# Performances of Microbial Fuel Cells Treating Organic Wastewater at Various COD: sulfate ratio

Witchayut Niyom, Decharthorn Komolyothin, and Benjaporn Boonchayaanant Suwannasilp

***Abstract***—This study investigates the effects of COD: $SO_4^{2-}$ ratio on two-compartment single-chamber air-breathing microbial fuel cells (MFCs) under continuous mode of operation. Glucose was added into synthetic wastewater at the approximate concentration of 3,000 mgCOD/L, whereas $SO_4^{2-}$ concentration of 3,000, 1,000, and 500 mg$SO_4^{2-}$/L was added into MFC1, MFC3, and MFC6, respectively, corresponding to the COD: $SO_4^{2-}$ ratio of 1, 3, and 6, respectively. Initial sludge was inoculated in the first compartment of each MFC with hydraulic retention time (HRT) of 1 day. The electricity equipment was installed in the second compartment of MFC after the system reaching steady state. Maximum COD removal efficiency of 47.66% was observed in the first compartment of the MFC operated at the COD:$SO_4^{2-}$ ratio of 6. On the other hand, the MFC operated at COD: $SO_4^{2-}$ ratio of 3 was the most effective in sulfate removal with the sulfate removal efficiency of 93.25% in the first compartment. After installing electricity equipment in the second compartment, the maximum power density of 9.33 mW/m$^2$ was achieved in MFC1 after one day of operation. The voltage across 1,000 ohm external resistance decreased over time during the first three days of operation then remained rather constant. The main mechanism of electricity generation in MFC3 and MFC6 was abiotic sulfide oxidation. On the other hand, both microbial processes and abiotic sulfide oxidation appeared to be involved in electricity generation in MFC1.

***Keywords***—Microbial fuel cell, Sulfate, COD:$SO_4^{2-}$ ratio, Sulfide.

## I. Introduction

SEVERAL types of industries have generated organic wastewater containing high-sulfate, for example, paper mill industry, rubber industry, pharmaceutical industry, mining industry, and tannery industry [1]. Treatment of sulfate-rich wastewater with biological anaerobic process generates hydrogen sulfide as a final product via sulfate-reducing bacteria (SRB), resulting in many adverse effects such as sulfide toxicity, bad odor, corrosion in concrete pipes, and decreases in quantity and quality of biogas produced from anaerobic processes. Microbial fuel cells (MFCs) are capable of treating organic wastewater containing sulfate should therefore be developed as an alternative for the treatment and energy recovery from this type of wastewater.

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MFCs have been developed to treat wastewater simultaneously with electricity generation. Several studies have investigated the treatment of sulfide using microbial fuel cells [2-5]. The results suggest that sulfide can be oxidized at the anode by abiotic sulfide oxidation and/or microbial mediated sulfide oxidation resulting in electricity production in MFCs. In addition, MFCs have been used to treat organic wastewater containing sulfate with simultaneous electricity generation [2,3,6]. The results show that the MFCs can remove sulfide by the oxidation process in the anodic chambers. Previous research mostly focused on sulfide removal and sulfate removal using MFCs in a batch mode of operation [2,4-5,7-10]. However, studies on sulfate removal using MFCs with continuous mode of operation are still lacking. The continuous mode of operation should be studied to represent actual situation in the wastewater treatment process.

COD:$SO_4^{2-}$ ratio is considered to be one of the key factors affecting MFC performance. COD:$SO_4^{2-}$ ratio has been shown to have an influence on microbial activities in the MFCs [11]. Treatment and electricity generation mechanisms in MFCs are, therefore, hypothesized to be depended on the COD:$SO_4^{2-}$ ratio.

In this study, two-compartment single-chamber air-breathing MFCs were used to investigate the treatment of organic wastewater with different COD:$SO_4^{2-}$ ratio, including the treatment and electricity generation mechanisms at continuous mode of operation.

## II. Materials and Methods

### *A. Two-compartment single-chamber air-breathing MFC*

Three MFCs (Fig.1), made of acrylics, with the dimensions of 10 cm x 20.5 cm x 22 cm (width x length x height) were separated into two compartments by three baffles. The first and the second compartment of each MFC have an active volume of 2.025 L (10 cm x 13.5 cm x 15 cm) and 0.63 L (10 cm x 7 cm x 9 cm), respectively. A 5 cm x 5 cm activated carbon cloth (Zorflex Knit FM50K, Calgon Carbon Corporation, USA) was used as an anode. A 5 cm × 5 cm carbon cloth (30% wt PTFE wet-proofed and loaded with Pt 0.5 mg/cm$^2$, Fuel Cell Store, USA) was hot-pressed with a proton exchange membrane (Nafion N117, Fuel Cell Store, USA). Silver mesh was attached to the cathode and connected with titanium wire to improve the electron transfer. The MFCs were operated with the distance of 1 cm between the anode and the cathode.

