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Article

Important Role of Abiotic Sulfide Oxidation in Microbial Fuel Cells Treating High-Sulfate Wastewater

Witchayut Niyom[1,a], Decharthorn Komolyothin[1], and Benjaporn Boonchayaanant Suwannasilp[1,2,3,b,*]

1 Department of Environmental Engineering, Faculty of Engineering, Chulalongkorn University, Bangkok, Thailand
2 Center of Excellence on Hazardous Substance Management, Chulalongkorn University, Bangkok, Thailand
3 Biotechnology for Wastewater Engineering Research Group, Chulalongkorn University, Bangkok, Thailand
E-mail: [a]witchayut_yen@hotmail.com, [b]benjaporn.bo@chula.ac.th (Corresponding author)

Abstract. Microbial fuel cells (MFCs) have been considered as an alternative for the treatment and energy recovery of organic wastewater containing sulfate, which cannot be achieved via conventional anaerobic treatment processes. This study investigates the performances and mechanisms of two-compartment single-chamber MFCs treating sulfate-rich organic wastewater at the chemical oxygen demand (COD) to sulfate ratio of 1, 3, and 6 in MFC1, MFC3, and MFC6, respectively. The first compartments functioned as anaerobic bioreactors. In the second compartments where fuel cell apparatuses were installed, sulfide removal was 49.51 ± 57.74, 24.08 ± 13.74, and 15.69 ± 21.30 mgS^{2-}/L in MFC1, MFC3, and MFC6, respectively. The maximum power generation amounts of 9.33, 1.79, and 1.41 mW/m^2 were achieved in MFC1, MFC3, and MFC6, respectively. A higher sulfide concentration in MFC1 contributed to higher power generation in MFC1. The main mechanism of electrical generation in all MFCs was abiotic sulfide oxidation.

Keywords: Microbial fuel cell, sulfate, sulfide, sulfate-reducing bacteria, COD:sulfate ratio.

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1. Introduction

Organic wastewater containing high sulfate has been generated in various types of industries, such as the paper mill industry, rubber industry, pharmaceutical industry, mining industry, and tannery industry [1]. High-strength organic wastewater is typically treated using anaerobic treatment since it can receive a high organic loading rate while recovering energy from wastewater in the form of biogas. However, in cases of organic wastewater contaminated with sulfate, sulfate reduction and sulfide production by sulfate-reducing bacteria (SRB) also occur in anaerobic process, thereby lowering the quantity and quality of biogas. Therefore, microbial fuel cells (MFCs) capable of treating organic wastewater containing sulfate have been developed as an alternative for treatment and energy recovery from this type of wastewater.

Several studies have investigated the treatment of sulfide using MFCs [2-5]. The results suggest that sulfide can be oxidized at anode electrodes by abiotic sulfide oxidation and/or microbial mediated sulfide oxidation. In addition, MFCs have been used to treat organic wastewater containing sulfate with simultaneous electricity generation [2, 3]. However, research on the effects of the COD:SO_4^{2-} ratio on MFC performances is still limited, especially under continuous operation [6, 7]. Since the COD:SO_4^{2-} ratio can have an influence on microbial activities in MFCs [6], treatment and electricity generation mechanisms in MFCs tend to depend greatly on the COD:SO_4^{2-} ratio.

In this study, two-compartment single-chamber air-breathing MFCs will be used to investigate the effects of the COD:SO_4^{2-} ratio on treatment and electricity generation mechanisms as well as microbial communities in MFCs. The results from this study will provide a better understanding of the treatment of sulfate-rich organic wastewater in MFC systems.

2. Material and Methods

2.1. MFC Configuration

Two-compartment single-chamber air-breathing MFCs were used in this study (Fig. 1). The first and second compartments had the working volumes of 2,025 mL and 630 mL, respectively. The first compartment functioned as an anaerobic bioreactor whereas fuel cell components were installed in the second compartment. The cathode electrode was a piece of 5 cm × 5 cm of 30% polytetrafluoroethylene (PTFE) wet-proof carbon cloth (0.5 mgPt/cm^2 Pt loading). The anode electrode was a piece of 5 cm × 5 cm rectangular activated carbon cloth, which connected to the cathode electrode through an external resistor with a 1 mm-diameter of titanium wire. The distances between the anode and the cathode electrodes were 2 cm. Nafion117 was used as a proton exchange membrane (PEM). The cathode and the PEM were assembled by hot-pressing. Silver mesh was attached to the cathode for electrical current collection.

2.2. MFC Operation

Three MFCs were used in this study to investigate the effects of the COD:SO_4^{2-} ratio on their performances and mechanisms. The first compartments seeded with anaerobic sludge (8,000 mgMLSS/L) were operated to enrich microbial communities at three different COD:SO_4^{2-} ratios (1, 3, and 6). Synthetic wastewater with the COD:SO_4^{2-} ratios of 1, 3, and, 6 were fed into the first compartments of MFC1, MFC3, MFC6, respectively, at a flow rate of 0.084 L/hr with a hydraulic retention time (HRT) of 1 day. Glucose with a COD concentration of approximately 3,000 mg/L was fed into all MFCs, whereas sulfate concentrations of 3,000, 1,000, and 500 mg/L were fed continuously into MFC1, MFC3, and MFC6, respectively. Macronutrients in the synthetic wastewater consisted of NH_4Cl 221.6 mg/L; $NaH_2PO_4.2H_2O$ 58.9 mg/L; NaCl 381.5 mg/L; KCl 573.8 mg/L; $CaCl_2$ 416.3 mg/L; and $MgCl_2$ 633.3 mg/L. Trace elements consisting of $FeSO_4.7H_2O$ 1 mg/L; $NiSO_4.6H_2O$ 0.526 mg/L; $MnSO_4.H_2O$ 0.526 mg/L; $ZnSO_4.7H_2O$ 0.106 mg/L; H_3BO_3 0.106 mg/L, $CoCl_2.6H_2O$ 52.6 μg/L; and $CuSO_4.5H_2O$ 4.5 μg/L were added into the synthetic wastewater. Sodium bicarbonate (3,000 mg/L as $CaCO_3$) was added as a source of alkalinity. Then, electricity equipment consisting of anodes, proton exchange membranes, cathodes, titanium wire, and external resistances was installed in the second compartments of the MFCs after the enrichment of microbial communities in the first compartments for 61 days for MFC1 and 58 days for MFC3 and MFC6. For each MFC, the effluent from the first compartment flowed continuously into the second compartment. The HRT of the second compartment was 7.5 hr. The second compartments were mixed using magnetic stirrers. All

MFCs were operated at an external resistance of 1,000 ohm. The open circuit voltages (OCVs) and voltages across the electrodes of all MFCs were measured over time using a multimeter (Fluke 115). The current that occurred in the MFCs was calculated by the relation of Ohm's law (I=V/R). Current densities (j) were calculated by dividing the current by the anode surface area. The polarization curves (I-V curves) and power density curves of each MFC were constructed on days 1, 2, 4, 8, 11, 18, 21, and 24 of MFC operation by varying the external resistances from 47 Ω to 150,000 Ω. The maximum power densities of MFCs were estimated from the power density curves by polynomial regression. Then, samples of microorganisms in the first compartments, in the second compartments, and on the anode electrodes were collected on day 33 in MFC1 and on day 40 in MFC3 and MFC6 for microbial community analysis. The anode electrodes in all MFCs were replaced by new ones, which were identical to the previous ones, on day 33 in MFC1 and on day 40 in MFC3 and MFC6. Polarization curves and power densities curves were constructed on days 1, 2, 3, 4, 5, 6, and 8 after the anode installation. The replacement of anode electrodes could help with identifying the influence of biofilm formation and sulfur accumulation on the deterioration of the anode electrodes in MFCs. Samples of anode electrodes before and after 8 days of MFC operation after the replacement of anodes were collected. The surfaces of the anode electrode samples were analyzed with scanning electron microscopy equipped with an energy-dispersive X-ray (SEM [JSM-6400] with EDX).

