm protein values in the cathode were 35.9 ± 3.23, 44.1 ± 3.23, 46.1 ± 4.67, and 70.2 ± 1.44 μg/cm^2, respectively. The biomass in the chambers increased with the applied voltages. In the PEM-MES, the highest protein values in the anode and cathode of 250 ± 27.8 and 45.2 ± 4.04 μg/cm^2 were obtained at 1.2 and 1.4 V, respectively. Compared with the PEM-MES, the biomass on the surface of anode and cathode of the BPM-MES improved by 190 ± 24.5% and 56.3 ± 17.2%, respectively, at 1.4 V. This result was well consistent with the enhanced performance in the BPM-MES as a result of pH changes. It was worth to note that the biomass on the anode was significant higher than that on the cathode. For example, the anode biomass values were 7.96 ± 0.95, 8.26 ± 0.53, 9.23 ± 1.6, and 8.48 ± 0.51 times higher than the cathode biomass values in the BPM-MES at the applied voltages. The relatively thin biofilm on the cathode has also been described for other biocathodes using pure or mixed cultures(Strycharz et al., 2010; Strycharz et al., 2008), which can be attributed to direct electrode-to-cell electron transfer respiration of the cathode cells.

The long-term viability of electrode biofilm was further evident from confocal scanning laser microscopy of biofilm under a condition of fixing CO_2 for over 48 d (Fig. 4B). Cells in the biofilm treated with LIVE/DEAD BacLight viability stain, stained green, suggesting that they were healthy and metabolically active (Nevin et al., 2010). With 0.8 V applied voltage, scattered green cells were observed on the anode surface of BPM-MES. The green cells covered most of the anode surface with the applied voltages from 1.0 to 1.4 V, which was consistent with the biomass results. As comparison, the green cells distributed lightly on the anode of PEM-MES, and the lowest was observed at 1.4 V. Viability staining showed that the average viability results of anode biofilm in the BPM-MES (0.723 ± 0.002, 0.958 ± 0.01, 0.961 ± 0.0159, and 0.897 ± 0.021) were higher than those in the PEM-MES (0.287 ± 0.042, 0.68 ± 0.003, 0.789 ± 0.013, and 0.363 ± 0.018) with 0.8, 1, 1.2, and 1.4 V, respectively. The significant difference between the anode biofilm of MESs was most likely attributable to the effect of pH changes, as the pH value in anode decreased to 5 in the PEM-MES and could significantly affect the metabolically active of bacteria. Similarly, the number of active cells on the cathode of BPM-MES was higher than that of PEM-MES (Fig. S4).

3.3.3 Bacterial community structure

To better understand the mechanism of MES and the difference between the BPM-MES and PEM-MES, the microbial community composition on inocula and the cathode biofilm were analyzed with pyrosequencing (Fig. 5). In the inoculum of biocathode, the *Firmicutes* accounted for absolute dominance with the abundance of

346 90% and no *Euryarchaeota* was observed at the phyla level. The predominant
347 bacterial phyla at cathode biofilm were *Euryarchaeota* (37.5%-59.3%), *Firmicutes*
348 (5.5%-16.2%), *Proteobacteria* (8.8%-19.6%), *Bacteroidetes* (10.3%-20.3%), and
349 *Synergistetes* (6.6%-24.2%). The similar structure of microbial communities was
350 observed previously on microbial biocathodes that catalyzed acetate production
351 (Marshall et al., 2012). The difference between the inocula and biofilm could be
352 attributed to the change of electron donor supplying. For the inocula, H_2 was offered
353 as the sole electron donor and supplied in batch mode. For the biofilm, cathode was
354 used as the electron donor and the bacteria accept the electron from the cathode
355 through direct or indirect pathways as described in literatures previously (Bajracharya
356 et al., 2015; Blanchet et al., 2015; Nevin et al., 2010).
357 At the genus level, the predominant species on the cathode biofilm mainly included
358 *Methanobrevibacter*, *Acetobacterium*, *Desulfovibrio*, *Aminivibrio*, and *Petrimonas*.
359 *Acetobacterium* is a well-known homoacetogenic bacteria and can convert CO_2 into
360 acetate using electrode or H_2 as electron donor directly (LaBelle et al., 2014; Marshall
361 et al., 2012; Marshall et al., 2013; Patil et al., 2015a). With applied voltages of 0.8,
362 1, 1.2 and 1.4 V, the relative abundance of *Acetobacterium* in the cathode of
363 BPM-MES was 12.6%, 13.6%, 9.6% and 12.1%, respectively, which decreased to
364 2.7%, 3.6%, 2.8% and 9.1% in the PEM-MES, respectively. This results combined
365 with the biomass data well explained the higher acetate production rate in the
366 BPM-MES than in the PEM-MES. *Methanobrevibacter* accounted for 16.5%-59.3%
367 on the biofilm, which implied that some of the electron could be loss for the