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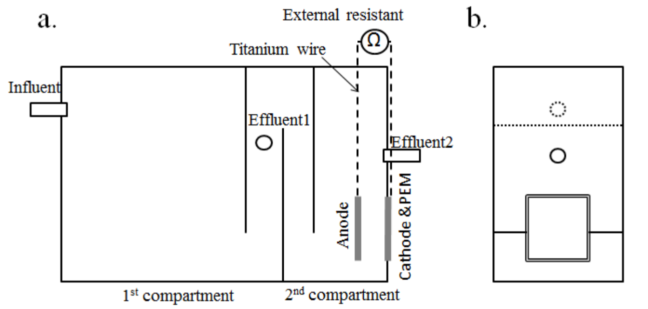


Fig.1 Schematic diagram of two-compartment single-chamber air-breathing MFC (a.) front view, (b.) side view.

### *B. Synthetic wastewater and Microbial inoculation*

Synthetic wastewater with three different COD: sulfate ratio (1, 3, and 6) were fed into three MFCs, MFC1, MFC3, MFC6, respectively, to enrich microbial community in each reactor. The synthetic wastewater was continuously fed into the first compartment of the MFC at the flow rate of 0.084 L/hr with the hydraulic retention time (HRT) of 1 day. Glucose with COD concentration of approximately 3,000 mg/L was fed into all MFCs, whereas sulfate concentration of 3,000, 1,000, and 500 mg/L were fed continuously into MFC1, MFC3, and MFC6, respectively. Macronutrients in the synthetic wastewater consist 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 $FeSO_4.7H_2O$ 10 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.

### *C. MFC operation and Electrical Measurement*

The electricity equipment consisting of anodes, proton exchange membranes, cathodes, titanium wire, and external resistances were installed in the second compartments of the MFCs after the enrichment of microbial communities in the first compartments. 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 compartment was mixed using a magnetic stirrer to avoid death zone and short circuit. In this study, the external resistance was set to 1,000 ohm. The voltages across the electrodes were monitored over time using a multimeter (Fluke 115, Fluke Corporation). Current density and power density were calculated using equation I = (V x A)/R and P = (I x V)/A, respectively, where V = voltage across electrode, R = external resistance, and A = projected cross-section area of the electrode (A = 25 $m^2$). The polarization curves and power density curves were constructed on the 1st, 2nd , and 4th day of operation. Eq.1 was used to fit to the polarization curve to describe the ohmic loss, of the MFCs. It should be noted that activation loss and mass transfer loss were not observed in all polarization curves.

$$V = V_{ocv} - jR; \qquad (1)$$

where $V_{OCV}$ = open circuit voltage (V); R = internal resistance (Ohm-$m^2$); j = current density (A/$m^2$).

### *D. Analyses*

Samples from the influent and the effluent of the first and the second compartment of the MFCs were collected to analyze COD, sulfate, pH, and oxidation-reduction potential (ORP) over time. The COD and sulfate were analyzed using the close reflux method and the turbidimetric method, respectively [12]. A pH meter and an ORP probe were used to measure pH and ORP, respectively.

## III. RESULTS AND DISCUSSIONS

### *A. Effects of COD:$SO_4^{2-}$ ratio on COD and sulfate removal efficiencies*

To investigate the effects of COD:$SO_4^{2-}$ ratio on COD and sulfate removal efficiencies in the first compartments of the MFCs, synthetic wastewater with the COD:$SO_4^{2-}$ ratio of 1, 3, and 6 was continuously fed into MFC1, MFC3, and MFC6, respectively. The average pH in each MFCs were 7.26 ± 0.2, 7.04 ± 0.19, and 6.99 ± 0.18 for MFC1, MFC3, and MFC6, respectively, which was suitable for anaerobic microorganisms in the systems. The ORP values were in the range of -320 to -370 for all MFCs. For MFC1 (Fig.1a), the COD concentration in the effluent of the first compartment was rather constant at 1,900 ± 300 mg/L, which was equivalent to the COD removal efficiency of 39.55 ± 9.77%. On the other hand, the average concentration of sulfate in the effluent was 2,490 ± 280 mg/L. Sulfate removal gradually increased over time, which might be due to a shift in microbial community in the system toward SRB. Sulfate was removed by SRB and generated sulfide which can cause inhibition to microorganisms.

