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Methanogenesis Control using 2-Bromoethanesulfonate for Enhanced Power Recovery from Sewage Sludge in Air-cathode Microbial Fuel Cells

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Coulombic efficiencies (CE) of microbial fuel cells (MFCs) treating real organic wastes are generally low. Methanogenesis is a major factor reducing CE by diverting electrons to methanogens rather than electrochemically active bacteria. In this study, batch tests were conducted to enhance power recovery in sludge-fed MFCs by using 2-bromoethanesulfonate (BES) to control methanogenesis. Results showed that 0.5 mM BES effectively inhibited methane production in the sludge MFCs. In the presence of BES, the MFC reactor yielded a maximum voltage of 0.62 V and a maximum power density of 115 mW/m^2, and CE increased from 4.1% to 7.8%. The cyclic voltammetry and DGGE analyses showed different catalytic behavior and microbial community of anode biofilm as a consequence of BES addition. As a first report using a complex feed, this study demonstrated that power recovery from complex organic wastes in MFCs can be improved by methanogenesis suppression with low dosage of BES.

Keywords: Microbial fuel cell; sewage sludge; methanogenesis; 2-bromoethanesulfonate (BES); Coulombic efficiency

1. INTRODUCTION

Microbial fuel cells (MFCs) can generate electrical energy from oxidation of organic matter through the catalytic activity of electrochemically active bacteria. A large number of substrates have been explored as fuel for MFCs, ranging from pure compounds (acetate [1]; glucose [2]) to complex mixtures of organic matter present in wastewater (brewery wastewater [3]; starch processing

wastewater [4]; landfill leachates [5]). Substrate is regarded as an important biological factor affecting not only the integral composition of the bacterial community in the anode biofilm, but also the MFC performance including current, power density and coulombic efficiency (CE) [6, 7].

Sewage sludge contains high levels of organic matter and its utilization as influent for MFCs has been demonstrated [8-12]. However, the power density of the MFC using sewage sludge as substrate was usually lower than that of pure compounds [9-11, 13]. For example, with a similar design of MFC, a maximum power density of 161 mW/m^2 was produced with glucose, but a limited power of 0.3 mW/m^2 with anaerobic sludge [14]. Like many other complex substrates, the CEs of sewage sludge based MFCs were rather low compared with those synthetic wastewaters [15]. The CE could be diminished by the competing microbial processes such as methanogenesis and biomass growth, or by the competitive utilization of alternative electron acceptor by the bacteria (e.g., oxygen, nitrate and sulfate [16]), also by the presence of electron acceptors that can be chemically reduced at the electrode surface.

In MFCs, bacteria unable to utilize the electrode as electron acceptor are likely to use substrate for fermentation and/or methanogenesis [17]. However, fermentations are not inherently detrimental to coulombic loss since they not only convert carbohydrates into ideal substrates for anodophiles, and also enriching the biofilm with electroactive compounds [18]. While methanogenesis is intrinsically detrimental to the anodic process and the coulombic loss for MFCs is irreversible. Methanogenesis is frequently reported in most MFC reactors [19-23], and caused a severe decrease in CE. In particular, methane production is very common in the MFCs using anaerobic sludge as source inocula because of the ubiquity of methanogens in anaerobic sludge.

To increase the CE, it is important to reduce substrate oxidation by biomass other than electrochemically active bacteria and thus increase the competitiveness of electrochemically active bacteria. This can be done by elimination of methanogens. Attempts have been made with periodic aeration of the anode compartment to kill methanogens, however, this is not very successful for thick biofilm since oxygen is hard to reach the deepest layer where methanogens continue to thrive [18, 24]. This study challenged to control methanogenesis in a sludge-fed MFC using 2-bromoethanesulfonate (BES), which is a structural analogue of coenzyme M and regarded as an effective methanogen-specific inhibitor [25-26]. Though the addition of BES to suppress methane production has been carried out in MFC reactors [24], this study appears to be the first report using a complex feed. In the air-cathode single-chamber MFC, the MFC performance and methane production were examined in the presence and absence of BES, and the effect of BES on the anode biofilm was further analyzed using cyclic voltammetry and denaturing gradient gel electrophoresis (DGGE).