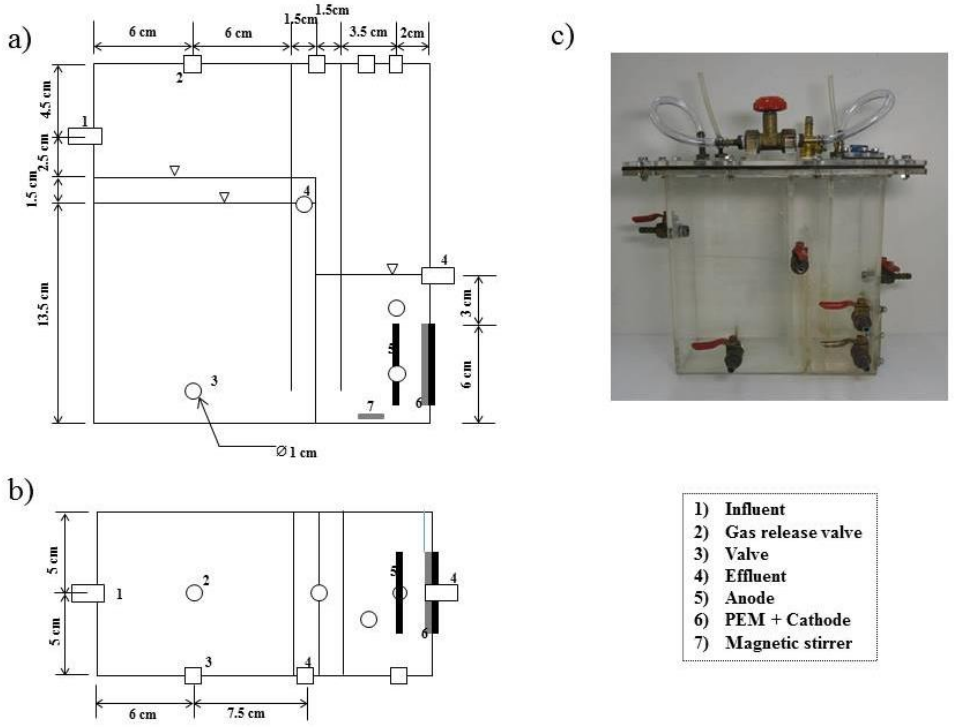

Fig. 1. MFC configuration: a) front view, b) top view, and c) MFC.

2.3. Abiotic Fuel Cell Operation

Abiotic fuel cells (AFCs) fed with sulfide were operated under similar conditions to the second compartments of MFCs to investigate the mechanisms of abiotic sulfide oxidation in the MFCs. Synthetic wastewater containing sulfide of 393.9 ± 10.6 mgS^{2-}/L, 252.3 ± 11.1 mgS^{2-}/L, and 119.7 ± 5.2 mgS^{2-}/L were fed directly into AFC1, AFC3, and AFC6 with the same flow rate (0.084 L/hr) and HRT (7.5 hr) as in the MFC operation. These sulfide concentrations were close to the sulfide concentrations in the effluent of the first compartments of MFC1, MFC3, and MFC6, respectively. Sodium bicarbonate of about 3,000 mg/L as $CaCO_3$ was added into the synthetic wastewater as a source of alkalinity. The pH in the synthetic wastewater was adjusted to 7, which was close to the average pH in the effluent of the first compartments of MFCs. Sulfate, sulfide, OCVs, and voltages across the electrodes were measured over time. Polarization curves and power densities curves were constructed on days 1-7 of abiotic fuel cell operation.

2.4. Analytical Measurement

Samples from the influent and effluent of the first and second compartments of the MFCs were collected for COD, sulfate, sulfide, and pH measurement over time. The COD and sulfate were analyzed using the close reflux method and the turbidimetric method, respectively [8]. Sulfide was measured using a sulfide ion-selective electrode (PerfectION™ Combination Silver/Sulfide Electrode, Mettler Toledo). A pH meter (InLab® Expert Pro-ISM electrode, Mettler Toledo) was used for pH measurement.

2.5. Microbial Community Analysis

Sludge in the first compartments, suspended microorganisms in the second compartments, and biofilms on the anode electrodes were collected for microbial community analysis. For suspended microorganisms in the second compartment, the samples were filtered with 0.2 μm filters and then used for DNA extraction. For the biofilms on the anode electrodes, deionized water was used to remove biofilms from the anode electrodes. The DNA was extracted from all of the samples using FastDNA® SPIN Kit (MP Biomedicals). Microbial communities were analyzed using 16S rRNA amplicon sequencing by the MiSeq system (Illumina). The 16S rRNA genes were amplified with polymerase chain reactions (PCRs) using universal primers for bacteria and archaea (515F: 5'-GTGYCAGCMGCCGCGGTAA-3' and 806R: 5'-GGACTACHVGGGTWTCTAAT-3') [9]. The PCR products were purified using AMPure XP beads. Dual indices and Illumina sequencing adapters were attached to the PCR products using the Nextera XT Index Kit. The PCR products were purified again using AMPure XP beads. Library quantification was conducted using a Qubit® 2.0 Fluorometer (Life Technologies), and then the DNA samples were diluted to 4 nM with 10 mM Tris-HCl pH 8.5 and pooled as a sample library. The sample sequencing was performed in the MiSeq system (Illumina) at the Faculty of Medicine, Chulalongkorn University, Thailand. Sequencing quality was checked using an online FastQC application in BaseSpace (http://basespace.illumina.com). The paired-end sequences were assembled using PANDASeq [10]. The sequences with a similarity greater than 99.7% were clustered into one operational taxonomic unit via the UPARSE algorithm [11]. The Basic Local Alignment Search Tool (BLAST) [12] was used to assign a taxonomy to each operational taxonomic unit (OTU) according to bacterial and archaeal 16S rRNA sequences from Bioprojects database from National Center for Biotechnology Information (NCBI) (ftp://ftp.ncbi.nlm.nih.gov/blast/db/). The information on taxonomy and OTU clustering was combined to create OTU tables using our own scripts. The results were then analyzed in the MEGAN5-MetaGenome Analyzer (http://ab.inf.unituebingen.de/ software/megan5/).

3. Results and Discussion

3.1. Treatment of COD, Sulfate, and Sulfide in MFCs

Treatment efficiencies and average concentrations of COD, sulfate, and sulfide during MFC operation are summarized in Table 1. COD removal efficiencies in the first compartments of MFC1, MFC3, and MFC6 were 56.06 ± 10.67 %, 62.49 ± 11.21 %, and 63.22 ± 11.57 %, respectively, which were not significantly different. However, the sulfate removal of 1,209 ± 455 mgSO_4^{2-}/L, 964 ± 93 mgSO_4^{2-}/L, and 492 ± 44 mgSO_4^{2-}/L were observed in MFC1, MFC3, and MFC6, respectively. Since the theoretical COD:SO_4^{2-} ratio based on electron equivalents assuming a negligible biomass yield was 0.67, COD removal via sulfate reduction was 870 mgCOD/L, 644 mgCOD/L, and 330 mgCOD/L for MFC1, MFC3, and MFC6, respectively. The results show different extents of COD removal via sulfate reduction among these MFCs.