TABLE I
CONCENTRATIONS OF COD, $SO_4^{2-}$, PH, AND ORP IN COMPARTMENT1 AND COMPARTMENT2

| Parameter | MFC1 | MFC3 | MFC6 |
|---|---|---|---|
| | Compartment 1 (Compartment2) | Compartment 1 (Compartment2) | Compartment 1 (Compartment2) |
| Influent COD (mg/L) | 3,130 ± 195 (1,831 ± 172) | 3,120 ± 250 (1,140 ± 169) | 3,150 ± 225 (1,483 ± 76.5) |
| Effluent COD (mg/L) | 1,900 ± 300 (1,762 ± 165) | 1,715 ±260 (1,087 ± 113) | 1,640 ± 300 (1,424 ± 104) |
| COD removal | 39.55 ± 9.77 % (3.64 ± 4.58 %) | 41.26 ± 7.94 % (0.04 ± 0.08 %) | 47.66 ± 9.08 % (3.80 ± 8.1 %) |
| Influent $SO_4^{2-}$ (mg/L) | 3,200 ± 125 (2,012 ± 144) | 1,090 ± 25 (61.62 ± 91.57) | 537 ± 38 (1.1 ± 2.16) |
| Effluent $SO_4^{2-}$ (mg/L) | 2490 ± 280 (1,912 ± 95) | 190 ± 178 (116.87 ± 160) | 22.79 ± 8.20 (N.D.) |
| $SO_4^{2-}$ removal | 29.45 ± 7.76% (4.83 ± 3.39%) | 93.24 ± 24.29% (N.A.) | 79.64 ± 19.16% (N.A.) |
| pH | 7.26 ± 0.20 | 7.04 ± 0.19 | 6.99 ± 0.18 |
| ORP | 320 – 380 | 320 – 380 | 320 – 380 |

Note: N.D. = Not detectable, N.A. = Not applicable

For MFC3 (Fig.2b), the average COD and sulfate concentration in the effluent of the first compartment over 55 days of operation were 1,715 ± 260 mgCOD/L and 190 ± 178 $mgSO_4^{2-}/L$, respectively. Sulfate concentrations in the initial stage (5 days) were excluded from the calculation to avoid the effects of remaining sulfate in the initial sludge. After 35 days of the operation, the system removed sulfate effectively (70 – 99%).