2. EXPERIMENTAL

2.1 Chemicals and Materials

The sewage sludge used as influent in this study was collected from Liede Wastewater Treatment Plant in Guangzhou, China. Using APHA standard methods [27], the main characteristics of sludge are given in Table 1. The sludge was stored at 4±1ºC before use.

Table1. Characteristics of raw sewage sludge

Parameters	Value
Total suspended solids (TSS) (%)	3.91
Volatile suspended solids (VSS) (%)	2.35
pH	6.8~7.0
Total chemical oxygen demand (TCOD) (mg/L)	24,750
Soluble chemical oxygen demand (SCOD) (mg/L)	785
NH_4-N (mg/L)	270
Total nitrogen (mg/L)	546

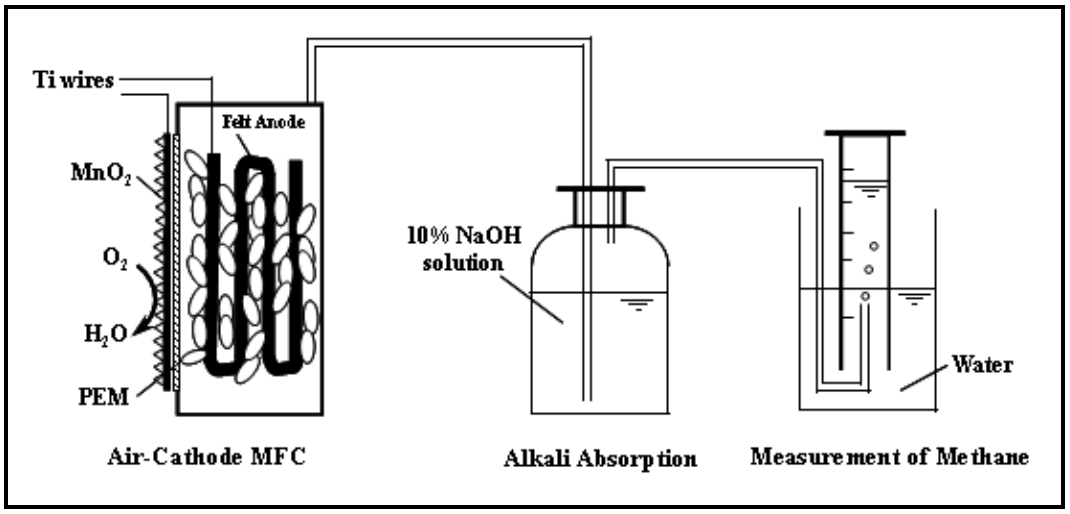

Figure 1. MFC configuration and the measurement system for methane production.

The single-chamber, air-cathode MFCs were fabricated using polyvinyl chloride (PVC) plastic tube, as previously described [28]. The schematic details of MFC along with the methane measurement are depicted in Fig.1. Graphite felt (14.5×17.5 cm, 5.0 mm in thickness, Liaoyang Jingu Carbon Fiber Sci-Tech Co., Ltd., China), folded as roll form, was placed inside the chamber, functioning as the anodic electrode. The cathode assembly was built using a piece of carbon cloth (15×12.5 cm) and a same size of proton exchange membrane (Zhejiang Qianqiu Group Co., Ltd., China), and the construction procedure followed our previous work [28]. Cathode had a projected surface area of 187.5 cm^2 and a catalyst loading of 5.0 ± 0.1 mg cm^{-2} MnO_2. The liquid volume of the reactor was 170 ml.

The anode chamber of the MFC was inoculated with pre-acclimated bacterial solution from an MFC reactor that had been operated with sewage sludge under fed-batch mode over 6 months. MFC experiments were initiated after verifying reproducible current generation. During the entire experiments, the MFCs were loaded with a fixed external resistance of 1000 Ω (except as noted) and conducted in batch mode at 30±1ºC in a temperature-controlled chamber.