methanogenesis during the operation. The high abundance of methanogenic bacteria was found commonly in the biocathode of mixed culture MES (Marshall et al., 2012; Patil et al., 2015a). Methanogenic bacteria have the ability to use the cathode as the sole electron donor that was named by electromethanogenesis as reported by Cheng et al. (Cheng et al., 2009). Methane production from H_2 consumption is one of the most critical causes of low H_2 yield in the microbial electrolysis system. In this study, the acetate prodution increased gradually with the operation time, suggesting that electro-methanogenesis and hydrogenotrophic methanogenesis were the major metabolic pathway of *Methanobrevibacter*. Several studies have reported that acetate cannot be utilized by some genus of *Methanobrevibacter* (Ferrari et al., 1994; Leadbetter & Breznak, 1996; Miller et al., 1982; Savant et al., 2002). And some strains of *Methanosarcina* can excrete acetate during growth on H_2 plus CO_2 (Westermann et al., 1989). Besides, hydrogen-producing bacteria *Petrimonas* reached 15% in the BPM-MES, indicating the hydrogenotrophic metabolism might occurred for acetate production (Blanchet et al., 2015). *Desulfovibrio* has been found commonly in the bioelectrochemical system, which can play a positive role on electron transfer between the electrode and bacteria (Luo et al., 2014). The content of gases including H_2 and methane was not detected as the air pressure became negative at every 2 d (before bubbling 100% CO_2). To investigate the relationship between species such as *Methanobrevibacter* and *Acetobacterium*, further study should be conducted under continuous air supplying mode which is outside the scope of this study.

4. Conclusions

Bipolar membrane was successfully utilized in the MES, in which bioanode was used to supply electrons to the cathode. The BPM could dissociate H_2O into H^+ and OH^- in situ to minimize the pH imbalance in the MES chambers, which resulted in the increase of acetate yield, current efficiency, and biomass and bacterial activity of biofilm, compared with those in the PEM-MES. The relative abundance of *Acetobacterium* in the cathode of BPM-MES was higher than that in the PEM-MES. Further study is needed to investigate the relationship between species in the mixed culture biocathode of MES.

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Table 1. Performance of MES of each test

Anode	Separator Types	Applied voltage (V)	Current Density (A/m^2)	Electrons Harvesting (C)	P_v (mM/d)	Electron recovery in product (%)	*CE* in anodic side (%)	η_{E+C} (%)	Total energy consumption (%)	Ref.
Mixed culture	BPM	0.8	0.101 ± 0.009	1033.5 ± 102.0	0.41 ± 0.075	46.5 ± 6.6	41.2 ± 2.5	14.8 ± 1.7	27.6 ± 2.47	This study
		1.0	0.190 ± 0.026	1943.3 ± 269.7	0.82 ± 0.032	51.8 ± 6.6	62.6 ± 3.8	20.7 ± 1.8	19.6 ± 1.59	
		1.2	0.272 ± 0.033	2785.1 ± 339.3	1.02 ± 0.025	44.7 ± 6.2	80.0 ± 4.9	19.3 ± 2.1	21.1 ± 2.16	
		1.4	0.369 ± 0.041	3780.9 ± 424.2	1.39 ± 0.089	44.6 ± 2.5	89.1 ± 6.1	18.8 ± 0.4	21.4 ± 0.43	
Mixed culture	PEM	0.8	0.072 ± 0.002	737.1 ± 18.8	0.36 ± 0.063	60.0 ±12.3	33.5 ± 1.4	16.1 ± 3.4	26.0 ± 2.04	This study
		1.0	0.075 ± 0.002	765.6 ± 20.4	0.41 ± 0.062	64.3 ± 10.8	34.4 ± 1.2	16.9 ± 2.6	24.3 ± 2.17	
		1.2	0.078 ± 0.001	800.9 ± 9.4	0.43 ± 0.045	64.9 ± 7.5	34.3 ± 0.7	16.3 ± 1.9	25.0 ± 2.27	
		1.4	0.073 ± 0.002	749.3 ± 12.6	0.41 ± 0.022	66.4 ± 4	34.1 ± 0.9	15.9 ± 0.8	25.4 ± 1.32	
Abiotic	CEM	-0.4[a]	0.208	-	0.17	85	-	-	-	(Nevin et al., 2010)
Abiotic	CEM	-1.26[a]	5[b]	-	1.0 ± 0.09	58 ± 5	-	-	-	(Patil et al., 2015a)
Abiotic	PEM	-0.60[a]	39.11	-	1.125	26.26	-	-	-	(Mohanakrishna et al., 2016)
Granular activated sludge	None[c]	-0.4[a]	0.142	-	0.949	29.91	-	-	-	(Mohanakrishna et al., 2015)
Desulfobulbus propionicus	PEM	+0.498[d]	0.15	-	0.23	>90	-	-	-	(Gong et al., 2013)