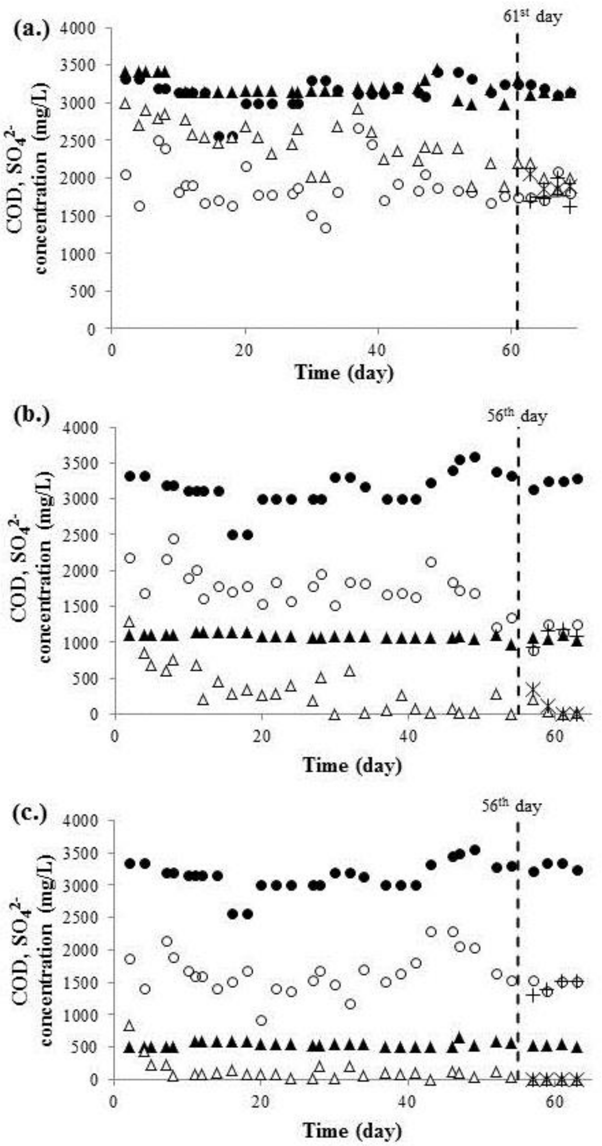


Fig. 2 COD and sulfate concentrations of influent and effluent of (a.) MFC1, (b.) MFC3, and (c.) MFC6 in the first and the second compartment; ● Influent COD, ○ Effluent COD of the first compartment, + Effluent COD of the second compartment, ▲ Influent sulfate, Δ Effluent sulfate of the first compartment, and ✳ Effluent sulfate of the second compartment.

For MFC6 (Fig.2c), the average COD concentration of 1,600 ± 300 mgCOD/L was observed in the effluent of the first compartment. On the other hand, effluent sulfate concentration was approximately 100 $mgSO_4^{2-}/L$ after 10 days of operation. The average pH in the system was 6.99 ± 0.18. The COD removal efficiency was the highest in MFC6 (average of 47.66%) compared to the other MFCs.

The sulfate removal of 697, 900, and 511 $mgSO_4^{2-}/L$ were observed in MFC1, MFC3, and MFC6, respectively. From the results, $COD:SO_4^{2-}$ of 3 was the most effective to remove sulfate in wastewater. Total sulfide production from each MFC can be estimated from sulfate removal with the assumption that all sulfate removed was converted into total sulfide; therefore, sulfate 96 $mgSO_4^{2-}/L$ is equivalent to $32mgS^{2-}/L$. As a result, the total sulfide productions were 232, 300, and 170 $mgS^{2-}/L$ for MFC1, MFC3, and MFC6, respectively. A previous study has reported that 50% inhibition of SRB was found when aqueous $H_2S$ concentration was 250 $mgH_2S/L$, and 50% inhibition of other anaerobic bacteria was also found when $H_2S$ concentration in the range of 50 – 130 $mgH_2S/L$ [13]

### *B. Effects of $COD:SO_4^{2-}$ ratio on MFC performance*

Both open circuit voltages (OCVs) and the voltages across the electrodes at 1,000 ohm external resistance of all MFCs decreased over time (Fig.3). The OCVs and the voltages across 1,000 ohm external resistance of all MFCs were initially at maximum values right after the installation of anodes and cathodes. On the first day of operation, the OCV values of 635, 475, and 460 mV were observed for MFC1, MFC3, and MFC6, respectively, which led to the voltages across 1,000 ohm external resistance of 300, 110, and 183 mV for MFC1, MFC3, and MFC6, respectively. For all MFCs, the OCVs and voltages decreased during the first three days of operation, after that the values became rather constant.

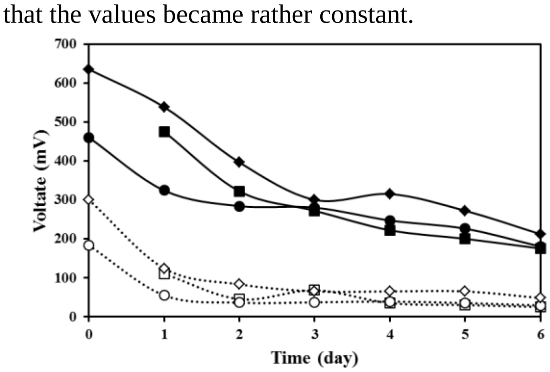


Fig. 3 The OCVs and voltages across the electrodes when operated at 1,000 ohm external resistance; ◆OCV of MFC1, ■ OCV of MFC3, ● OCV of MFC6, ◇ voltage of MFC1, □ voltage of MFC3, ○ voltage of MFC6.