The chemical inhibitor, 2-Bromoethanesulfonate (BES) was dissolve in deionized water to make 20 mM stock solution, and the target amount of the stock solution was injected directly into the anodic compartment of the MFC reactors to achieve the concentrations of 0.3 and 0.5 mM.

2.2 Analysis and Calculations

The output voltage was recorded directly using a 16-channel voltage collection instrument (AD8223, China) and a potentiostat connected to a personal computer. Upon a stable power generation, the external resistor was varied over the range of 20–10,000 Ω to obtain a polarization curve. Data for each resistor was collected after the MFC reactor produced stable power over a minimum period of 5 h. Voltage was converted to power density based on the projected cathode surface area. CE (η) was calculated from $\eta = \frac{M\int_0^t Idt}{FbV_{an}\Delta COD}$, where M = 32 (the molecular weight of oxygen), F is Faraday's constant, b = 4 is the number of electrons exchanged per mole of oxygen, V_{an} is the volume of liquid in the anode compartment, and ΔCOD is the change in COD over time *t* [17].

The concentrations of soluble COD (SCOD) were measured by a COD digital reactor block (DRB200, HACH, USA) equipped with a spectrophotometer (DR2700, HACH). Soluble volatile fatty acids (SVFAs) (SVFA) (acetate, propionate, butyrate) were measured in triplicate using a gas chromatograph (Techcomp 7900) and a fused-silica capillary column as described by Velasquez-Orta et al. [29].

The biogas from the MFC was bubbled through a NaOH solution (10%) in an airtight bottle to strip CO_2. The gases exiting from the bottle were collected by the displacement of water in a graduated cylinder. Methane in headspace was analyzed using a Hewlett-Packard 5890 Series II gas chromatograph with a thermal conductivity detector equipped with a HP-624 capillary column.

Cyclic voltammetries (CVs) were conducted with a CHI660A system (CH Instruments, Inc.) in a conventional three-electrode cell. The MFC anode, cathode, and Ag/AgCl served as the working electrode, counter electrode, and reference electrode, respectively. Potentials from −0.6 V to +0.6 V (vs. Ag/AgCl) were applied at a scan rate of 1 mV s^{-1} with continuous monitoring of the current response.

The graphite felt electrodes from the anode compartment of the MFCs were used for DNA extraction. DNA was extracted using a PowerSoil DNA isolation Kit (Mo Bio Labs, Inc. Carlsbad, CA) according to the manufacturer's instruction. The nearly complete 16S rRNA gene was used as template to amplify the V3 region of 16S rRNA gene with the primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') with a GC clamp (CGCCCGCCGCGCCCCGCGCCCGGCCCGCCGCCCCCGCCCC) at the 5'-end and 518R (5'-ATTACCGCGGCTGCTGG-3'). Each PCR mixture (25 μl) contained 2.5 μl 10 × PCR reaction buffer, 200 μM of each deoxyribonucleoside triphosphate, 1 μM each of the forward and reverse primer, and approximately 20 ng of DNA. The PCR was incubated for 5 min at 94°C, followed by 20 cycles of 40 s at 94°C, 30 s at 65°C, and 30 s at 72°C, then 15 cycles of 40 s at 94°C, 30 s at 55°C, and 30 s at 72°C, and a final extension of 72°C for 10 min.

A DCode Universal Mutation Detection System (Bio-Rad, USA) was used to perform DGGE analysis. A gradient of 35–65% denaturant (100% of denaturation corresponds to 7 M urea and 40% formamide) was constructed in an 8% polyacrylamide gel. Samples containing approximately equal amounts of PCR products (200 ng) were loaded in wells of the gel. Electrophoresis was run at 60 ºC for 14 h at a constant voltage of 75 V in 1×TAE. After electrophoresis, gels images were captured using ImageQuant 350 (GE Healthcare, USA).

Bands of interest (designated with numbers) were excised and rinsed in 0.2 ml sterile deionized water. And each band in gel was crushed with a sterile pipette tip and resolved in 20 µl sterile deionized water overnight at 4ºC. DNA solution was used as template for re-amplification with the primer 338F and 518R. The sequences of both excised DGGE bands and clones were manually checked and modified with BioEdit v7.0.9 and then compared with the GenBank database by the BLAST.

