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Microbial fuel cells using *Cellulomonas* spp. with cellulose as fuel

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***Cellulomonas fimi*, *Cellulomonas biazotea*, and *Cellulomonas flavigena* are cellulose-degrading microorganisms chosen to compare the degradation of cellulose. *C. fimi* degraded 2.5 g/L of cellulose within 4 days, which was the highest quantity among the three microorganisms. The electric current generation by the microbial fuel cell (MFC) using the cellulose-containing medium with *C. fimi* was measured over 7 days. The medium in the MFC was sampled every 24 h to quantify the degradation of cellulose, and the results showed that the electric current increased with the degradation of cellulose. The maximum electric power generated by the MFC was 38.7 mW/m^2, and this numeric value was 63% of the electric power generated by an MFC with *Shewanella oneidensis* MR-1, a well-known current-generating microorganism. Our results showed that *C. fimi* was an excellent candidate to produce the electric current from cellulose via MFCs.**

[**Key words:** Air-cathode; Carbon felt; Cellulose; *Cellulomonas*; Microbial fuel cell]

Cellulose is the main component of cell walls and fibers of plant cells. It is the most abundant carbohydrate source and comprises glucose polymers with a Gibbs energy of approximately 2890 kJ/mol. Electrical energy is the foundation of our everyday lives, and thus, methods involving efficient conversion of energy from various sources to electrical energy have grown in importance. For example, in the conversion of thermal energy into electrical energy, the efficiency of the conversion process is limited by the Carnot cycle and the efficiency is determined by the ratio of temperature of burnt fuels to atmospheric temperature. Given this limitation, thermal power plants cannot convert more than 40% of chemical energy from the burnt materials to electrical energy. A few recent studies attempted to overcome the limitation of this energy conversion. Studies on fuel cells reported the production of electrical energy using enzyme reactions on cellulose (1–3). However, several processes are required for the production and purification of enzymes; moreover, using enzymes over long periods is difficult because of denaturation. Hence, microbial fuel cells (MFCs) utilize the self-propagation abilities of microorganisms instead of using enzymes (4). In an MFC, respiration activities of microorganisms release electrons and these electrons are transferred to the anode, which is used as an electron acceptor. This technique is extensively used to transfer microbial electrons to an anode for electric current production in MFCs under anaerobic conditions with the reduction of oxygen at the cathode (5). Ren et al. (6) analyzed electricity generation and the microbial ecology of cellulose-fed MFCs of a two-chamber type by using a defined co-culture of *Clostridium cellulolyticum* and *Geobacter sulfurreducens*. However, *Clostridium* spp. is an anaerobic bacterium, and some of its strains exhibit pathogenicity. Rezaei et al. (7) reported on a sediment-based system for chitin and cellulose as substrate. The sediment used in their study was an anaerobic sediment obtained from the Delaware Bay, and it contained several types of microorganisms. Rismani-Yazdi et al. (8) examined the generation of electricity with rumen microorganisms as biocatalysts and cellulose as the electron donor in two-compartment MFCs. Ishii et al. (9) inoculated with rice paddy field soil and fed cellulose as a carbon and energy source for a two-chamber type MFC. Their results indicated that subculturing biofilms attached on anode electrodes enriched the electricity-generating microorganisms and resulted in phylotypes frequently detected by clone library analyses as being affiliated with *Clostridiales*, *Chloroflexi*, *Rhizobiales*, and *Methanobacterium*.

Cellulomonas spp. is a well-known microorganism that utilizes cellulose as a carbon source (10–15). Furthermore, its strains are not pathogenic. There is a good review about microbes used in MFCs (16). Beside this review, to the best of our knowledge, there are no studies on MFCs with *Cellulomonas* spp. that consume cellulose as fuel. Despite there being many studies regarding cellulose degradation with *Cellulomonas* spp., there is no information as to which species is suitable for medium condition of MFC. Therefore, in this study, three types of cellulose-degrading bacteria, namely, *Cellulomonas fimi*, *Cellulomonas biazotea*, and *Cellulomonas flavigena*, were selected and compared in terms of their respective microbial degradations of cellulose and development of MFCs.

