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Microbial fuel cells equipped with an iron-plated carbon-felt anode and *Shewanella oneidensis* MR-1 with corn steep liquor as a fuel

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A single chamber type microbial fuel cell (MFC) with 100 mL of chamber volume and 50 cm^2 of air-cathode was developed in this study wherein a developed iron-plated carbon-felt anode and *Shewanella oneidensis* MR-1 were used. The performance of the iron-plated carbon-felt anode and the possibility of corn steep liquor (CSL) as a fuel, which was the byproduct of corn wet milling and contained lactic acid, was investigated here. MFCs equipped with iron-plated or non-plated carbon-felt anodes exhibited maximum current densities of 443 or 302 mA/m^2 using 10 g/L of reagent-grade lactic acid, respectively. In addition, using centrifuged CSL without insoluble ingredients or non-centrifuged CSL as a fuel, the maximum current densities of the MFCs with iron-plated carbon-felt anode were 321 or 158 mA/m^2, respectively. This report demonstrated the effect of iron-plated carbon-felt anode for electricity generation of MFC using *S. oneidensis* MR-1 and the performance of CSL as a fuel.

[**Key words:** Corn steep liquor; *Shewanella oneidensis* MR-1; Microbial fuel cell; Lactic acid; Iron-plated carbon-felt anode]

Mankind is facing serious energy issue, and technologies with high energy conversion efficiency are needed. Microbial fuel cell (MFC) that converts biomass energy into electricity by microbial metabolism is one of the important technologies for sustainable society. *Shewanella oneidensis* MR-1 has excellent characteristics for MFCs (1), because the strain can be used with various compounds such as manganese (IV) oxide cobalt and uranium as terminal electron acceptors. In particular, the strain can utilize Fe_2O_3 as an electron acceptor, which is insoluble in water, indicating a possibility that this microorganism can transfer electrons to solid electrodes (2,3). Therefore, it is important to improve the anode for high performance MFCs using *S. oneidensis* MR-1, particularly by anode manipulation (4). MFCs using reticulated vitreous carbon (5) and solid graphite (6) as anode materials with *S. oneidensis* have been reported. An MFC with a carbon-felt electrode interposed between iron nets has been shown to generate a power density that is 1.5 times higher than that without iron nets (7). Another study demonstrated that the addition of Fe_3O_4 to activated carbon with a stainless steel mesh enhanced the performance and capacitance of the anode (8).

Although the development of carbon-felt coated with Fe_3O_4 is important for improving the current density of MFCs, few coating techniques have been developed, e.g., chemical vapor deposition and thermal deposition (9,10), which are expensive and require large-scale, complex apparatus. In a previous report, we demonstrated the various conditions of applying iron plating to carbon-felt anodes by using $FeSO_4 7H_2O$. X-ray photoelectron spectroscopy and X-ray diffraction analyses confirmed that the surfaces of the fibers of felt were covered with Fe_2O_3; the iron-plated carbon-fiber surface area was found to be very large due to the microcrystalline of Fe_2O_3 on the basis of observations made by scanning electron microscopy (SEM). Moreover, the iron-plated carbon-felt anodes were found to facilitate the formation of a biofilm of *S. oneidensis* MR-1 by measuring colony-forming units and using crystal violet staining (11). However, the effectiveness of these iron-plated carbon-felt anodes for electricity generation in MFCs has yet to be characterized.

Corn steep liquor (CSL) is a potential low-cost fuel for MFCs using *S. oneidensis* MR-1. It is a byproduct of corn starch and contains large concentrations of lactic acid because wild-type bacteria produce lactic acid during the wet milling process (12,13). We further show the potential for using CSL as a fuel in MFCs with *S. oneidensis* MR-1.

MATERIALS AND METHODS

Strain, medium, and fuel solutions *S. oneidensis* MR-1 (ATCC 700550) was used for this study. Luria–Bertani (LB) medium was composed of 1 g of Tryptone (Nacalai Tesque, Kyoto, Japan), 0.5 g of Yeast extract (Nacalai Tesque), and 1 g of NaCl, dissolved in 100 mL of distilled water. LB medium was used for making biofilm of *S. oneidensis*. While fuel solutions for electric generation contained 12.4 g/L of sodium lactate (70% aqueous solution, Wako Pure Chemical Industries, Ltd., Osaka, Japan) in 100 mM phosphate buffer (pH 7.0). This concentration of sodium lactate fuels, which using reagent grade lactic acid, was equivalent to 10 g/L of lactic acid fuel solution (R-LA). Another feed solution was prepared with CSL (Sigma–Aldrich, MO, USA). According to specification sheet, CSL contains 40–60% unsolvable components. Therefore, CSL was centrifuged (MX-301, Tomy Digital Biology Co., Tokyo, Japan) at 9100 × *g* for 40 min. The supernatant was centrifuged again at 9100 × *g* for 10 min and was adjusted to pH 7.0 with 10 N NaOH. Then the solution was added to 100 mM phosphate buffer pH 7.0 and used