3. RESULTS AND DISCUSSION

3.1 Effect of BES addition on MFC performance

BES is selected as the methanogen-specific inhibitor in this study and its inhibition effect on methanogensis in the anode chamber of the MFC was investigated with two concentration levels (0.3 and 0.5 mM). Over an operational time of 7 days, the methane yield from the MFC without BES addition was 1.1 mmol, and the methane production from the MFCs with 0.3 mmol/L BES was measured to be 0.46 mmol. With a higher BES concentration of 0.5 mM, nearly no methane was detected, suggesting the complete suppression of methanogensis in the anode chamber. The injection concentration of BES was thus set at 0.5 mM for the following experiments.

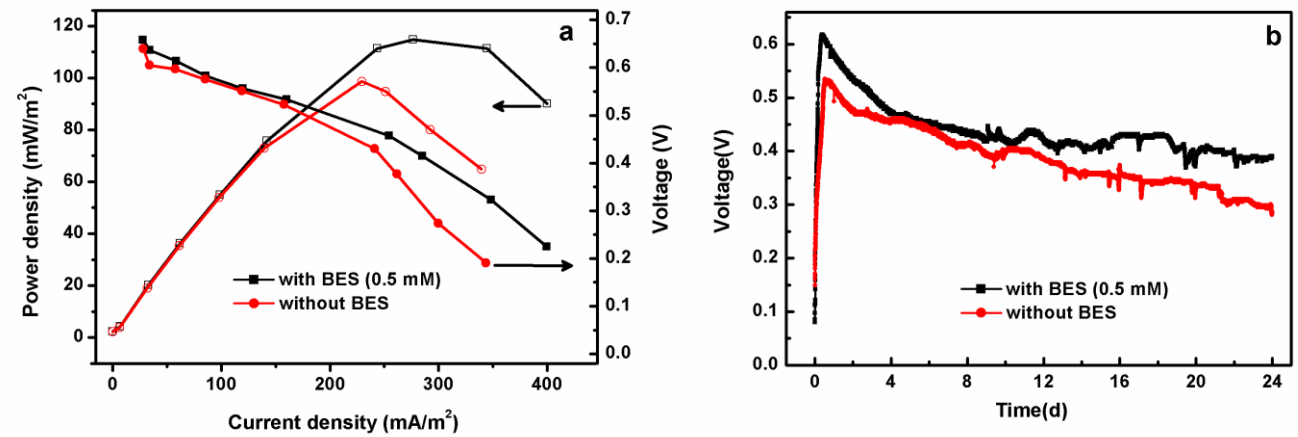

Figure 2. Polarization curves for the MFCs in the presence and absence of BES (a); Comparison of the cell voltage output in the presence and absence of BES (b).

For the two MFCs with and without BES addition, Fig.2 shows the polarization curves as a function of current density, the output voltage and power density measured at variable external resistance (20~10,000 Ω). The curves (Fig.2a) depicted the maximum power densities of 115 and 99 mW/m^2 for the MFCs with and without BES, respectively, indicating a 16.4% increase of power output by the BES addition. Over a 24-d operation period, the output voltages topped at 0.62 and 0.54 V for the MFCs with and without BES, respectively, and voltage generated from the BES-added MFC was constantly greater than that from the MFC without BES.

