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Effects of applied voltage on the anode biofilm formation and extracellular polymers substances in a single chamber microbial electrolysis cell

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ABSTRACT

The aim of this study was to clarify the effects of applied voltage on the biofilm formation and extracellular polymeric substances (EPS) production in microbial electrolysis cell (MEC). It was found that the applied voltage affected the anode microfilm and EPS components in MEC. Thus, the effects of different voltage levels (0.4, 0.8, 1.2 and 1.6 V) on hydrogen production potential, active biomass and the content of EPS production of anode biofilm were assessed here. According to the results, the maximum hydrogen production of the MEC anode biofilm increased from 2.4 ± 0.34 to 6.25 ± 0.95 mol/mol-glucose with the increase in the applied voltage from 0.4 to 0.8 V. Subsequently, the hydrogen production rate decreased with the consistent increase in the voltage. It was found that a dense anode biofilm had higher current density since the biofilm matrix could immobilize more electrochemically active microorganisms together on the anode surface. Fourier transform infrared (FTIR) spectrophotometry analysis demonstrated the presence of proteins and carbohydrates in the biofilm. Furthermore, anode-attached bacteria could secrete more $EPS_{protein}$ and $EPS_{carbohydrate}$ when stimulated by electric current.

Keywords: Microbial electrolysis cell (MEC); Anode biofilm; Hydrogen; applied voltage; extracellular polymeric substances (EPS)

1. Introduction

Microbial electrolysis cells (MECs) are ideal for organic wastewater treatment, simultaneously with the generation of bioenergy resources (hydrogen and methane). In MECs, the microorganisms attaching on the working electrode can oxidize biodegradable organic compounds which are present in biomass and transmit electrons to a solid electron acceptor to complete the energy recovery [1,2]. These bacteria are known as exoelectrogens [3]. Many researchers reported that exoelectrogens can form an anode biofilm in MEC [4,5]. To improve the performance of MEC, the amount of active electrogenic bacteria should be increased.

In recent years, the volumetric efficiency of MEC was still insufficient for the practical applications [6]. To improve the MEC performance, many efforts have been made to enhance electrochemically active of anode biofilm, e.g., optimizing process parameters [7–9]. According to several studies, the applied voltage in MEC is one of the major factors affecting the performance, and it was significantly correlated with the hydrogen product [10,11]. Low external voltage (≤0.4 V), will result in a slow rate of hydrogen production and a decrease in pH due to substrate oxidation produced proton accumulation in solution. However, microorganism may also be suppressed in the case of the excessive supplied voltage (≥ 1.4 V) [11].

Anode biofilm is the main carrier for the growth and propagation of MEC electrochemically active bacteria. In fact, the key feature of MEC system is the bacteria-catalyzed electron transfer from the organic substrates to solid electrodes [12]. The external voltage required in the MEC

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promotes hydrogen production, which also affects the formation of the anode biofilmsystem. Ding et al. [10]proved that variations in the external resistance affected not only the methane generation but also the microbial metabolism. Whether electrode-attached microbial growth or suspended microbial growth are probability responsible for electron transfer, and their activity varies with the culture conditions [13].

The biofilm matrix on the anode surface is highly correlated with the extracellular polymer substance (EPS) [14]. EPS are major components of biofilm, taking up more than 90% of dry biomass [15]. Thus, the characteristics of EPS are vital to the understanding of MEC. The varied surrounding environment conditions may significantly affect the content and component of EPS biopolymers, e.g., protein, carbohydrate, nucleic acids and lipids. The composition and quantity of EPS will determine the immobilization and growth of microorganisms on the anode surface as well as the electron transfer [16].

The aim of this study was to gain an insight into the effects of applied voltage on biofilm enrichment in four anodic samples of a single-chamber MEC using glucose as substrate. Anaerobic activated sludge served as inoculantat an external voltage of 0.4, 0.8, 1.2 and 1.6 V, respectively, in which the positive pole was connected from a power supply to the anode and the negative pole to the cathode. Also, the effects of applied voltage on the start-up process, biofilm formation and structure, and the hydrogen generation of the MECs were assessed.