Previous research has shown that methanogens can usually outcompete SRB when the COD:SO_4^{2-} ratio is greater than 2; meanwhile, SRB tend to outcompete methanogens when the COD:SO_4^{2-} ratio is lower than 1.3 [13]. However, a recent study by Hu et al. [14] reported the coexistence of SRB and methanogens even with a COD:SO_4^{2-} ratio as low as 1 when the COD concentration was high enough (3,000 mgCOD/L), which was similar to what was observed in our study. In terms of treatment efficiencies, sulfate removal efficiencies in MFC3 (95.01 ± 8.88 %) and MFC6 (96.65 ± 7.44 %) were higher than in MFC1 (42.96 ± 10.45 %).

In the second compartment, the COD removal efficiencies were 0.15 ± 9.83 %, 7.98 ± 10.23 %, and 9.98 ± 16.50 % for MFC1, MFC3, and MFC6, respectively, which corresponded to the COD removal of 1.62 ± 114.03 mgCOD/L, 85.89 ± 111.51 mgCOD/L, and 101.68 ± 229.90 mgCOD/L, respectively. On the other hand, sulfate removal in the second compartments of MFC1 and MFC6 were 23.33 ± 140 and 3.49 ±

30.76 $mgSO_4^{2-}/L$, respectively, while no sulfate removal was observed in the second compartment of MFC3. Sulfide removal in the second compartments of MFC1, MFC3, and MFC6 were 49.51 ± 57.74 mgS^{2-}/L, 24.08 ± 13.74 mgS^{2-}/L, and 15.69 ± 21.30 mgS^{2-}/L, respectively. A high soluble sulfide concentration in MFC1 (342 ± 80 mgS^{2-}/L) was in the range that could inhibit microorganisms in the systems [15]. Therefore, no significant removal of both sulfate and COD occurred in the second compartment of MFC1.

Table 1. COD, sulfate, and sulfide concentrations and pH in MFC1, MFC3, and MFC6.

Parameters	MFC1		MFC3		MFC6	
	Compart. 1	Compart. 2	Compart. 1	Compart. 2	Compart. 1	Compart. 2
Influent COD (mgCOD/L)	3,043 ± 139	1,344 ± 359	2,999 ± 427	1,084 ± 127	3,033 ± 349	1,110 ± 359
Effluent COD (mgCOD/L)	1,344 ± 359	1,342 ± 303	1,084 ± 127	994 ± 131	1,110 ± 359	1,003 ± 391
COD removal efficiency (%)	56.06 ± 10.67	0.15 ± 9.8	62.49 ± 11.21	7.98 ± 10.23	63.22 ± 11.57	9.98 ± 16.5
Influent sulfate ($mgSO_4^{2-}/L$)	3,036 ± 60	1,736 ± 334	1,015 ± 29	53.8 ± 91.9	509 ± 17	16.85 ± 37.16
Effluent sulfate ($mgSO_4^{2-}/L$)	1,736 ± 334	1,709 ± 249	53.8 ± 91.9	59.22 ± 93.70	16.85 ± 37.16	14.06 ± 18.28
Sulfate removal efficiency (%)	42.96 ± 10.45	N.A.	95.01 ± 8.88	N.A.	96.65 ± 7.44	N.A.
pH	7.37 ± 0.17	7.57 ± 0.32	7.08 ± 0.12	7.22 ± 0.20	7.00 ± 0.16	7.12 ± 0.20
Sulfide (mgS^{2-}/L)	400 ± 69	342 ± 80	265 ± 59	237 ± 59	119 ± 32	100 ± 34
Sulfide removal efficiency (%)	N.A.	14.23 ± 15.71	N.A.	10.32 ± 5.01	N.A.	15.69 ± 21.30

3.2. Electricity Generation of MFCs

The OCVs and voltages across the electrodes at the 1,000 Ω external resistances of all MFCs are shown in Fig. 2. The OCVs and voltages of all MFCs decreased during the first week of operation (Fig. 2 (a)). All MFCs generated maximum values of both OCVs and voltages across the electrodes at a 1,000 Ω external resistance on the first day after the installation of electrical equipment. The maximum OCVs of 635 mV, 475 mV, and 460 mV were observed in MFC1, MFC3, and MFC6, respectively. For the voltage across the electrodes at 1,000 Ω external resistance, the maximum values of 300 mV, 110 mV, and 183 mV were also found on the same day as the maximum OCV values in MFC1, MFC3, and MFC6, respectively.

The decrease in OCVs in all MFCs was likely due to the deterioration of the cathode electrodes (activation loss) since the replacement of anode electrodes did not improve the OCVs in all MFCs (Fig. 2 (b)). On the other hand, the results showed that the voltage across the electrodes at 1,000 Ω external resistances were suddenly increased after replacing with the new anode electrodes, which was a similar observation to that of Sangcharoen et al. [16]. As the new anode electrode had no sulfur accumulation, no biofilm formation of non-exoelectrogenic microorganisms, and a higher active surface area than that of the old one, ohmic and activation losses were likely to decrease, thereby increasing power-density production. In addition, sulfide concentrations after the anode-electrode replacement were higher than they were before the replacement, which could also contribute to lower voltage losses; this will be discussed further.

Both OCVs and voltages across the 1,000 Ω external resistance of MFC1 were higher than those associated with MFC3 and MFC6, respectively. The higher voltage in MFC1 than those in the other MFCs was probable due to two factors: 1) a higher dissolved sulfide concentration in MFC1 and 2) a higher level of ionic strength in MFC1 since a large amount of sulfate was added to the system compared to in MFC3 and MFC6. The increase in the sulfide concentration can increase the OCV and voltages in abiotic fuel cells fed with sulfide (results shown in 3.4). Moreover, the increase in the ionic strength in MFCs can improve the proton transfers, thereby decreasing the ohmic loss of the systems [17].

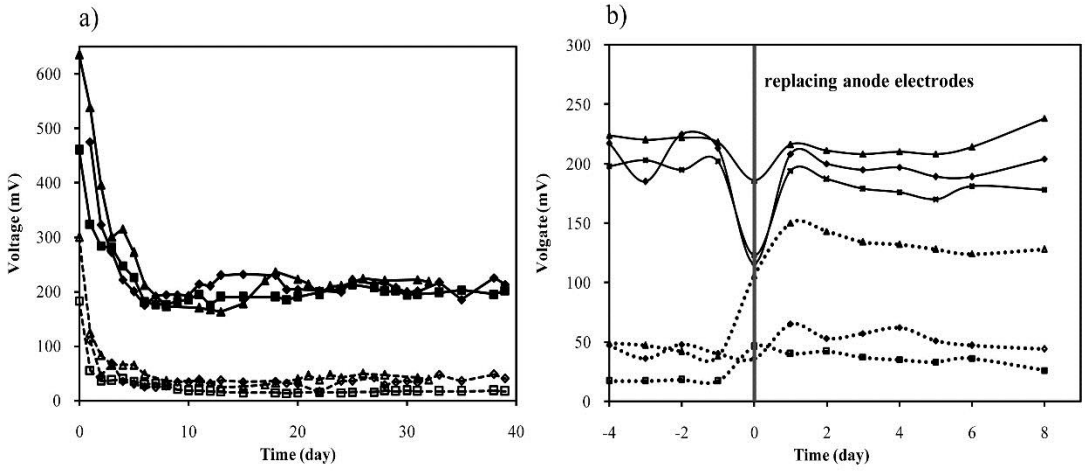

Fig. 2. OCVs and voltages across the electrodes at 1,000 Ω external resistances: a) before replacing anode electrodes and b) after replacing anode electrodes: OCVs in ▲MFC1, ◆MFC3, and ■MFC6 and the voltage across the electrodes at 1,000 Ω external resistances in △MFC1, ◇MFC3, and □MFC6.