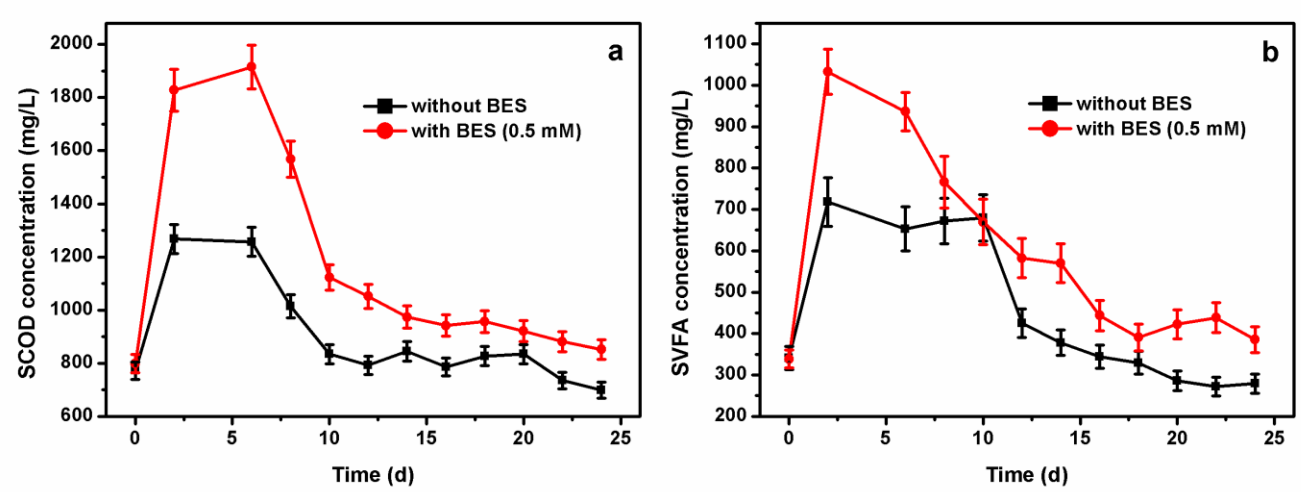

Figure 3. Comparison of soluble chemical oxygen demand (SCOD) (a) and soluble short-chain fatty acids (SFCAs) (b) in the anode chamber in the presence and absence of BES.

There was a distinct difference between the control and BES-added MFCs with respect to both SCOD and SVFA concentrations (Fig.3). With the function of available microbes in MFC, the particulate COD in sludge was hydrolyzed into soluble COD which was further converted to electricity and metabolites by microbes. Within the first 6 days, SCOD in the MFCs with and without BES increased from 780 mg/L to the maximum value of 1920 and 1260 mg/L, respectively (Fig.3a). At day 2, SVFAs in the BES-added MFC achieved the maximum concentration of 1032 mg/L, which was nearly 3 times higher compared with the control MFC (Fig.3b). It seemed that SVFAs made the major contribution to SCOD considering their similar dynamic trend.

In a study of microbial response of anaerobic sludge digestion to the addition of $CHCl_3$ and BES [30], SVFAs such as propionate, *n*-butyrate and iso-valerate accumulation were reported to be found in both inhibitors-added sets while none of them was detectable in the control. Chen et al. [31] found that the addition of BES during anaerobic fermentation of sludge resulted in the accumulation of acetate, propionate, butyrate, in which acetate concentration was 4-fold higher than that of the control. In this experiment, the main component in SVFAs was identified to be acetate, suggesting that the presence of BES facilitated the acetate accumulation in the sludge MFC. It has been demonstrated that the growth rate of electrochemically active bacteria, the biocatalyst for electricity generation, can be limited by substrate availability and consequently reducing power [32]. The enhanced concentration of SVFAs provided higher substrate availability for electrochemically active bacteria to produce

electricity, which explained the higher power output in the BES-added MFC in this study. The maximum power density (115 mW/m^2) produced from BES-added MFC was of the same order of magnitude as that obtained from other sludge based MFCs. Liu et al. [9] reached a maximum power density of 220.7 mW/m^2 by using a single-chamber floating-cathode MFC with surplus sludge as fuel. Jiang et al. [10] reported a maximum power density of 8.5 W/m^3 (302 mW/m^2) in a two-chambered MFC with potassium ferricyanide as the electron acceptor and utilizing sewage sludge.