In addition, pH is one of the important factors that can affect the solubility of sulfide in wastewater. Since the $pK_a1$ of hydrogen sulfide is 7.04 [14], the predominant species of sulfide would be $HS^-$, not $H_2S$, at high pH, resulting in higher solubility of sulfide in MFC1 (pH =7.26) than in the other MFCs (7.03 and 6.99 for MFC3 and MFC6, respectively). Sulfide concentrations might affect the power generation in MFCs from abiotic and/or biotic reactions. The percentages of $[HS^-]$ from total sulfide can be roughly estimated from Eq.2, which were 62.4 %, 50 %, and 47.1 % for MFC1, MFC3, and MFC6, respectively.

$$H_2S \longleftrightarrow HS^- + H^+ \qquad pK_a1 = 7.04 \qquad (2)$$

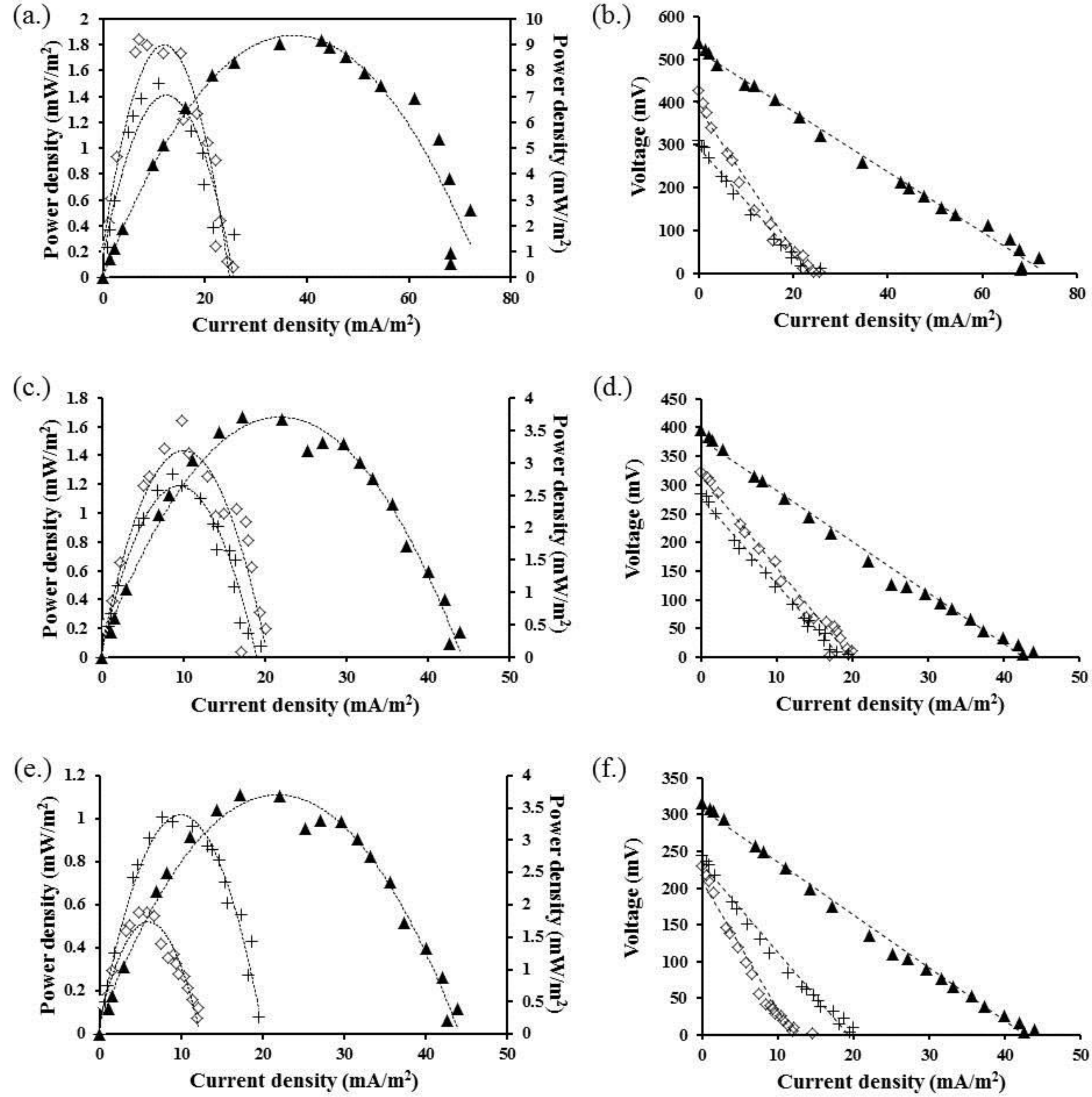


Fig.4. Polarization curves of (a.) day 1, (c.) day 2, and (e.) day 4 and power density curves of (b.) day 1, (d.) day 2, and (f.) day 4. ▲MFC1, ◊ MFC3, and + MFC6.