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as the fuel solution (C-CSL). In this study non-centrifuged CSL was used for fuel (N-CSL) as same preparation of C-CSL. Both the concentration of lactic acid in C-CSL and N-CSL were to 10 g/L.

MFC configuration The MFC chamber, current and voltage measurement device methods in this study are same as we have previously reported (14). The following are the points: The MFC anode was composed of carbon-felt (LFP-210, Osaka Gas Chemicals Co., Osaka, Japan), and the air-cathode. The volume of the chamber was 100 mL and carbon-felt occupies half of chamber. The effective area of air-cathode was 50 cm^2. The air-cathode had three layers, namely a catalyst layer consisting of Pt-supported carbon (IFPC40-III, Ishifuku Metal Industry Co., Tokyo, Japan) with perfluorinated resin (Nafion 510211, Sigma–Aldrich), a carbon-paper layer (TGP-120, Toray Co., Tokyo, Japan), and a polytetrafluoroethylene layer (PTFE, 60% dispersion, 31-JR, Du Pont–Mitsui Fluorochemicals Co., Tokyo, Japan).

Iron-plating In previous report, we reported the methods for iron-plating for anode of MFC (11). The following are the points: A 4 g of $FeSO_4 7H_2O$ was dissolved in 400 mL of distilled water and used as electrolyze of electroplating method. The carbon-felt immersed in the beaker and connected to negative and positive side of power supply (SN-5B, Kenis Ltd., Osaka, Japan). Electroplates was carried out under current at 0.2 A (22 V) for 10 min. After electroplating, the sample was washing with pure water (approximately 50 mL) till the pH became neutral promptly, because in the process of electroplating, the H_2SO_4 is produced in the solution and soaked in the carbon-felt. In this study, non-plated carbon-felt anode was also used as a reference.

Biofilm formation Carbon-felts were autoclaved at 121 °C for 20 min then dried at room temperature. After positioning the sterilized iron- and non-plated carbon-felt anode in the MFC chamber, the chamber was filled with 100 mL of LB medium. Pre-cultured *S. oneidensis* MR-1 was inoculated in the LB medium at $OD_{600} = 0.2$, and incubated for 5 days at 30 °C for making biofilm on iron-plated carbon-felt anode.

MFC operation After biofilm formation on iron- and non-plated carbon-felt anode, the culture medium for making biofilm was replaced with the 70 mL of R-LA. The MFC was incubated at 30 °C, and current measurements were started.

Measurements of electric generation The electric currents generated by the MFC were monitored using a digital multimeter (KEW 1062, Kyoritsu Electrical Instruments, Tokyo, Japan) and were automatically recorded by application software (model 8241, Kyoritsu Electrical Instruments). The accuracy of the meter for current measurement is ±0.2% of indicated values ±5 least-significant digits. Then it was directly connected between an anode and a cathode of MFC to measure the current. Polarization and power density curves were obtained using the rheostat at various external resistances (0–20 kΩ) when the maximum current generated and the potential were recorded after it had stabilized approximately 5 min. Maximum power density and ohmic resistance of MFCs were acquired by polarization curves (14).

Analysis of lactic acid The consumption rates of lactic acid were carried out using high-performance liquid chromatography (HPLC) equipped with a UV detector monitored at 210 nm (Prominence, Shimadzu Co., Kyoto, Japan) and SCR-102H column (Shimadzu). The elution solvent was 0.1 v/v% perchloric acid at a flow rate 0.9 mL/min. The type of column is an ion-exclusion chromatography column that detects the lactate as lactic acid (15). All the experiments were performed three times independently.

RESULTS AND DISCUSSION

Electricity generation during biofilm formation Fig. 1 shows the current generation by MFCs with iron- and non-plated carbon-felt anodes over 5 days of incubation with *S. oneidensis* MR-1. With the non-plated carbon-felt anode, the current density increased gradually and reached a maximum of 248 mA/m^2. However, the MFC with an iron-plated carbon-felt anode exhibited a maximum current density of 486 mA/m^2, which was approximately two times higher than that with the non-plated anode.