2. Materials and methods

2.1 Reactor design

The MEC was made of glass with a cylindrical shape, 90 mm in diameter and 100 mm in height (empty bed volume of 500 mL) (Fig. 1). In MEC, the electrode chamber had a water inlet and an outlet, while at the top of it, there was a gas outlet and a socket of electrode. The anode was made of a graphite flake (30*30*2 mm) intertwined through holes drilled into a stainless-steel frame, which served as the anode module placed in the center of the chamber. The cathode electrode was a sheet electrode made of platinum with a surface area of 18 cm^2, and it was placed on the opposite side of the anode. Electrode slice were held together by plastic screws and spaced for 3 cm between each other. Furthermore, they were connected to an external circuit with titanium wires. The positive pole was connected to the anode by series connection with a programmable power supply and the negative pole was connected to the cathode. An online recorder instrument was applied here to record the electric current.

2.2. MEC inoculation and operation

The inflow in the MEC reactors were given the same substrate (1.5 g/L glucose), and sludge in the reactor was taken from the secondary sediment of pig manure as raw material of anaerobic fermentation (College of energy and envi-

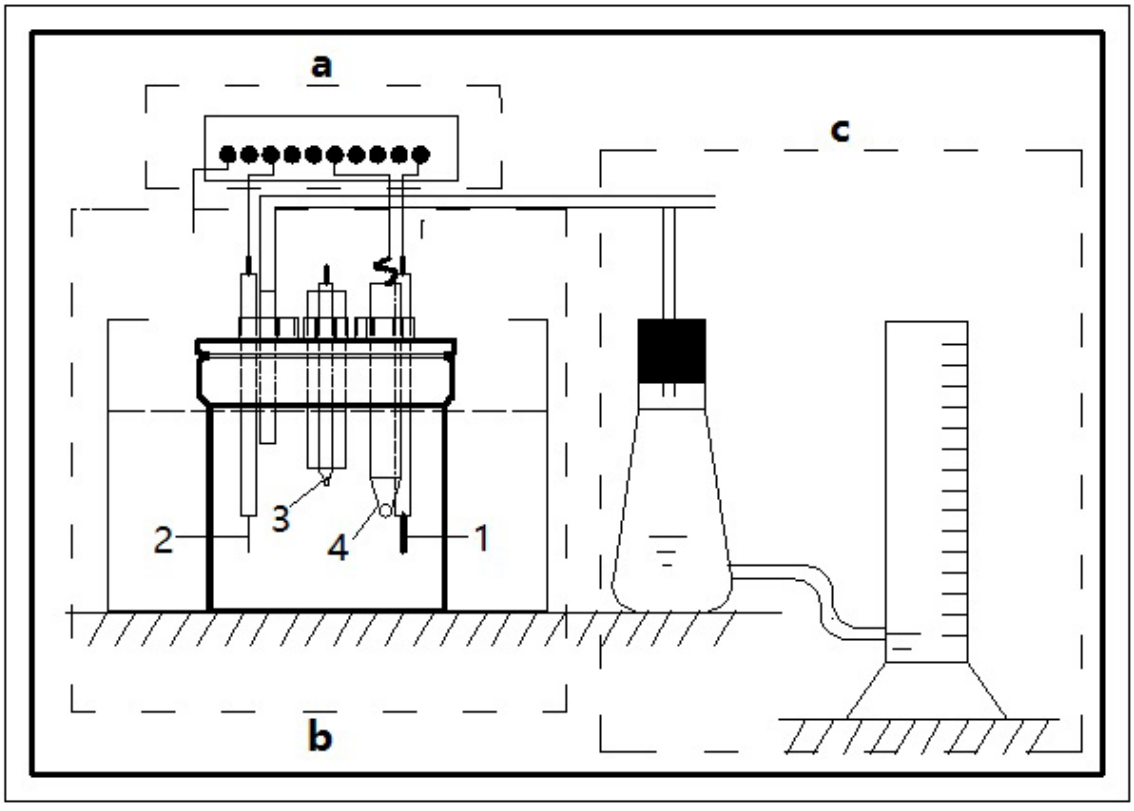

Fig. 1. Single chamber MEC shown with online recorder (a), fermentation chamber (b), gas collection unit (c), anode electrode (1), cathode electrode (2), pH electrode (4), Ag/AgCl-KCl reference electrode (3).