The polarization curves (I-V curve) and power density curves of all MFCs were constructed on days 1, 2, 4, 11, 18, 21, and 24 of the MFC operation as shown in Fig. 3. The maximum power densities of MFC1, MFC3, and MFC6 on day 1 were 9.33 mW/m^2, 1.79 mW/m^2, and 1.41 mW/m^2, respectively.

Since the polarization curve appears to be the straight lines, it cannot be clearly distinguished between the ohmic losses and the activation losses. However, considering the slopes of the polarization curves, which represent the voltage losses in the systems, MFC1 appeared to have less voltage losses compared to MFC3 and MFC6. Possible explanations could be that sulfide concentrations in MFC1 were higher than in MFC3 and MFC6, resulting in less activation losses. Moreover, a higher ionic strength in MFC1 due to a higher amount of sulfate added could also contribute to lower ohmic losses in MFC1. The lower voltage losses in MFC1 could explain the higher voltages achieved in MFC1 during the MFC operation. In addition, activation losses could result from 1) the sulfur accumulation and biofilm formation of non-exoelectrogenic microorganisms on the anode electrodes, which can decrease the active surface areas of the anode electrodes and 2) the deterioration of the cathode electrodes, which could decrease the OCVs and voltages across the electrodes. On the other hand, ohmic losses could result from 1) the sulfur accumulation and biofilm formation of non-exoelectrogenic microorganisms on the anode electrodes, which can also increase the ohmic losses by obstructing the electron transfers in the systems and 2) biofilm formation on PEM, which could impede the proton transfers from the anode chamber to the cathode chamber.

3.3. Surface Analysis of Anode Electrodes

The surfaces of anode electrodes before and after eight days of MFC operation were analyzed by SEM/EDX to investigate the elements that had attached to and accumulated on the anode electrodes. The results of the SEM/EDX analysis are shown in Fig. 4. The results show that a large amount of elemental sulfur accumulated on the anode electrode surfaces after eight days of MFC operation, which supported our explanation that the increase in voltage losses was partly due to sulfur accumulation on the anode electrodes.

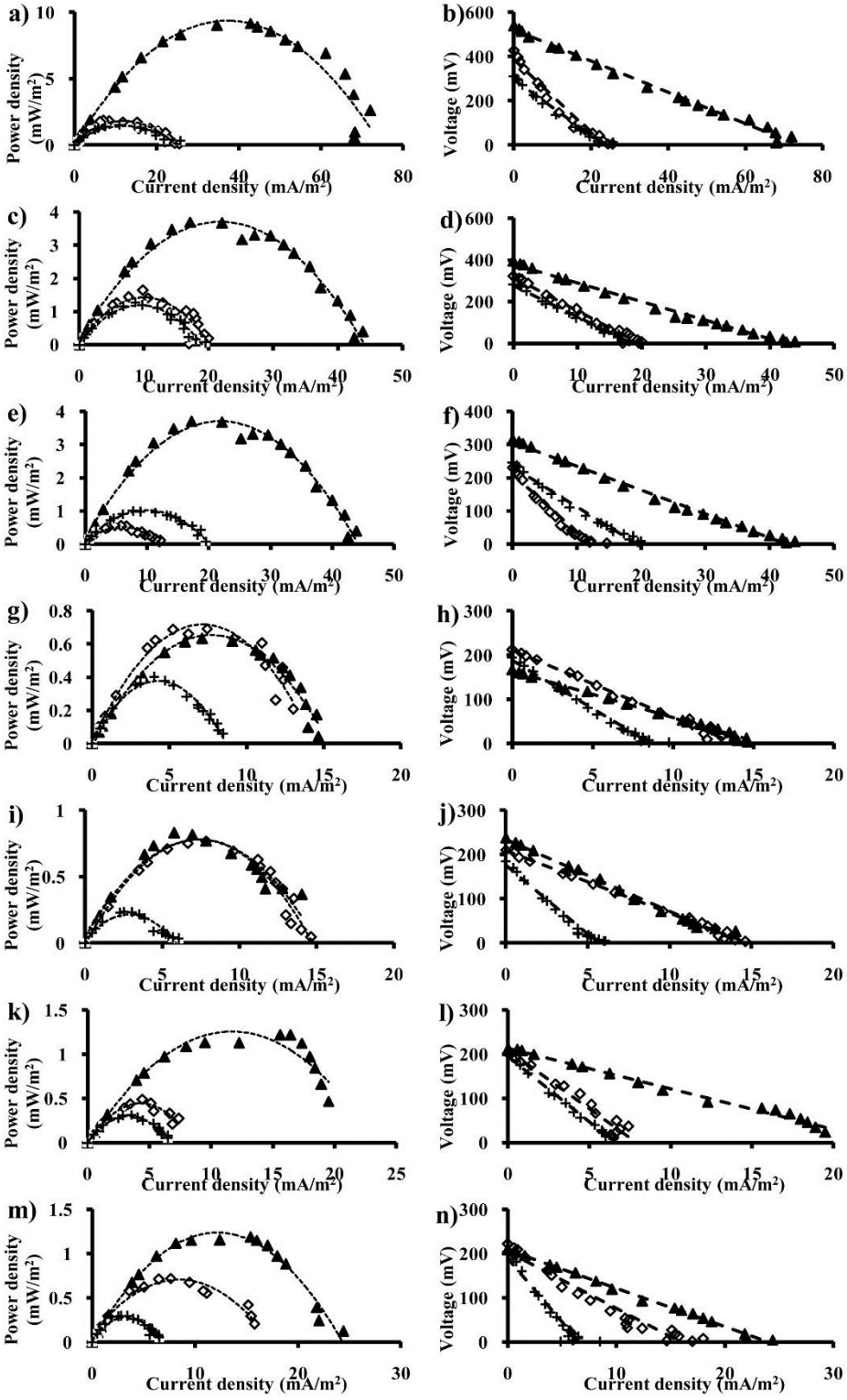

Fig. 3. Polarization curve on day: a) 1, c) 2, e) 4, g) 11, i) 18, k) 21, and m) 24 and power density curve on day b) 1, d) 2, f) 4, h) 11, j) 18, l) 21, and n) 24 of operation, ▲ MFC1, ◇ MFC3, and + MFC6.