3.2 Enhanced power recovery

As a parameter measures how much of the available "fuel" has been converted into electrical current in the MFC, CE in this study was determined to be 7.8% and 4.1% for the MFCs with and without BES (Fig.4), respectively. The low CE was anticipated for MFCs using sewage sludge as fuel. CE of MFCs varies widely and depends on the types of substrate. Table 2 showed the effect of substrate type and concentration on the CEs for MFCs. The relatively high CE levels have been achieved in the MFCs fed with pure compound or synthetic wastewater that was rich in carboxylic acid substrates, such as acetate that can be directly used by bacteria for releasing electrons. CE levels for real wastewater treatment are generally low [3, 33-35]. For many real wastewaters, the presence of recalcitrant organic matter that likely elevates the estimate of total organic carbon always has very little contribution to power production, especially for complex substrates and high concentrations.

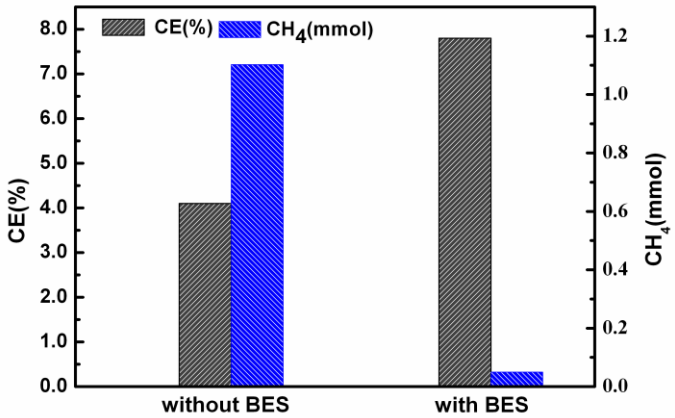

Figure 4. Coulombic efficiency (CE) and methane production from the MFCs in the presence and absence of BES.

On the other hand, the CE was decreased by the produced fermentation products from sludge fermentation. Sewage sludge is a complex carbon source mainly consisting of carbohydrates, proteins and lipids. During hydrolysis, the carbohydrate metabolism generates fermentation products such as organic acids (formic and lactic acids) and alcohols (ethanol, butanol, propanol). Some fermentation products may not be suitable substrates for current-producing bacteria [37], and the CEs of the MFCs utilizing fermentable substrates were rather lower than that of the MFCs using non-fermentable substrates. For example, the CEs of the MFCs fed with acetate and glucose were extensively

compared. In the MFC using ferricyanide as the final electron acceptor, the CE was 71% for acetate [38] but was only 20% with glucose [39]. The high CE of 65% with acetate and low CE of 14% with glucose were also recorded in the flat plate dry air-cathode MFC [34]. Recently, Chae et al. [21] also reported that acetate-fed-MFC generated the highest CE (72.3%), followed by butyrate (43.0%), propionate (36.0%) and glucose (15.0%) using the same MFC configuration. Lee et al. [40] reported the energy conversion efficiency (ECE) of acetate and glucose, and the ECE was 42% with acetate, but only 3% with glucose.

Table 2. The effect of substrate type and concentration on the Coulomic efficiency for MFCs

Substrate	COD (mg/L)	Coulombic efficiency (%)	Reference
Sewage sludge	24,750	7.8	This study
Swine waste	8,320	8	[33]
Beer brewery	2,240	10	[3]
Slaughterhouse	1,420	5	[35]
Paper recycling	723	22	[36]
Municipal	379	6	[34]
Acetate	1,000	65	[34]
Glucose	1,000	14	[34]

In the sludge-fed MFC, biomass, residual organic products, and CH_4 are possible non-electricity sinks. Table 3 depicts the distribution of electron equivalents in the MFCs expressed as COD at the end of the experiments, established as previously described [40]. Because of the presence of recalcitrant carbon in sludge and high strength of the sludge, the residual organic compounds were the largest non-electricity sink for both MFCs: 66.5% of the initial COD for the control MFC and 67.0% for the BES-added MFC. The unknown portion comprising of biomass formation, instrumental loss, and other electron acceptors consumption was the second largest non-electricity sink for both MFCs. Fermentation involves diverse microorganisms that have higher growth yields than anode-respiring bacteria [41], thus microbial biomass likely accounts for very great proportion of unknown. CH_4 was only detected in the MFC without BES (a yield of 1.1 mmol) and it was negligible in the BES-added MFC (Fig.4). As shown in Table 3, the diversion of electron flow from initial COD to methanogenesis was 1.5%, which approximately equaled to the electricity sink (1.4%). As methanogenesis was effectively suppressed by BES injection, the CE was improved from 4.1% to 7.8%, which was almost corresponded to the amount of saved electrons by inhibiting methane production. The increased CE by methanogenesis control with BES was more substantial in the MFCs using simple organic matter, for example, Chae et al. [24] found that the addition of 0.1~0.27 mM BES increased the CE from 35% to 70% in the acetate-fed MFC.