[a] Fixed cathode potential;
[b] Fixed current;
[c] Single chamber MES;
[d] Fixed anode potential.

Figures List

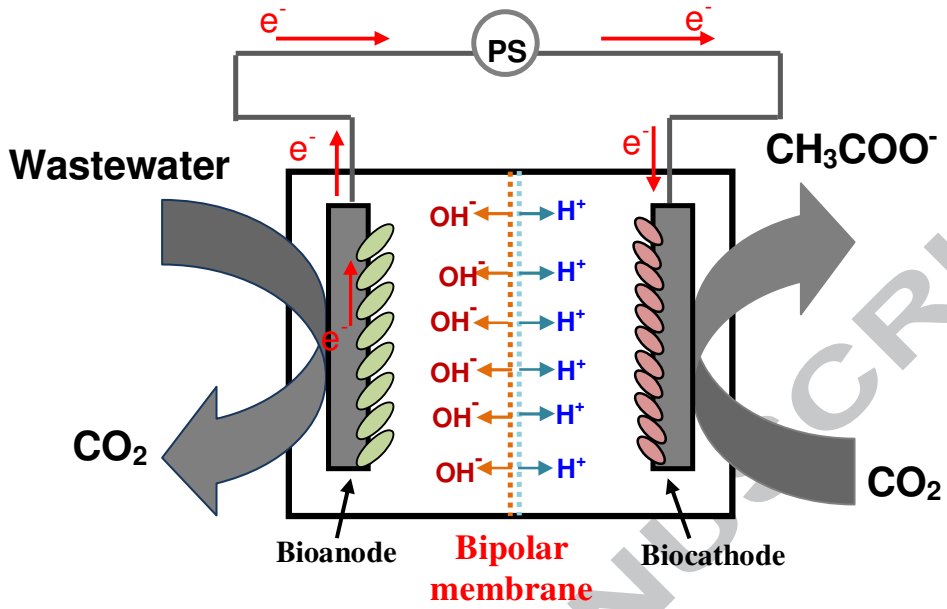


Fig. 1.