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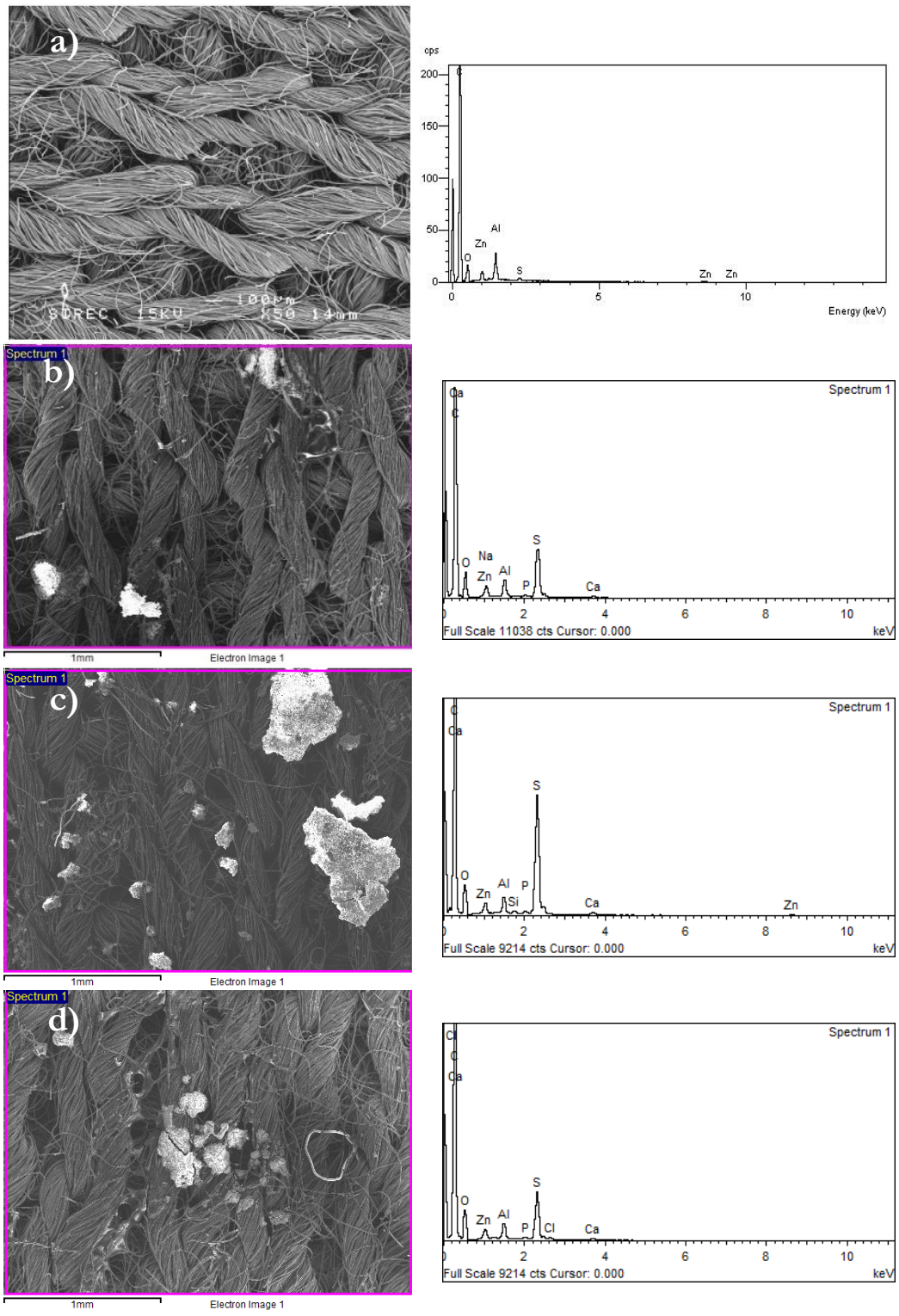


Fig. 4. SEM/EDX analysis of an activated carbon cloth a) before using as the anode electrode and at the end of operation in b) MFC1, c) MFC3, and d) MFC6.

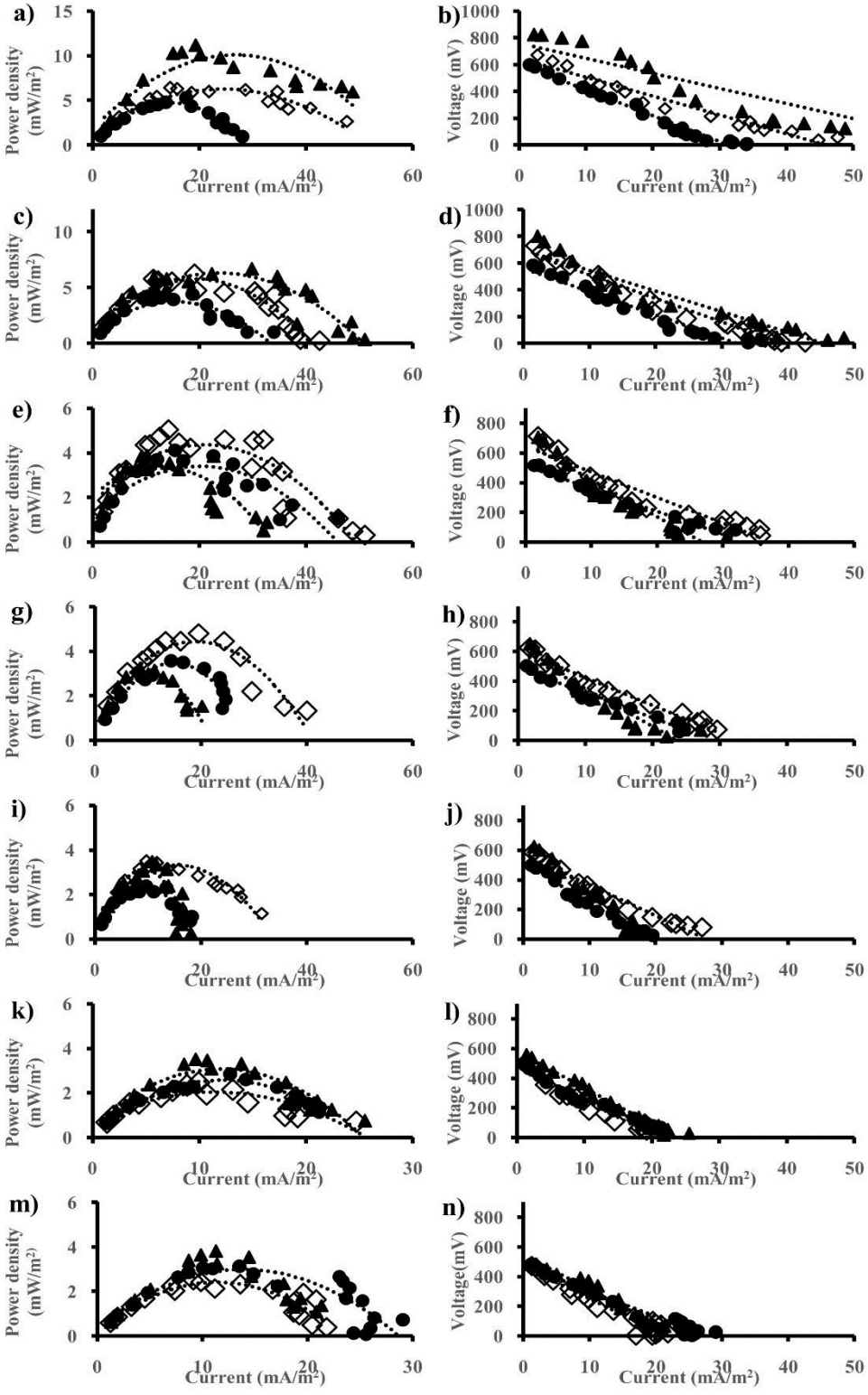


Fig. 5. Polarization curve on day: a) 1, c) 2, e) 3, g) 4, i) 5, k) 6, and m) 7 and power density curve on day b) 1, d) 2, f) 3, h) 4, j) 5, l) 6, and n) 7 of operation, ▲ AFC1, ◇ AFC3, and ● AFC6.