The polarization curves, power density, and ohmic resistance in the MFCs with iron- and non-plated carbon-felt anodes during biofilm formation are shown in Fig. 2. The maximum power densities from the MFCs with iron- and non-plated carbon-felt anodes were 65 and 30 mW/m^2, respectively (Fig. 2a). A polarization curve for MFC includes three characteristic regions located at different current ranges: The region of change transfer overpotentioanls at low currents; ohmic overpotentials (ohmic resistance) manifest at intermediate current; mass transport overpotentials at relatively high current densities. Ohmic resistance is caused by ionic resistances in the electrolyte, membrane, biofilm, and by electronic resistances in the electrodes, current collectors, interconnects, and the electronic components (16).

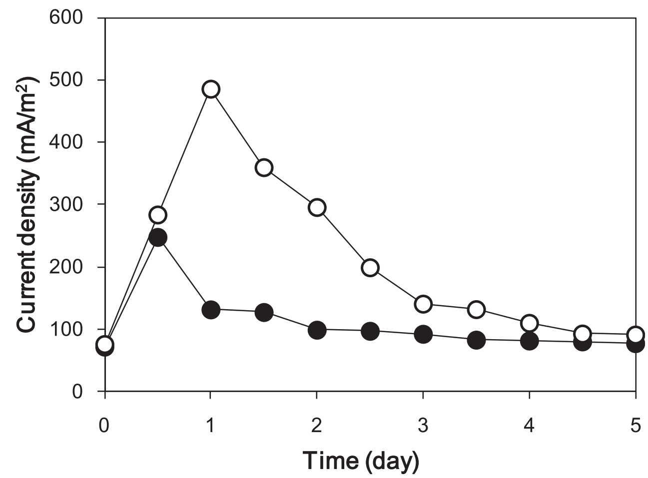

FIG. 1. Current generation during biofilm formation of *S. oneidensis* MR-1 with iron-plated carbon felt anode (open circles) and non-plated carbon felt anode (closed circles) during 5 days incubation at 30 °C in LB medium.

The ohmic resistance of MFC can be calculated from the linear portion of the slope of the straight-line approximation of the voltage versus current density of the MFC as an evaluation parameter. The ohmic resistance of the MFC was 101 Ω with the iron-plated carbon-felt anode and 124 Ω with the non-plated anodes (Fig. 2b). These results indicate that *S. oneidensis* MR-1 generated electricity while producing a biofilm in an LB medium and that more electricity was generated using the iron-plated carbon-felt anode than the non-plated carbon-felt anode. The results of three times experiments are summarized in Table 1.

MFC operation When R-LA was used as a new energy source for the MFC, the maximum current density in the MFC was 514 mA/m^2 with the iron-plated carbon-felt anode but only 333 mA/m^2 with the non-plated carbon-felt anode (Fig. 3a). Over 5 days of incubation with R-LA, the pH of the fuel solution increased from 6.9 to 8.5 in the MFC with the iron-plated carbon-felt anode and from 7.0 to 8.2 in the MFC with the non-plated anode as the lactic acid was consumed, leaving sodium hydrate in the solution. This observed increase in pH decreased cell activity because of the decrease in the transfer of H^+ to the cathode for reacting with O_2 and e^-. Therefore, electricity generation gradually ceased.

The power output and polarization curves with R-LA are shown in Fig. 4. The maximum power densities obtained with the iron- and non-plated carbon-felt anodes were 175 and 58 mW/m^2, respectively (Fig. 4a). On the basis of the linear portions of the slopes (Fig. 4b), the ohmic resistance of the MFC was 39 Ω with the iron-plated carbon-felt anode but 190 Ω with the non-plated carbon-felt anode.

CSL is a cloudy liquid that contains insoluble components. The production of corn starch via wet milling involves a preliminary soaking or steeping of the whole grains in a dilute sulfurous acid solution. During the steeping period and other phases of the process, a process of active natural fermentation occurs that is essentially lactic in nature (17). According to HPLC analysis, the stock CSL contains 140 g/L of lactic acid. After 11.8 wt% of the precipitate was removed by centrifugation, a brownish-colored solution was obtained. By operating the MFC with an iron-plated carbon-felt anode in the same manner as the R-LA, the maximum current density was 189 mA/m^2 by using N-CSL and reached 236 mA/m^2 by using C-CSL (Fig. 5a). The pH of the N-CSL decreased from 7.0 to 5.4 after 5 days

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