ronmental sciences, Yunnan normal university, Kunming, China). The inoculated sludge for each chamber was 120 mL in volume. The medium was a 100 mM PBS buffer consisted of $NaH_2PO_4 \cdot 2H_2O$, 5.54 g/L; $Na_2HPO_4 \cdot 12H_2O$, 23.088 g/L; KCl, 0.26 g/L; NH_4Cl, 0.62 g/L. Trace element formula referred to previous research results and the detailed compositions are shown as follows: NTA 1.5 g/L; $MgSO_4$ 3.0 g/L; $MnSO_4 \cdot H_2O$ 0.5 g/L; NaCl 1.0 g/L; $FeSO_4 \cdot 7H_2O$ 0.1 g/L; $CaCl_2 \cdot 2H_2O$ 0.1 g/L; $CoCl_2 \cdot 6H_2O$ 0.1 g/L; $ZnCl_2$ 0.13 g/L; $CuSO_4 \cdot 5H_2O$ 0.01 g/L; $AlK(SO_4)_2 \cdot 12H_2O$ 0.01 g/L; H_3BO_3 0.01 g/L; Na_2MoO_4 0.025 g/L; $NiCl_2 \cdot 6H_2O$ 0.024 g/L; $Na_2WO_4 \cdot 2H_2O$ 0.025 g/L and a multivitamin [17]. The solution formula in the reactor was added to the 100 mM PBS buffer with the trace element solution of 12.5 ml/L and the substrate of the corresponding concentration. The medium was pH 7.2, purged with N_2 gas for 10 min and maintained on anaerobic conditions at 35 ± 1°C. MECs were operated in batch mode and the hydraulic retention time (HRT) was 2 d. The mixed liquor from the reactor were sampled into a beaker with discarding the liquid supernatant and adding the fresh medium. During the cultivation of anode biofilm, the applied voltage was 0.4, 0.8, 1.2 and 1.6 V, respectively.

2.3. Anode biofilm characterization study

When each batch reached to a steady-state, and the electrolytic current was maximal and stable, the graphite anode was sampled. The anode biofilm was examined for hydrogen production by control MECs applied voltage uniformly at 0.8 V and other external conditions were consistent (without inoculation). The biomass of the anode biofilm and EPS content were also analyzed in those treatments.

2.4. Analytical methods

The applied voltage was controlled by dc stabilized voltage supply (TXN-1502D, Shenzhen, China), and electric current was recorded by online recorder (R7100-A08, Shanghai, China). The total volumes of biogas produced from the MECs were measured using the water displacement method. The biogas composition including hydrogen, methane and carbon dioxide were measured with a gas chromatograph (GC9790II, Zhejiang, China) using a thermal conductivity detector (TCD) and volatile fatty acids (VFAs) content were measured with a gas chromatograph using a flame ionization detector (FID).

To determine the electroactivity of the anode biofilm in MEC, a cyclic voltammetry (CV) scan in a three-electrode system was applied, and the anode enriched with bacteria was the working electrode. Ag/AgCl-KCl was taken as the reference electrode and the cathode as the counter electrode, the reference electrode was placed in the test chamber. Electrode potentials will be referred to the standard hydrogen electrode (SHE) (reference electrode) in this study.

The graphite anode was cleaned with 0.9 % NaCl solution 3 times, and EPS was extracted by ultrasound from anode biofilm [18]. MLSS and MLVSS were determined using the weighing method after being dried at 103–105 °C and burnt to ash at 550°C. The extracted proteins in EPS were further determined by coomassie brilliant blue method (A045-2, nanjing, China) [19]. Polysaccharide was determined using the anthrone method [20]. Extracellular polymer molecule structure was analyzed by FTIR (NICOLET-IS10, America). After pretreatment and digestion, the phosphorus content in the anode biomass membrane was determined under the phosphorus molybdenum blue spectrophotometry [21].