3.4. Abiotic Fuel Cell (AFC) Operation

Sulfide concentrations in all AFCs are summarized in Table 2. The results show that sulfide removal was rather constant in AFC6 (93.4 ± 9.0 mgS^{2-}/L) during seven days of operation. In contrast, sulfide removal continually decreased over time in AFC1 and AFC3. It should be noted that AFC1 and AFC3 received higher amounts of sulfide loading than AFC6 did, which could lead to the faster accumulation of elemental sulfur on the anode electrodes, resulting in a decrease in the active surface areas of the anode electrodes and a decrease in sulfide removal over time. For sulfate production, a very low amount of sulfate (<2% of sulfide removal) was generated in all AFCs. The results suggest that the main final product of abiotic sulfide oxidation in the MFCs was elemental sulfur.

Figure 5 shows the polarization curves and power density curves of all AFCs constructed on days 1-7. The results demonstrate the relationship between the OCVs and sulfide concentrations in the influent. The higher the sulfide concentration, the higher the OCV achieved. The OCVs in AFCs also decreased over time, similar to the observation in MFC operation, which was probably due to the deterioration of the cathode electrode as discussed in the previous section. Moreover, the results from polarization curves suggest an increase in voltage losses in AFC1 and AFC3 over time, resulting in a decrease in the maximum power densities in these AFCs. In contrast, AFC6 appears to have lower voltage losses compared to AFC1 and AFC3, which was likely due to less sulfide removal in AFC6, resulting in less sulfur accumulation on the anode electrodes of AFC6 compared to those of AFC1 and AFC3.

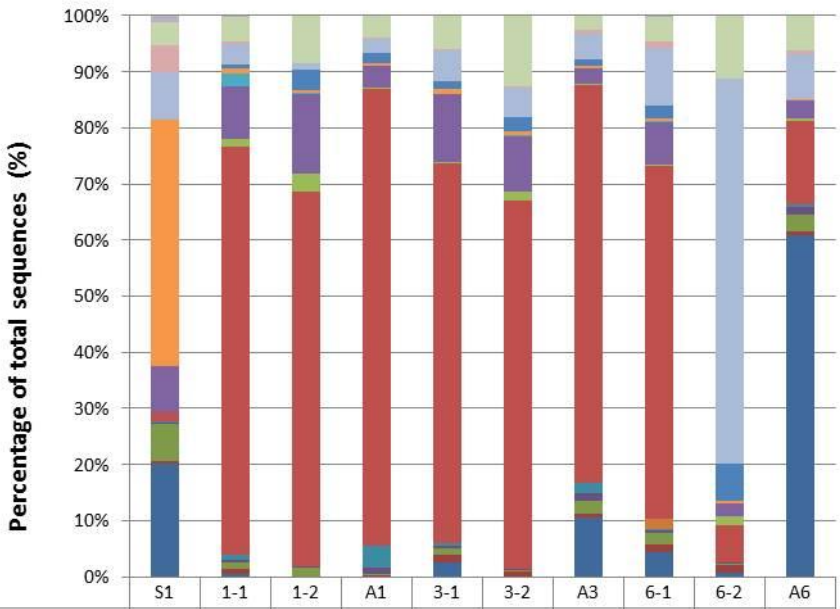
The results from the power density curves show that the maximum power density observed in the AFCs were higher than that in the MFCs. It suggests that the presence of biofilms on the anode electrodes had detrimental effects on the power generation of MFCs. The results from microbial community analysis (3.5) also show that most of the microorganisms found on the anode electrodes were non-exoelectrogenic microorganisms. Therefore, the main mechanism of electrical generation in all MFCs appears to be from abiotic sulfide oxidation.

Table 2. Sulfide removal and sulfate production in abiotic fuel cells.

Abiotic fuel cells	Sulfide		
	Influent (mgS^{2-}/L)	Effluent (mgS^{2-}/L)	Removal efficiency (%)
AFC1	393.9 ± 10.6	205.4 ± 54.5	48.1 ± 12.7
AFC3	252.3 ± 11.1	141.3 ± 15.0	43.9 ± 6.7
AFC6	119.7 ± 5.2	26.3 ± 4.7	77.9 ± 4.6

3.5. Microbial Community Analysis and Mechanisms of MFCs

Seed sludge, sludge in the first compartment, suspended solids in the second compartment, and biofilms on the anode electrodes in all MFCs were analyzed using 16S rRNA gene amplicon sequencing by the MiSeq system (Illumina) using universal primers for bacteria and archaea. Figure 6 shows microbial communities as a percent relative abundance of the microbial phyla of the samples obtained in this study. The results show great differences in the microbial community of the seed sludge when compared with the other samples, suggesting the selection of microbial communities after MFC operation. Table 3 shows the top five predominant genera of microbial communities observed in this study. *Tolumonas*, fermentative bacteria [18], was the predominant genus in the first compartments of all MFCs. Since glucose was the sole organic substrate in the influent, fermentative bacteria were necessary for fermenting glucose into acetate and other volatile fatty acids (VFAs), which methanogens and SRB can further use in the systems. Figure 7 summarizes possible treatment and electricity generation mechanisms in the MFCs. The possible microbial processes in the first compartments are shown in Fig. 7(a). The differences in the sulfate and sulfide concentrations among the MFCs appear to affect the relative abundance of the SRB and methanogens in the systems. The percentages of SRB in the first compartment of MFC1, MFC3, and MFC6 were 9.34 %, 12.11 %, and 5.65 % of total sequences, respectively. On the other hand, the percentages of methanogens in the first compartments of MFC1, MFC3, and MFC6 were 0.55 %, 2.56 %, and 4.36 % of total sequences, respectively.



	S1	1-1	1-2	A1	3-1	3-2	A3	6-1	6-2	A6
Actinobacteria	1.195	0.237	0.007	0.002	0.070	0.004	0.088	0.166	0.022	0.105
Bacteroidetes	4.048	4.419	8.493	3.828	5.894	12.700	2.558	4.415	11.267	6.009
Chloroflexi	4.915	0.328	0.015	0.271	0.323	0.020	0.513	1.129	0.048	0.788
Firmicutes	8.374	3.740	1.094	2.491	5.468	5.283	4.738	10.405	68.444	7.937
Lentisphaerae	0.030	0.705	3.663	1.869	1.274	2.498	0.990	2.231	6.697	0.108
Proteobacteria (alpha)	43.836	0.939	0.554	0.486	0.908	0.835	0.599	0.406	0.380	0.188
Proteobacteria (beta)	0.013	2.204	0.154	0.053	0.016	0.077	0.010	0.146	0.114	0.032
Proteobacteria (delta)	8.364	9.442	14.093	3.923	12.207	9.976	2.655	7.574	2.344	3.224
Proteobacteria (epsilon)	0.005	1.322	3.186	0.102	0.189	1.486	0.147	0.367	1.415	0.449
Proteobacteria (gamma)	1.433	72.776	66.780	81.490	67.602	65.631	71.049	62.834	6.614	14.874
Proteobacteria (unclassified)	0.026	0.008	0.000	0.000	0.000	0.003	0.001	1.930	0.000	0.000
Spirochaetes	0.255	0.853	0.185	3.896	0.602	0.065	1.764	0.119	0.068	0.386
Synergistetes	0.257	0.464	0.149	1.112	0.455	0.152	1.317	0.551	0.316	1.304
Other bacteria	6.735	1.123	1.485	0.317	1.124	0.388	2.302	1.906	0.237	3.067
Unclassified bacteria	0.415	0.869	0.140	0.143	1.286	0.779	0.782	1.343	1.344	0.559
Archeae	20.10	0.57	0.002	0.02	2.58	0.10	10.49	4.48	0.69	60.97

Fig. 6. Microbial communities shown as percent relative abundance of microbial phyla: S1 = seed sludge, 1-1 = first compartment of MFC1, 1-2 = second compartment of MFC1, 3-1 = first compartment of MFC3, 3-2 = second compartment of MFC3, 6-1 = first compartment of MFC6, 6-2 = second compartment of MFC6, A1 = anode electrode of MFC1, A3 = anode electrode of MFC3, A6 = anode electrode of MFC6.