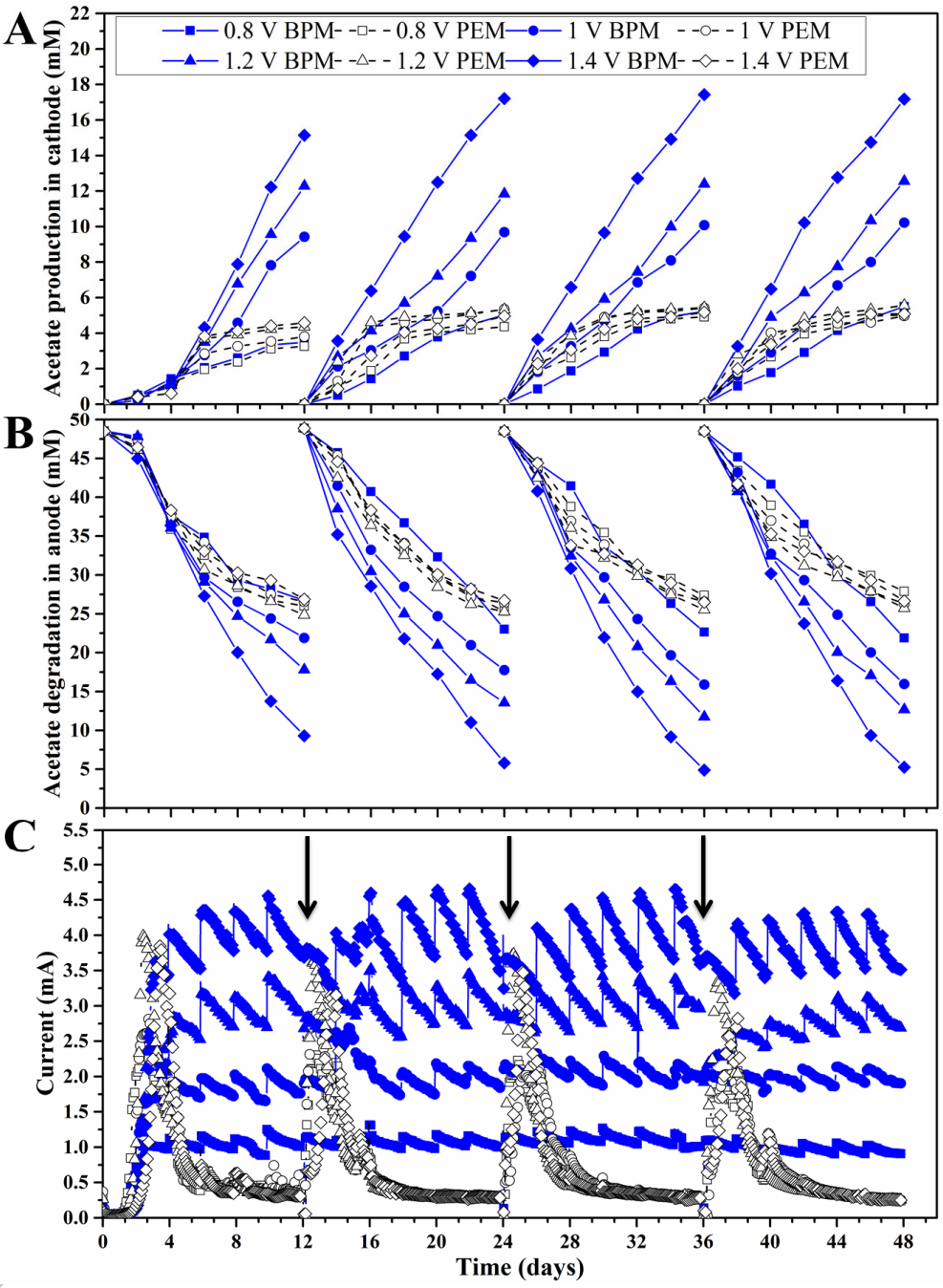
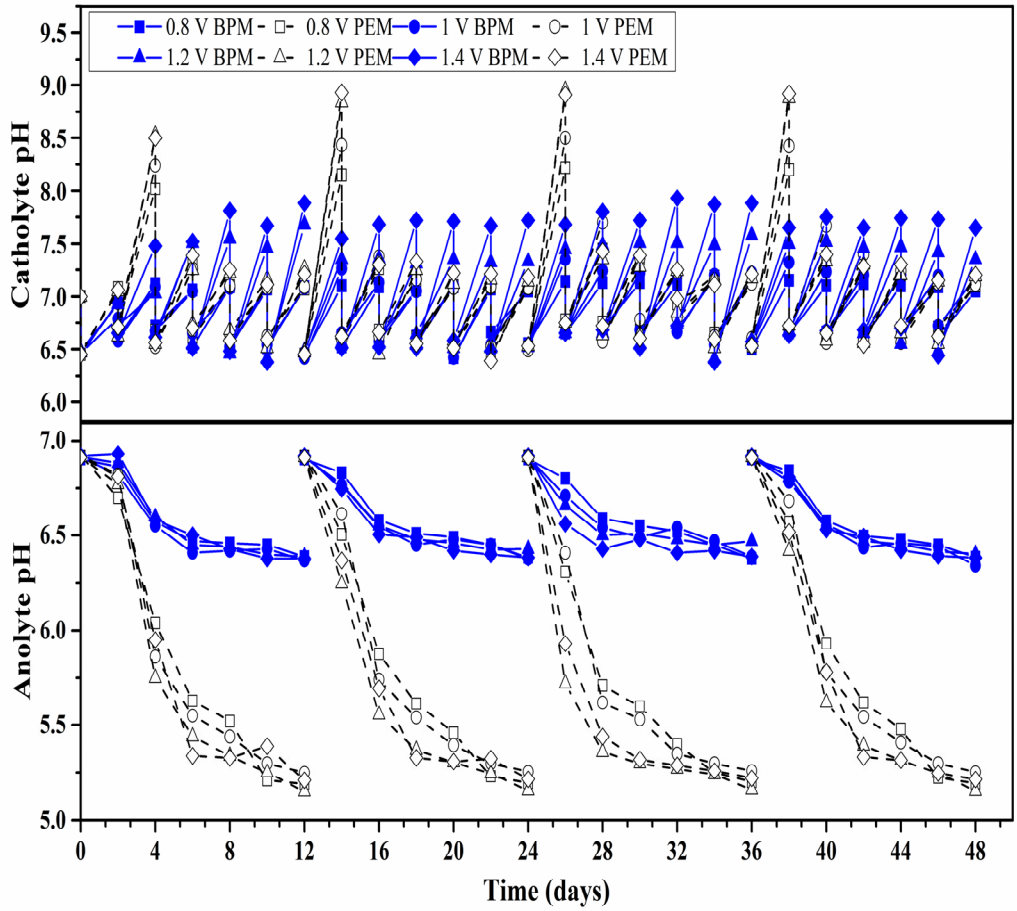