3. Results and discussion

3.1. MEC operation condition and performance

According to the results, the current density changed with time on the voltage of 0.4, 0.8, 1.2 and 1.6 V, respectively (Fig. 2). There was no electrolytic current at the initial stage of MEC anode biofilm cultured except that there was a weak current at the high voltage of 1.6 V. (0.1 ± 0.06 mA). The current stayed at zero for the first 48 h, and the subsequent 1.2 V app generated a current. The second was 0.8 V app, which generated an electrolytic current in 60 h. However, the current effects had been weak at 0.4 v and 1.6 v. The maximum current density was 0.22 ± 0.04, 5.9 ± 0.54, 4.9 ± 0.36 and 0.44 ± 0.06 A/m^2 at voltages of 0.4, 0.8, 1.2, and 1.6 V (The area of the graphite flake was 18 cm^2), respectively. It was obvious that the current density was relatively high at 0.8 and 1.2 V app. This indicated that the anode exoelectrogenic microbes were effectively attached to the anode [22]. In addition, the current on 0.4 and 1.6 V was similar while it became more different between 0.8 and 1.2 V.

Fig. 3 shows the biogas yield variation with different culture voltages. For non-applied voltage, 1.5 g/L of glucose was barely fermented to produce hydrogen. Different external voltage also had significant effects on the hydrogen and methane production rate. Similar to the trend of current density, the hydrogen production rate increased from voltage 0.4 V and reached the peak of 0.066 ± 0.009 mol/mol-glucose at 0.8 V. Subsequently, it decreased to 0.044 ± 0.006 mol/mol-glucose and 0.021± 0.004 mol/mol-glucose at 1.2 and 1.6 V, respectively. An appropriate increase in applied voltage would improve the activity of the anaerobic microorganisms and accelerate the generation of hydrogen by the proton and electron transfer rate [23]. However, the hydrogen production decreased when the applied voltage was 1.2 and 1.6 V respectively. This indicated that the over high voltage would had negative effect on the microorganisms. Ding [10] reported that higher voltage led to the greater plasmatorrhexis, lower growth rate and lower metabolic activity. Methanogens were present in the inoculation, and all the experiments were accompanied with a large amount of methane. It was also assumed that the methane-producing bacteria would had negative effects on hydrogen production in the cultured process.

The pH of MEC reaction solution varying with voltage is shown in Fig. 4. The initial pH value of the reaction solution in the electrolytic chamber was 7.2 ± 0.1. Under the effects of phosphate buffer and the glucose fermentation produced acid intermediates, the pH was obviously fluctuated at the applied voltage. Moreover, ph in the MEC system of the glucose matrix was lower than that of the system of anaerobic digestion (non-applied voltage). Acetate, propionate and butyrate were the main end-products of glucose fermentation. Through the following ion Eqns. (1)–(4), protons in the anaerobic digestion system were converted to methane in time, resulting in stable pH value. In the MEC

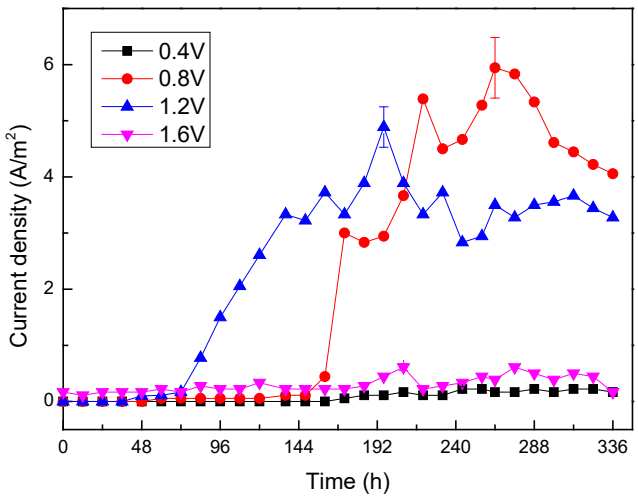

Fig. 2. Current density under different voltages.