Table 3. Dominant genera in seed sludge, MFC1, MFC3, and MFC6.

Seed sludge		Rhodovibrio (43.58%) Methanosaeta (10.34%) Methanolinea (6.05%) Alkaliflexus (2.72%) Nitrospira (2.20%)
MFC1	compartment 1	Tolumunas (55.23%) Desulfovibrio (4.41%) Klebsiella (4.20%) Desulforhabdus (2.85%) Desulfomicrobium (1.60%)
	compartment 2	Klebsiella (52.46%) Desulfovibrio (13.45%) Bacteroides (7.40%) Tolumonas (5.24%) Victivallis (3.66%)
	anode	Klebsiella (64.53%) Tolumonas (3.17%) Treponema (2.25%) Desulfovibrio (2.22%) Victivallis (1.87%)
MFC2	compartment 1	Tolumunas (60.99%) Desulfovibrio (9.51%) Bacteroides (3.64%) Trichococcus (2.04%) Desulforhabdus (1.94%)
	compartment 2	Bacteroides (11.38%) Desulfovibrio (9.75%) Klebsiella (9.81%) Tolumonas (3.96%) Victivallis (2.50%)
	anode	Tolumonas (57.38%) Methanosaeta (9.37%) Klebsiella (8.16%) Desulfovibrio (1.45%) Melioribacter (1.13%)
MFC3	compartment 1	Tolumunas (61.95%) Desulfovibrio (4.37%) Trichococcus (3.47%) Methanosaeta (2.53%) Victivallis (2.23%)
	compartment 2	Trichococcus (17.12%) Bacteroides (9.60%) Victivallis (6.70%) Tolumonas (2.42%) Desulfovibrio (2.18%)
	anode	Methanoseata (57.74%) Tolumonas (12.99%) Trichococcus (2.80%) Alkaliflexus (2.75%) Clostridium (1.27%)

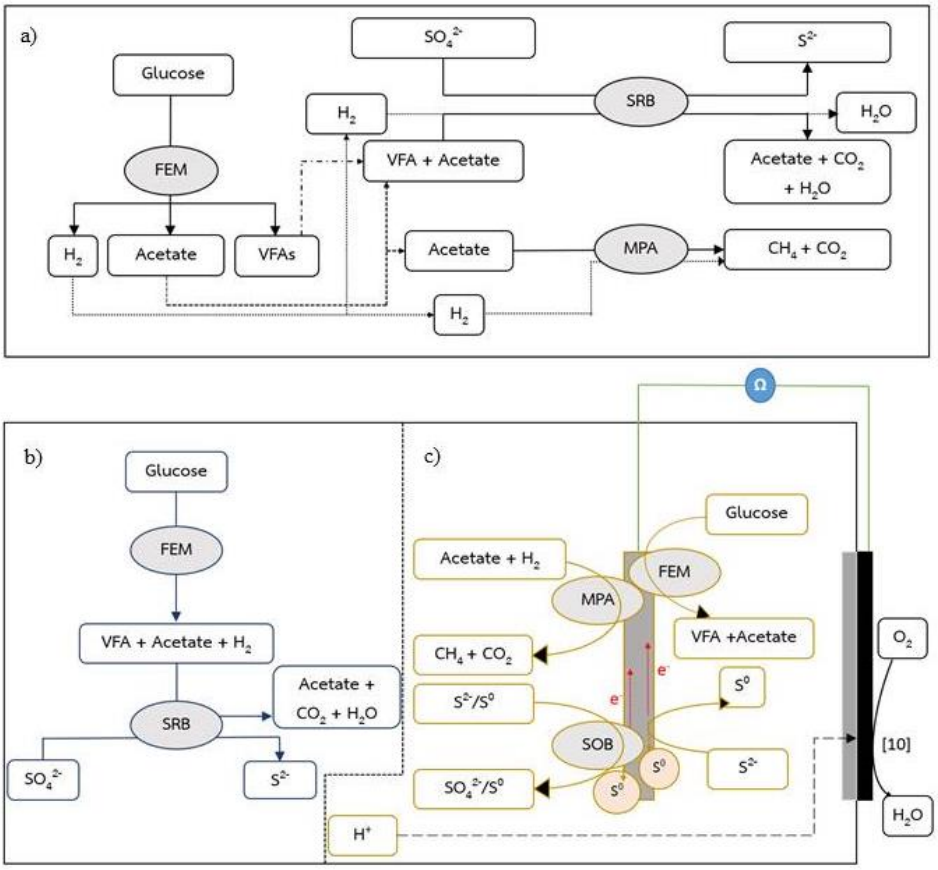


Fig. 7. Possible mechanisms of MFC: a) in the first compartment, b) in suspended solids in the second compartment, and (c) on the anode electrode. (Note: Methanogens (MPA) in the second compartment were found only in MFC3 and MFC6, and sulfur-oxidizing bacteria (SOB) were observed at very low amounts.)

For the suspended microorganisms in the second compartments of MFCs, *Klebsiella* was a predominant genus in MFC1 whereas *Bacteroides* and *Trichococcus* were predominant genera in MFC3 and MFC6, respectively. These groups of microorganisms are fermentative bacteria [19-21]. A greater abundance of SRB was observed in MFC1 than in MFC3 and MFC6, probably because the remaining sulfate concentration was still high in the second compartment of MFC1 compared to in MFC3 and MFC6. Nevertheless, the amount of suspended solids was very low in the second compartments of all MFCs, which could explain the low level of COD and sulfate removal in the second compartment. Possible mechanisms of suspended microorganisms in the second compartments are shown in Fig. 7(b).

For microbial communities of the biofilms on the anode electrodes, *Klebsiella* spp. were found to be predominant on the anode electrode of MFC1. Methanogens about 10.08 % and 60.72 % of total sequences were observed on the anode electrodes of MFC3 and MFC6, respectively. *Methanosaetaceae* was the main group of methanogens found in MFC3 and MFC6. For MFC6, sulfate and sulfide concentrations in the second compartment were the lowest compared to those in MFC1 and MFC3. Because of the low sulfate and sulfide concentrations, methanogens could become predominant on the anode electrode of MFC6. The presence of methanogens in both MFC3 and MFC6 suggests that COD removal in the second compartments of these MFCs might be derived from methanogenesis. Although the presence of methanogens on anode electrodes has long been expected, the evidences via molecular techniques have been rare since archaea was usually not the main interest of analysis. Until now, only one study by He et al. [22] observed methanogens on the anode using fluorescence insitu hybridization (FISH).

However, a low amount of known exoelectrogenic microorganisms was observed on the anode electrodes of all MFCs. The results show that known exoelectrogenic microorganisms of only 0.05 %, 0.12 %, and 0.23 % of total sequences were observed in MFC1, MFC3, and MFC6, respectively. High sulfide

concentrations in the MFCs might be unfavorable for the growth of exoelectrogenic microorganisms. In addition, sulfur-oxidizing bacteria, such as *Dyella thiooxydans* and *Sulfurovum,* were observed on all of the anode electrodes but at very low amounts (< 1.5% of total sequences).

Since most microorganisms on the anode electrodes are non-exoelectrogenic microorganisms, the main mechanism of electrical generation in all MFCs in this study was likely to be due to abiotic sulfide oxidation, which generated elemental sulfur as the final product. Figure 7c summarizes possible mechanisms on the anode electrodes in the second compartments of MFCs.