For MFC3 and MFC6, there were no significant changes in COD and sulfate concentrations in the second compartments of the MFCs. The results suggest that the electricity generation in these MFCs were likely due to the electron transfer from sulfide via abiotic sulfide oxidation process to elemental sulfur. Immediate production of electricity right after the electrode installation was one evidence which suggested that the electricity in both MFC3 and MFC6 should be generated from sulfide, not microbial activities. The decrease in OCVs and the voltages across the electrodes might be caused by the decrease in sulfide concentrations in the second compartments.

On the other hand, MFC1 has higher voltages than the other MFCs probably due to 1) COD and sulfate were slightly removed in the second compartment, suggesting the presence of SRB and the production of sulfide, which could generate electricity in the system; and 2) pH in MFC1 was higher than those in the other MFCs, resulting in higher aqueous sulfide concentration in the MFC, which could contribute to more electricity generation. Therefore, in MFC1, there could be co-activities between microorganisms and abiotic sulfide oxidation that were involved in electricity generation.

TABLE II
ELECTRICITY PARAMETER ESTIMATED FROM THE POLARIZATION AND POWER DENSITY CURVES

| Parameter | MFC1 | | | MFC3 | | | MFC6 | | |
|---|---|---|---|---|---|---|---|---|---|
| | Day 1 | Day 2 | Day 4 | Day 1 | Day 2 | Day 4 | Day 1 | Day 2 | Day 4 |
| Open circuit voltage (OCV) | 538 | 396 | 315 | 475 | 322 | 222 | 324 | 284 | 247 |
| Internal resistance (Ohm-$m^2$) | 7.05 | 9 | 7.19 | 16.57 | 16.06 | 17.41 | 12.54 | 15.12 | 11.96 |
| Total internal resistance (Ohm) | 2,820 | 3,600 | 2,876 | 6,628 | 6,424 | 6,964 | 5,016 | 6,048 | 4,784 |
| Maximum power density ($mW/m^2$) | 9.33 | 3.39 | 3.7 | 1.79 | 1.43 | 1.02 | 1.41 | 1.19 | 0.52 |
| The best external resistant | 2,000 | 1,800 | 1,800 | 5,000 | 6,800 | 6,800 | 3,000 | 6,800 | 6,800 |

The polarization curves (I-V curves) and power density curves were constructed on day 1, 2, and 4. Table 2 shows the estimated parameters from the polarization and power density curves. The maximum power densities on the first day of operation were 9.33, 1.79, and 1.41 $mW/m^2$ for MFC1, MFC3, and MFC6, respectively, whereas the operating power (at external resistance = 1,000 ohm) were 7.40 $mW/m^2$ for MFC1, 1.04 $mW/m^2$ for MFC3, and 0.96 $mW/m^2$ for MFC6. It should be noted that MFC1 was operated near the maximum power density, which occurred at the external resistance of approximately 2,000 ohm. The operating condition of MFC1 was considered to be suitable. However, the external resistance that corresponded to maximum power density for MFC3 and MFC6 were approximately at 5,000 and 3,000 ohm, which were far from the operating condition, which was at 1,000 ohm external resistance. Therefore, higher external resistances would be more suitable for MFC3 and MFC6 if higher power density is required.

Since the activation loss and mass transfer loss were not found in all MFCs, ohmic loss (internal resistance) was the main loss in all MFCs in this study, which can be estimated using Eq.1. The internal resistance of each MFC estimated from the polarization curves are summarized in Table 2. The results show that the internal resistance of each MFC did not significantly change over 4 days of operation. However, the ohmic loss of MFC3 and MFC6 were two times greater than that of the MFC1, which could be one factor contributing to a higher power density in MFC1 than in the other MFCs.

## IV. CONCLUSION

COD:$SO_4^{2-}$ ratio is one of the important factors affecting the performance of COD removal, sulfate removal, and electricity generation in the two-compartment single-chamber air-breathing MFC. The maximum COD removal efficiency of 47.66 % was observed at the COD:$SO_4^{2-}$ ratio of 6, whereas the maximum sulfate removal of 900 mg$SO_4^{2-}$/L was observed at the COD:$SO_4^{2-}$ ratio of 3. In the case of electricity production, the MFC operated at COD:$SO_4^{2-}$ ratio of 1 generated the highest power density compared to the other MFCs.

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