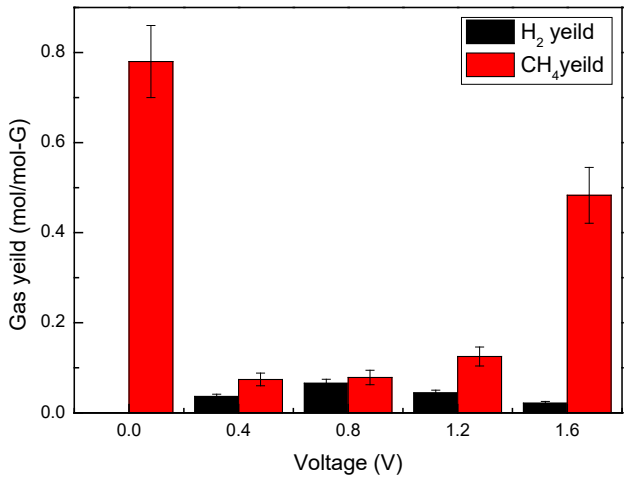

Fig. 3. Hydrogen and methane production under different culture voltages.

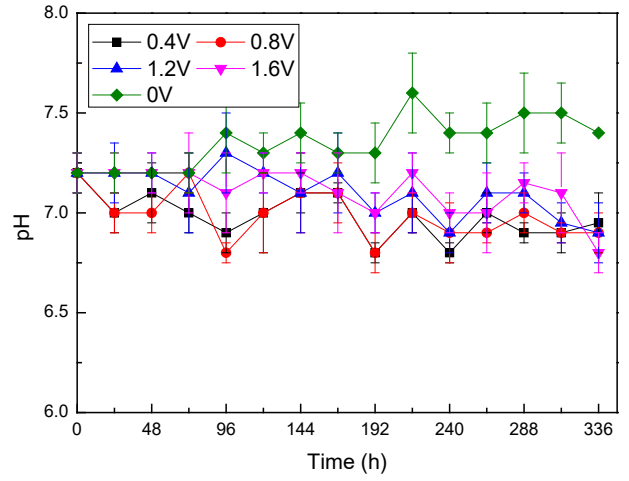

Fig. 4. The pH of MEC reaction solution varied with time under different external voltages.

and fermentation system, the anode microbial community could directly oxidize glucose or fermentation intermediates to more protons and electrons through a half-cell Eqns. (5)~(7) [24,25].

$$\text{Propionate}^- + 3H_2O \rightarrow \text{Acetate}^- + HCO_3^- + H^+ + 3H_2 \quad \Delta G'^0 = +76.1\ \text{kJ} \tag{1}$$

$$\text{Butyrate}^- + 2H_2O \rightarrow 2\text{Acetate}^- + H^+ + 3H_2 \quad \Delta G'^0 = +48.1\ \text{kJ} \tag{2}$$

$$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O \quad \Delta G'^0 = -135.6\ \text{kJ} \tag{3}$$

$$\text{Acetate}^- + H_2O \rightarrow CH_4 + HCO_3^- \quad \Delta G'^0 = -31\ \text{kJ} \tag{4}$$

$$\text{Glucose} + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^- \tag{5}$$

$$\text{Acetate}^- + 2H_2O \rightarrow 2CO_2 + 8H^+ + 8e^- \tag{6}$$

$$\text{Propionate}^- + 4H_2O \rightarrow 3CO_2 + 14H^+ + 14e^- \tag{7}$$

At different voltages, the end products of MEC anode biofilm enrichment are shown in Fig. 5, which helps to further understand the difference of fermentation mode between different voltages. Acetic acid, propionic acid and butyric acid were the main metabolites of glucose in oxidative degradation. Compared with the non-applied voltage, the content of the end products of MEC increased with the increase in applied voltage from 0 to 1.6 V. When the input voltages were 0.4, 0.8, 1.2 and 1.6 V, the residual amount of butyric acid were 735.6 ± 56.6, 933.5 ± 84.2, 661.7 ± 52.6 and 452.5 ± 46.5, respectively. According to the results, the highest concentration of butyric acid was found in the 0.8v experimental groups with the best electricity production. This revealed that the MEC-anaerobic fermentation system with glucose as substrate was mainly butyric acid fermentation, which was consistent with the research of Wang et al. [23].