4. Conclusion

This study investigated the performances and mechanisms of three microbial fuel cells, MFC1, MFC3, and MFC6, treating sulfate-rich wastewater at the COD:SO_4^{2-} ratios of 1, 3, and 6, respectively. For the first compartments, the COD removal efficiencies of all MFCs were similar. However, sulfate removal and sulfide production varied greatly among the three MFCs, which resulted in different amounts of power generation in MFC1, MFC3, and MFC6. The maximum power generation of 9.33, 1.79, and 1.41 mW/m^2 were achieved in MFC1, MFC3, and MFC6. Sulfur accumulation and the presence of non-exoelectrogenic microorganisms on the anode electrodes could contribute to the voltage losses in the systems. Abiotic sulfide oxidation was the main mechanisms for sulfide removal in all MFCs.

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References

[1] P. N. L. Lens, F. Omil, J. M. Lema, and L. W. Hulshoff Pol, "Biological treatment of organic sulfate-rich wastewater," in *Environmnetal Technologies to Treat Sulfur Pollution: Principles and Engineering*, P. N. L. Lens and L. W. Hulshoff Pol, Eds. IWA Publishing, 2000.

[2] K. Rabaey, P. Clauwaert, P. Aelterman, and W. Verstraete, "Tubular microbial fuel cells for efficient electricity generation," *Environ. Sci. Technol.*, vol. 39, pp. 8077-8082, 2005.

[3] K. Rabaey, K. Van de sompel, L. Maignien, N. Boon, P. Aelterman, P. Clauwaert, H. T. Pham, J. Vermeulen, M. Verhaege, P. Lens, and W. Verstraete, "Microbial fuel cells for sulfide removal," *Environ. Sci Technol.,* vol. 40, pp. 5218-5224, 2006.

[4] M. Sun, Z. H. Tong, G. P. Sheng, Y. Z. Chen, F. Zhang, Z. X. Mu, H. L. Wang, R. J. Zeng, X. W. Liu, H. Q. Yu, L. Wei, and F. Ma, "Microbial communities involved in electricity generation from sulfide oxidation in a microbial fuel cell," *Biosens. Bioelectron.*, vol. 26, no. 2, pp. 470-476, 2010.

[5] F. Zhao, N. Rahunen, J.R. Varcoe, A. Chandra, C. Avignone-Rossa, A. E. Thumser, and R. C. T. Slade, "Activated carbon cloth as anode for sulfate removal in a microbial fuel cell," *Environ. Sci. Technol.*, vol. 42, pp. 4971-4976, 2008.

[6] M. M. Ghangrekar, S. S. R. Murthy, M. Behera, and N. Duteanu, "Effect of sulfate concentration in the wastewater on microbial fuel cell performance," *Environ. Eng. Manag. J.*, vol. 9, no. 9, pp. 1227-34, 2010.

[7] B. Zhang, J. Zhang, Q. Yang, C. Feng, Y. Zhu, Z. Ye, and J. Ni, "Investigation and optimization of the novel UASB–MFC integrated system for sulfate removal and bioelectricity generation using the response surface methodology (RSM)," *Biores. Technol.,* vol. 124, pp. 1-7, 2012.

[8] APHA, AWWA, WEF, *Standard Methods for the Examination of Water and Wastewater*, 21st ed. Washington DC: American Public Health Association, 2005.

[9] K. Ding, X. Wen, Y. Li, B. Shen, and B. Zhang, "Ammonia-oxidizing archaea versus bacteria in two soil aquifer treatment systems," *Appl. Microbiol. Biotechnol.,* vol. 99, no. 3, 1337-1347, 2015.

[10] A. P. Masella, A. K. Bartram, J. M. Truszkowski, D. G. Brown, and J. D. Neufeld, "PANDAseq: Paired-end assembler for illumina sequences," *BMC bioinformatics*, vol. 13, no. 31, pp. 1-7, 2012.

[11] R. C. Edgar, "UPARSE: Highly accurate OTU sequences from microbial amplicon reads," *Nat. Methods.* vol. 10, no. 10, pp. 996-998, 2013.
[12] S. F. Altschul, W. Gish, W. Miller, E. W. Myers, and D. J. Lipman, "Basic local alignment search tool," *J. Mol. Biol.,* vol. 215, no. 3, pp. 403-10, 1990.
[13] H. H. Chou, J. S. Huang, W. G. Chen, and R. Ohara, "Competitive reaction kinetics of sulfate-reducing bacteria and methanogenic bacteria in anaerobic filters," *Biores. Technol.*, vol. 99, no. 17, pp. 8061-8067, 2008.
[14] Y. Hu, Z. Jing, Y. Sudo, Q. Niu, J. Du, J. Wu, and Y.-Y. Li, "Effect of influent COD/SO_4^{2-} ratios on UASB treatment of a synthetic sulfate-containing wastewater," *Chemosphere*, vol. 130, pp. 24-33, 2015.
[15] P. N. L. Lens, A. Visser, A. J. H. Janssen, L. W. H. Pol, and G. Lettinga, "Biotechnological treatment of sulfate-rich wastewaters," *Crit. Rev. Environ. Sci. Technol.*, vol. 28, no. 1, pp. 41-88, 1998.
[16] A. Sangcharoen, W. Niyom, and B. B. Suwannasilp, "A microbial fuel cell treating organic wastewater containing high sulfate under continuous operation: Performance and microbial community," in *Proc. Biochem.*, 2015, vol. 50, no. 10, pp. 1648-1655.
[17] H. Liu, S. Cheng, and B. E. Logan, "Power generation in fed-batch microbial fuel cells as a function of ionic strength, temperature, and reactor configuration," *Environ. Sci. Technol.*, vol. 39, no. 14, pp. 5488-5493, 2005.
[18] M. E. Caldwell, T. D. Allen, P. A. Lawson, and R. S. Tanner, "Tolumonas osonensis sp.nov., isolated from anoxic freshwater sediment, and emended description of the genus Tolumonas," *Int. J. Syst. Evol. Microbiol.,* vol. 61, no. 11, pp. 2659-2663, 2011.
[19] J. R. Liu, R. S. Tanner, P. Schumann, N. Weiss, C. A. McKenzie, P. H. Janssen, E. M. Seviour, P. A. Lawson, T. D. Allen, and R. J. Seviour, "Emended description of the genus *Trichococcus*, description of *Trichococcus collinsii* sp. nov., and reclassification of *Lactosphaera pasteurii* as *Trichococcus pasteurii* comb. nov. and of Ruminococcus palustris as Trichococcus palustris comb. nov. in the low-G+C Gram-positive bacteria," *Int. J. Syst. Evol. Microbiol.,* vol. 52, pp. 1113-1126, 2002.
[20] K. Niu, X. Zhang, W. S. Tan, and M. L. Zhu, "Characteristics of fermentative hydrogen production with Klebsiella pneumoniae ECU-15 isolated from anaerobic sewage sludge," *Int. J. Hydrogen Energy*, vol. 35, pp. 71-80, 2010.
[21] M. T. Madigan, J. M. Martinko, D. A. Stahl, and D. P. Clark, *Brock Biology of Microorganisms*, 13th ed. Pearson Education Inc., 2012.
[22] Z. He, S. D. Minteer, and L. T. Angenent, "Electricity generation from artificial wastewater using an upflow microbial fuel cell," *Environ. Sci. Technol.*, vol. 39, no. 14, pp. 5262-5267, 2005.