Table 3. Distribution of electron equivalents in the MFCs expressed as COD (mg)

	MFC without BES		MFC with BES	
	COD (mg)	Fraction (%)	COD (mg)	Fraction (%)
Initial COD	4,208	100	4,208	100
Final COD	2,800	66.5	2,860	67.0
Current	57.7	1.4	105.3	2.5
CH_4 gas	64.2	1.5	ND[a]	0
Unknown	1,286	30.6	1,243	30.5

[a] not detected

3.3 CV and DGGE analysis

To study the effect of BES on the catalytic behavior of the anaerobic consortia in the anode chamber, CV was performed on the MFCs with and without BES, along with a new carbon felt. As shown in Fig.5, no clear redox couples were observed for the new carbon felt, while significant oxidation and reduction peaks were found for the both MFCs.

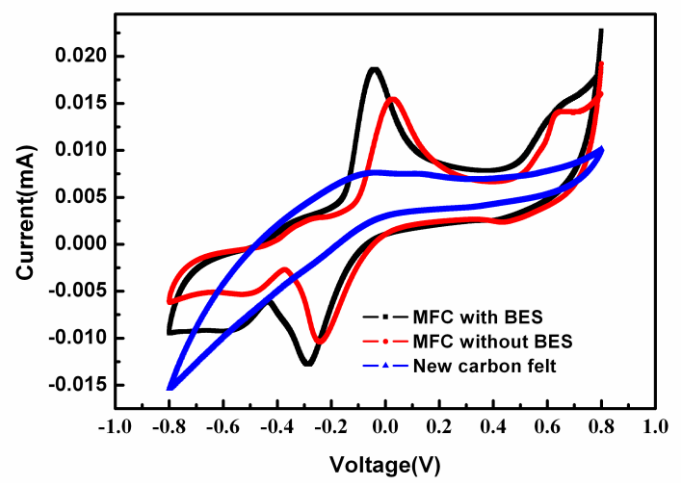

Figure 5. Cyclic voltammograms of anode biofilm in the MFC added with BES (black square), the MFC without BES (red circle), and a new carbon felt (blue triangle). Scan rate was 1 mV/s. Biofilm samples were taken on day 24.

For the MFC without BES addition, the CV recorded an oxidation peak at +0.026 mV in the forward scan and a reduction peak at –0.25 mV in the reverse scan, suggesting electrochemical activity of the biofilm on the anode surface. After BES addition, the voltammogram showed that both oxidation and reduction peaks shift towards the direction of negative potential (–0.03 and –0.29 V vs. Ag/AgCl), which lead to an enhanced open circuit voltage of the fuel cell. Compared with the control MFC, there was an evident increase in catalytic current response for both oxidation and reduction

peaks in the BES-added MFC, in which the maximum current reached 0.0186 mA for the oxidation peak. Current in the voltammogram is a visual signal of the release of e^- produced from the oxidation of substrate in the bacterial cell. A higher current observed on voltammogram can be correlated to higher electron discharge. The CV results suggested a higher activity of electrocatalytic biofilm in the BES-added MFC, which was consistent with enhanced power output in the BES-added MFC.