The CV curve of the anode biofilm in MEC can provide important information for understanding electron transfer mechanism. Thus, the electron velocity of the anode biofilm surface can be measured by cyclic voltammetry [26]. The performance of anodes before (orange line) and after the bacteria enrichment under different voltages is clearly observed in Fig. 6 at a scan rate of 10 mV/s. Compared with the anode of blank graphite, the CV curve of the anode biofilms produced an obvious oxidation current, suggesting that electron transfer capability occurred in the biofilm attached to the anode. In particular, the peak potential of the oxidation process which appearing at 0.1 ~ –0.1 V showed a stable value. The CV's behavior of the anode biofilms was consistent with the estimated equilibrium potential of *G. Sulfurreducens* cells that spanned a range of potentials from approximately –0.2 to +0.1 V Vs SHE [27]. According to the oxidation peak potentials in Fig. 6, biofilms could oxidize substrate to produce different electron transfer capacities. This result suggested that the utilization rate of the substrates for electricigens in biofilms followed the order: 0.8 V > 1.2 V > 0.4 V > 1.6 V.

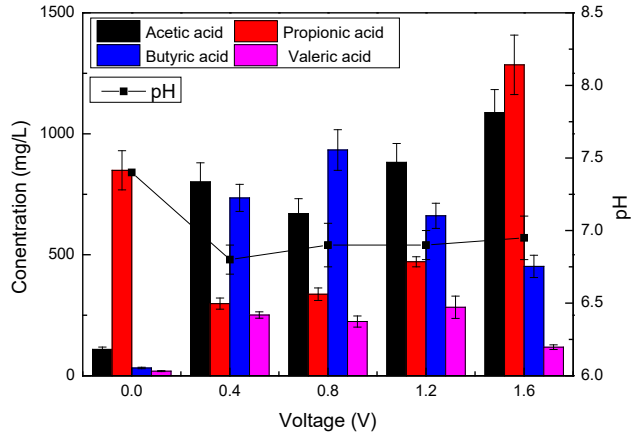

Fig. 5. End-production concentrations and pH variation of MECs with biofilm enriched at different culture voltages.

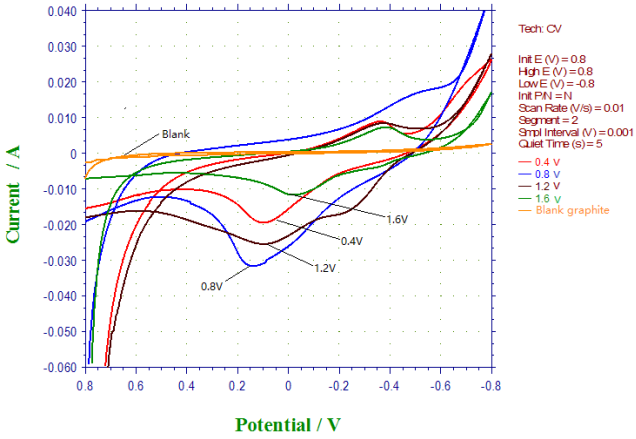

Fig. 6. The cyclic volt ammogram obtained for different enrichment voltages.

The cultured anode biofilm was transferred to the medium corresponding to anode bacteria enrichment process (without inoculation). Applied voltage of MECs were controlled uniformly at 0.8 v, and other external conditions were consistent. The hydrogen production of anode biofilms at different voltages are shown in Fig. 7. The hydrogen yield reached the top of 6.25 ± 0.95 mol/mol-glucose at 0.8 V, and then it decreased to 5.36 ± 0.74 and 1.36 ± 0.24 mol/mol-glucose at 1.2 and 1.6 V, respectively. The hydrogen production variation was similar to the current change and electrochemical activity. All data were optimal at 0.8 V. Given the variation of hydrogen production capacity of anode biofilm at different voltages, it was speculated that the anode biofilm was improved in the presence of voltage, and the positive effect reached its optimum at 0.8 V. However, the improvement became weakened with the continuous increase in voltage. It was assumed that the over high voltage would have negative effects on the enrichment of electrogenic bacteria at anode [28]. Accordingly, it was concluded that appropriate applied voltage can effectively enrich the anode biofilm, yet the over-dosed high voltage also suppressed the microorganisms in the anode biofilm formation.