Fig. 2.



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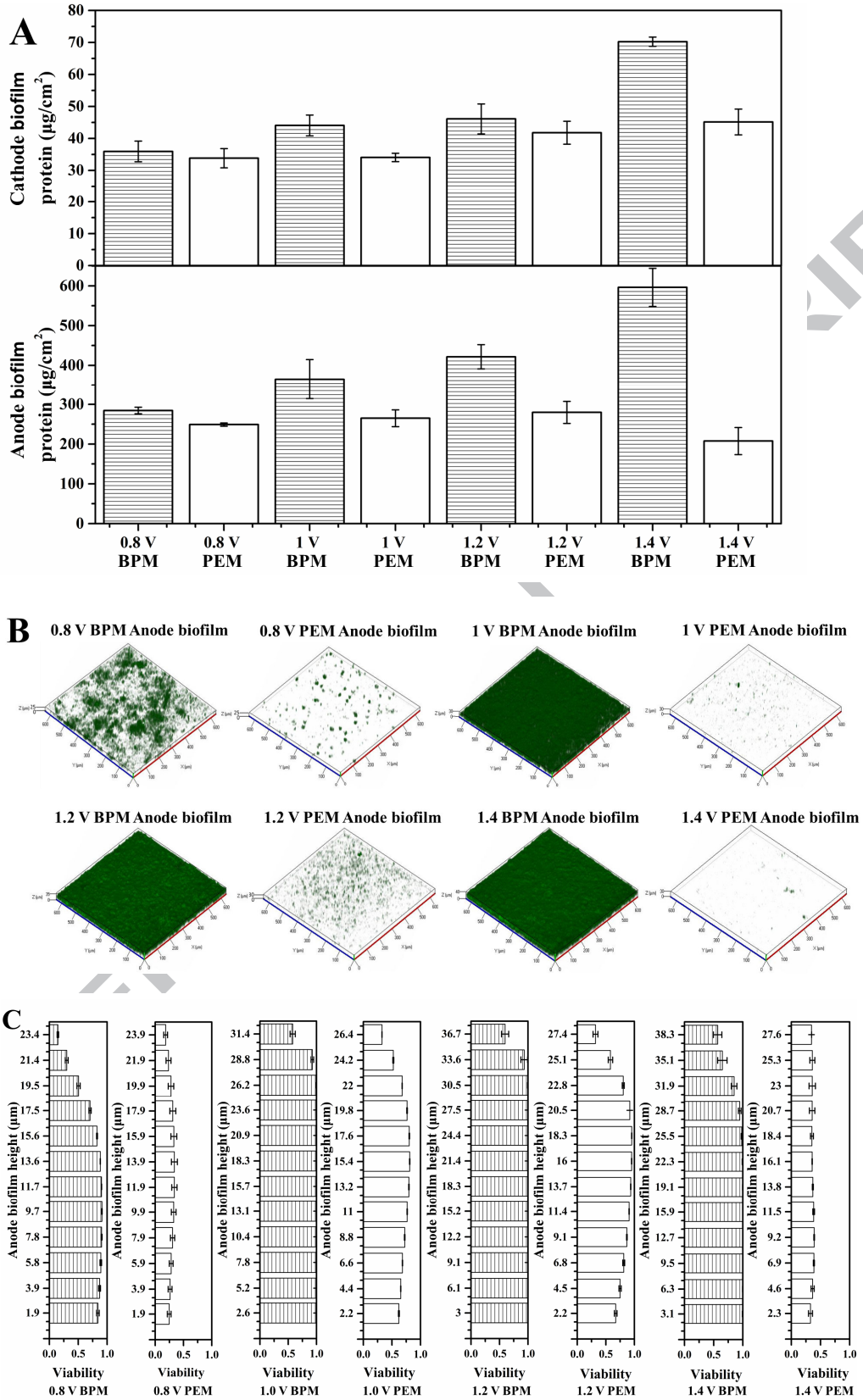