MATERIALS AND METHODS

Chemicals A Cellulose powder (38 μm, Nacalai Tesque Inc., Kyoto, Japan), Yeast extract (Kyokuto Pharmaceutical Industrial Co., Tokyo, Japan), Tryptone (Nacalai Tesque), Anthraquinone-2,6-disulfonic acid disodium salt (AQDS; Combi-Blocks Inc., CA, USA) and neutral red (Sigma–Aldrich Co., MO, USA) were used in this study.

Bacterial strains and medium The strains used in this study were *C. fimi* NBRC 15513, *C. biazotea* NBRC 12680, *C. flavigena* NBRC 3775, *Shewanella oneidensis*

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MR-1 (ATCC 700550). The cellulose-containing medium was composed of 0.5 g of cellulose powder, 0.05 g of yeast extract, and 1.0 g of NaCl, dissolved in 100 mL of 0.1 M phosphate buffer (pH 6.8), and was then autoclaved (121°C, 20 min). Luria–Bertani (LB) medium was composed of 1 g of Tryptone, 0.5 g of yeast extract, and 1 g of NaCl, dissolved in 100 mL of distilled water, and was then autoclaved (121°C, 20 min).

Degradation test of cellulose Three species of *Cellulomonas* spp. were precultured in a LB medium and then centrifuged (1800 ×*g*, 5 min). The concentration of the cellulose-containing medium was adjusted to an optical density (OD_{600}) of 2.0. This solution was added to a Sakaguchi flask and was shaken for 4 days (30°C, 120 rpm). Sampling was performed every 24 h. Then, 1 mL of this sample was added to 9 mL of distilled water. A filter paper (5A, Toyo Roshi Kaisha, Ltd., Tokyo, Japan) was used to filter the resulting solution. The obtained residue was washed with 10 mL of distilled water and heated at 80°C for 24 h. Finally, the dry weight of the sample was measured.

The chamber structure of MFC The setup of the MFC used in this study was modified according to that in a previous study (17) and is shown in Fig. 1. The internal volume of the MFC was 100 mL, the MFC anode was composed of carbon felt (LFP-210, Osaka Gas Chemicals Co., Osaka, Japan), and the cathode was an air-cathode fabricated according to specifications used in an extant study (18). The air-cathode had three layers (Fig. 1), namely a catalyst layer consisting of Pt-supported carbon (IFPC40-III, Ishifuku Metal Industry Co., Tokyo, Japan) with Nafion (510211, Sigma–Aldrich, Tokyo, Japan), 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). The voltage and electric current generated by the MFC were measured using a digital multimeter (KEW 1062, Kyoritsu Electrical Instruments, Tokyo, Japan). Internal resistance for DC voltage measurement of this meter is 100 MΩ, and the accuracy of this meter for current measurement is ±0.2% of indicated values ±5 least-significant digits. Therefore in this study, internal resistance for current measurement was considered to be negligibly small, and for voltage, it was considered to be infinite.

Evaluation of the MFC chamber A method to evaluate the battery is estimated by using the internal resistance, since the reaction speed of the battery had limitations, and it is expressed as resistance. In the case of the MFC, the reaction speed was limited by the metabolism of the microorganism and the chamber structure consisting of components including an air-cathode and anode. Additionally, factors such as the volume and surface area of the air-cathode affect the performance of the MFC (19). The purpose of this study includes demonstrating the generation of electrical energy by *Cellulomonas* spp. using cellulose and evaluating the generated values. Therefore, in order to validate the performance of the MFC chamber used in this study, the internal resistance of the MFC was measured by using the same microorganism previously reported for the MFC, and the values of internal resistance were evaluated. That is, the performance of the MFC chamber used in this study was evaluated using *S. oneidensis* MR-1 by measuring the electric current and voltage curves.