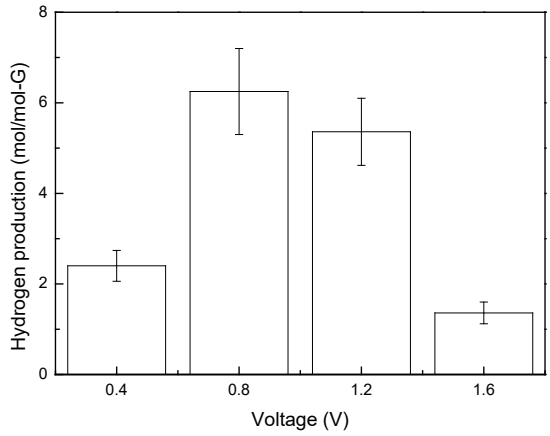

Fig. 7. Hydrogen production capacity of anode biofilm enriched at different voltages (no inoculants).

3.2. Effects on anode biomass and EPS contents of the biofilms

The effects of applied voltage on the accumulated anode biomass and EPS components of the biofilms formed at 0.4, 0.8, 1.2 and 1.6 V during the start-up is shown in Fig. 8. It is suggested that the anode biomass of the MECs decreased with the increase in applied voltage from 0.8 to 1.6 V, while increased as applied voltage increased from 0.4 to 0.8 V. This trend was consistent with previous reports in which high electric fields could negatively affect living organism [29,30]. The applied voltage could affect the electrical property of cell membrane. If the electrical property of cell membrane changed, the cell membrane was likely to be suppressed [31]. Accordingly, the applied voltage could have two different effects on exoelectrogens. A suitable voltage stimulated the rapid growth and reproduction of exoelectrogens, and high voltage was more effective than low voltage. However, excessive voltage (more than 0.8 V) may exert destructive effects which were more serious than the facilitation [28].

The EPS components were often referred to consolidating material for the entire biofilm, and they protect the biofilm from environmental effects [32]. Fig. 8 shows the distribution of total protein and total polysaccharide in EPS of biofilms at different applied voltages. As shown in Fig. 8, protein was more abundant than the polysaccharide in the EPS. The composition of EPS increased with the rise in applied voltage, and a maximum was obtained at 0.8 V, protein and polysaccharide in the biofilm increased from 18.65 ± 3.12 and 4.75 ± 1.56 mg g^{-1} VSS to 52.89 ± 8.96 and 5.65 ± 1.89 mg g^{-1} VSS. Subsequently, the protein and polysaccharide dropped sharply to 10.96 ± 2.16 and 2.68 ± 0.75 mg g^{-1} VSS at 1.6 V. According to the results of the analysis on the aerial yields of proteins and carbohydrates, there existed differences between the crude chemical natures of the EPS extracted from the anode biofilms in the various MECs. This suggested that electricigens in the various anode biofilms tend to produce more EPS components at higher anode biomass and ampere density. An explanation was given that bacteria under the high-power density condition tended to be stimulated and secreted more EPS contents in response to the stressful power condition [33].Another explanation was that EPS contained conductive nanowires during the

extraction, and the presence of bacterial nanowires had significant implications of electrogenic microorganism attachment and electron transfer to anodes [16,34]. However, excessive applied voltage would suppress or negatively affect the metabolic growth of electrogenic microorganisms and secreted EPS. When the applied voltage continued to increase to 1.6 V, the content of protein and total EPS all experienced different levels of decrease. EPS were originated from the metabolism or lysis of microorganisms, excessive voltage was go against for electrogenic microorganisms' propagation and metabolism at the anode than no more extracellular polymers were secreted.

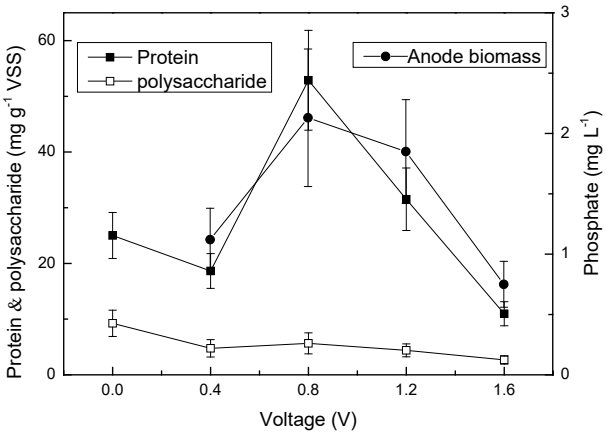

Fig. 8. Effect of applied voltage on anode biomass and EPS contents of the biofilms after start-up.