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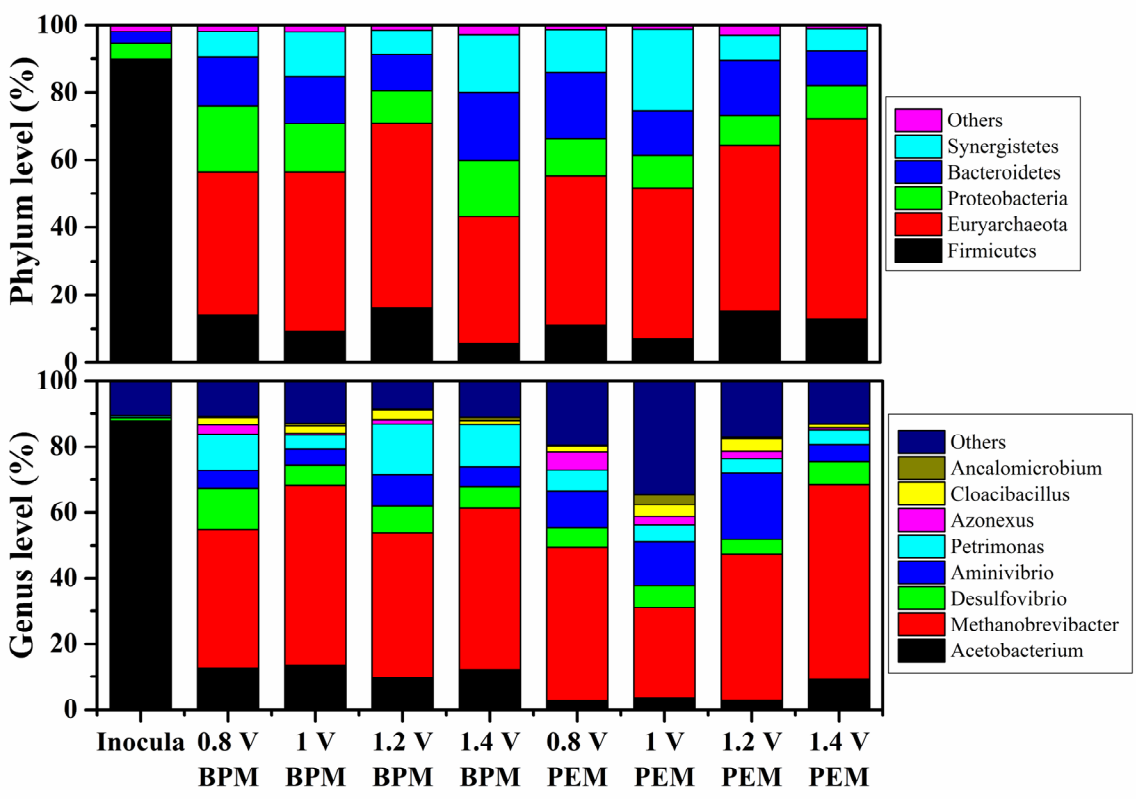

Fig. 5.

Highlights

1. Bioanode MES was constructed using BPM and PEM as separator, respectively.
2. BPM-MES achieved 238% improvement in acetate production rate compared to PEM-MES.
3. The biofilm biomass on the electrodes of BPM-MES were higher than the PEM-MES.
4. Higher abundance of *Acetobacterium* was observed in the cathode of BPM-MES.