At the end of the experiment, anode biofilm in both MFC reactors were collected to identify microbial communities. The PCR-amplified 16S rRNA gene fragments were used for DGGE. A band at about 230 bp for the 16S rRNA gene was obtained for both samples. Fig.6 illustrated the DGGE profiles of anode bacterial communities obtained from the control MFC and BES-added MFC. As known, each band appearing in DGGE profile represents different species present in the microbial population and the staining intensity of a band is a representation of the relative abundance of the corresponding microbial species. As seen from Fig.6, the banding pattern of both samples are rather similar, however, the staining intensities of same band showed differently. The comparative sequence analysis of excised DGGE bands revealed that band 1, 2 and 3 were related to methanogenic communities, and the other DNA fragments (band 4, 5 and 6) resembled the electrochemically active microorganisms. It is clear that band 1, 2 and 3 showed lower intensity in the sample from the BES-added MFC than that from the control MFC, while the staining intensities of band 4, 5 and 6 were significantly enhanced in the BES-added MFC. The results suggested that the application of BES suppressed the presence of methanogen species and simultaneously increased the abundance of electrochemically active bacteria. As the competitive metabolism of methanogenesis is suppressed, the substrate availability for electrochemically active bacteria is increased and consequently enhancing power recovery [42, 43].

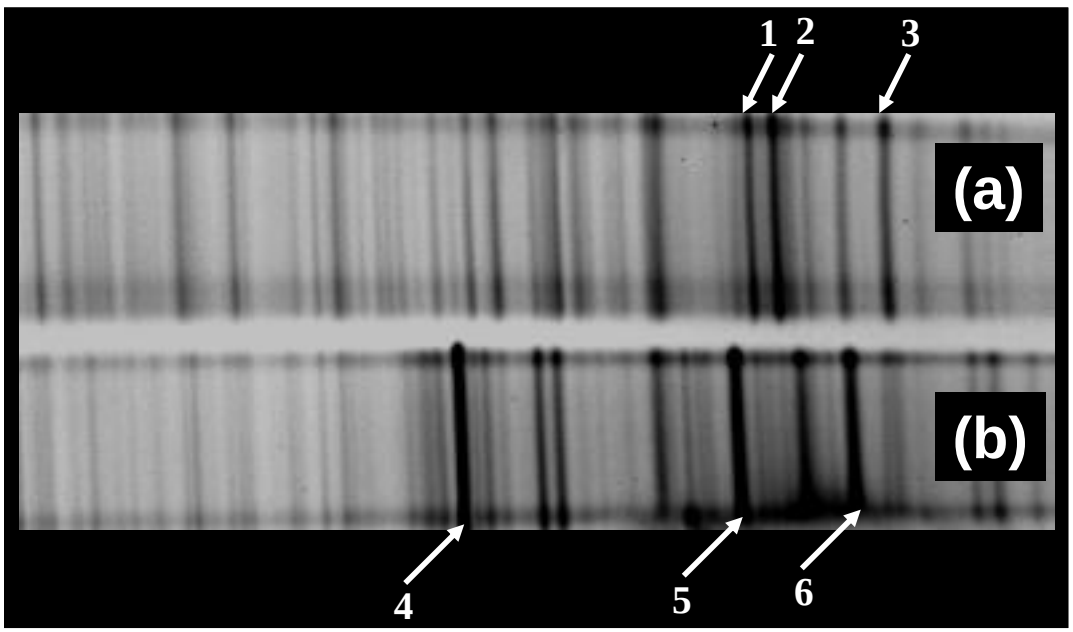

Figure 6. PCR-DGGE profiles of anode bacterial communities obtained from the MFC without BES (a) and the MFC with BES (b). Samples were taken on day 24

4. CONCLUSION

The competing effect of methanogensis is detrimental to the performance of MFCs inoculated with anaerobic sludge. This study demonstrated the effective suppression of methanogensis in the sludge MFC employing BES at a low dose of 0.5 mM. The addition of BES seemed to result in the accumulation of SVFAs that provided higher substrate availability for MFC to generate a higher maximum power density (115 mW/m^2). As a consequence of applying BES, the flow of electrons going to the methanogens were greatly reduced, which led to a 90% increase in CE. CV and DGGE analyses showed the different catalytic behavior and microbial communities of the anode electrode in the MFCs with and without BES.

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