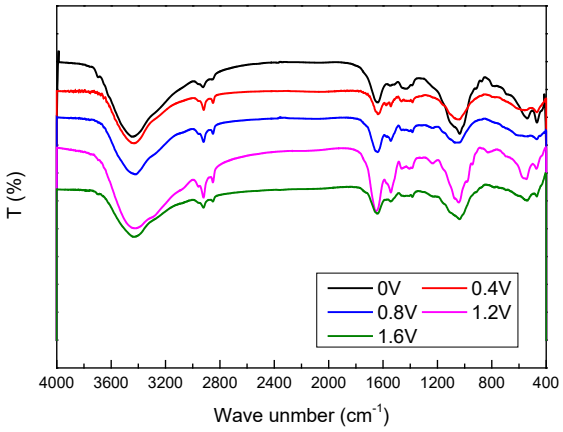

Fig. 9. FT-IR spectra of EPS in anode biofilms at different applied voltages.

The FT-IR spectra of the EPS extracted from anode biofilms, were made in quintuplicate, as shown in Fig. 9. Band assignments are listed in Table 1. The transmittance of the five spectra appeared to be fluctuated due to the difference of sample quantity for the determination of each voltage. In addition, the five spectra showed similar peaks. The spectra showed a broad region of adsorption around a peak at 3345–3420 cm^{-1}, which is attributed to stretching of the O-H and N-H bond in hydroxyl and amino functional groups [35]. The doublet peaks around 2920 and 2850 cm^{-1} were attributed to the signatures of aliphatic chains which were mostly contributed by lipids and proteins [36]. All sample spectra and those belonging to polysaccharides and nucleic acids showed protein-specific bands (amide I and amide II) at 1638 and 1542 cm^{-1} [37]. This indicated that proteins were one of the components of the anode biofilm EPS. The band at 1383 cm^{-1} was attributed to the stretching of C-H bonds which might be aliphatic chains or carbohydrates.Furthermore, a broad peak at 1221–1241 cm^{-1} showed the asymmetric PO^{2-} stretching of phosphodiesters, and it only occurred in the anode biofilms formed at 0.8 and 1.2 V. Shifts associated with this band were related to the high anode biomass (phosphodiester backbone of DNA/RNA) and to phosphate-sugar interaction [38]. The functional groups represented by the sharp peak at the range of 400–900 cm^{-1} belong to finger print region, and the peak in the region were specified as a phosphate or ester sulfate group [39].

4. Conclusions

The aim of this study was to investigate the effects of applied voltage on the anode biofilm formation, electricity and EPS components were investigated. With the increase in the applied voltage from 0.4 to 0.8 V, the enriched anode biofilm reached the optimal electricity generation and

Table 1
Assignment of infrared bands identified in the EPS

Vibration frequencies (cm^{-1})					Band assignments
0 V	0.4 V	0.8 V	1.2 V	1.6 V	
3445	3429	3420	3423	3432	OH and NH_2 stretching
2923	2919	2920	2920	2920	C-H stretching
2851	2850	2851	2851	2850	C-Hstretching
1640	1633	1640	1653	1639	-C=O stretching (Amide I)
1542	1542	1543	1542	1542	-NH in-plane bending and -CN stretching (Amide II)
–	1383	1384	1383	1384	$-CH_3$ umbrella mode or O-H in-plane bending
–	–	1234	1235	–	C-O stretching and O-H deformation in -COOH or C-N amide III vibration or ester sulfate band
1035	1040	1050	1044	1038	C-O-P stretching

hydrogen production capacity. Biomass and EPS components of the anode biofilms formed at various voltages suggested that a suitable biofilm structure is significant positively correlated with the electrical performance rather than the applied voltage. When the applied voltage was higher than the optimum value, a less biofilm structure was developed with a less active biomass and a lower EPS content. The contents of proteins and polysaccharides appeared similar trend with current and anode biomass, yet the variations of proteins were extremely more significant than those of polysaccharides. These results here indicated that abundant development of anode-attached bacteria could promote electricity generation, and bacteria secreted more $EPS_{protein}$ and $EPS_{carbohydrate}$ when stimulated by electric current.

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