The electric circuit was designed to estimate the internal resistance of the MFC (Fig. 2). In the figure, R_A, R_V, R_C, and R_{in} denote the internal resistance values of the ammeter, voltmeter, rheostat, and MFC, respectively. The "*I*" denotes the current value in the line and "*E*" denotes electromotive force. In the apparatus, R_A was

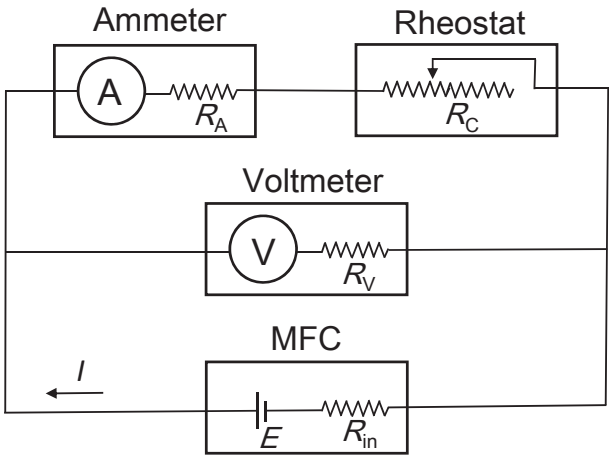

FIG. 2. The electric circuit to estimate an internal resistance of MFC. The R_A, R_V, R_C, and R_{in} stand for internal resistance values of ammeter, voltmeter, rheostat, and MFC, respectively. The *I* represents current value in the line and *E* electromotive force. In our apparatus, R_A is negligibly small and R_V is considered to be infinite.

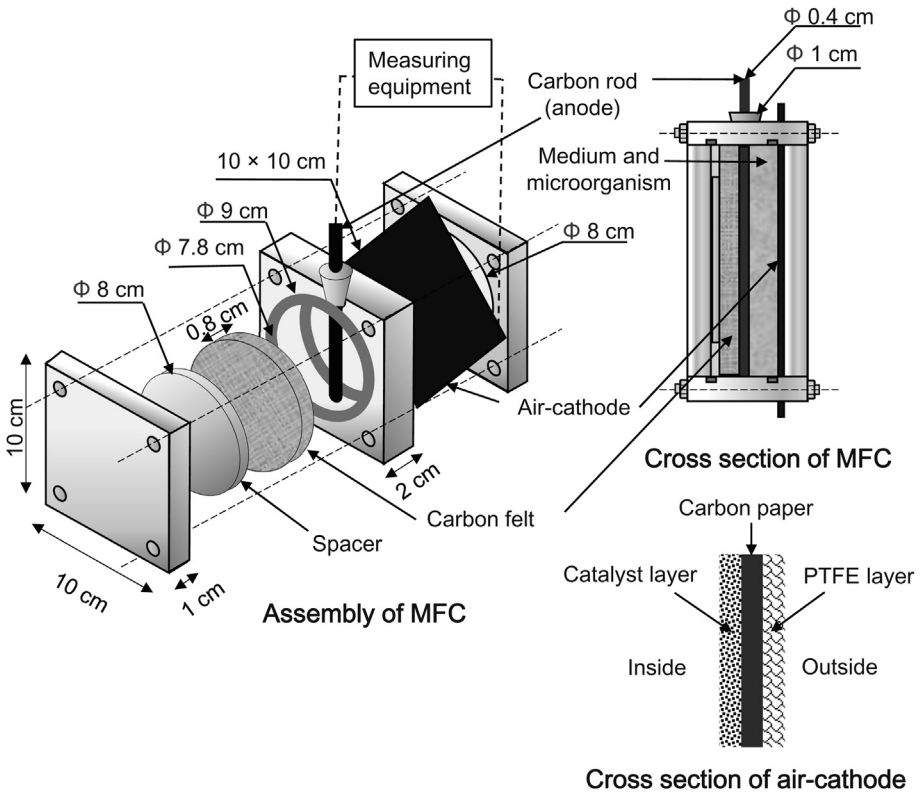

FIG. 1. Assembly and cross section of MFC and air-cathode. The vessel parts are made of polycarbonate. Spacer adjusts the volume of vessel (100 mL). The carbon rod conducted to carbon felt and did not to air-cathode.

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