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Evaluation of microbial fuel cells for electricity generation from oil-contaminated wastewater

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Large quantities of oils and fats are discharged into wastewater from food industries. We evaluated the possibility of using microbial fuel cells (MFCs) for the generation of electricity from food-industry wastewater containing vegetable oils. Single-chamber MFCs were supplied with artificial wastewater containing soybean oil, and oil removal and electric output were examined under several different conditions. We found that MFC performance could be improved by supplementing wastewater with an emulsifier, inoculating MFCs with oil-contaminated soil, and coating the graphite-felt anodes with carbon nanotubes, resulting in a power output of more than 2 W m^{-2} (based on the projected area of the anode). Sequencing of polymerase chain reaction (PCR)-amplified 16S rRNA gene fragments detected abundant amount of *Burkholderiales* bacteria (known to include oil degraders) in the oil-contaminated soil and anode biofilm, whereas those affiliated with the genus *Geobacter* were only detected in the anode biofilm. These results suggest that MFCs can be used for energy recovery from food industry wastewater containing vegetable oils.

[**Key words:** Food industry; Vegetable oil; Air cathode; Anode coating; Phylogenetic analyses]

Large amounts of oils and fats are used in food industries. These include a range of vegetable oils and related organic compounds such as fats (triacylglycerol), fatty acids, glycerin, hydrocarbons, and waxes, and substantial amounts of these compounds are discharged as wastes. It has been reported that the amount of cooking-oil waste in Japan reaches up to 500,000 tons per year (1). These compounds are potentially valuable as fuels because they contain considerable amounts of energy, e.g., 38 kJ per gram of lipids, more than those contained in carbohydrates and proteins. In many cases, however, it is difficult to use waste oils as fuels because they are mixed with large amounts of water or are present in wastewater.

Microbial fuel cells (MFCs), which exploit living microbes as electrode catalysts, have recently attracted considerable attention as green energy devices for generating electricity from various organic and inorganic materials (2,3). Workers are interested in using MFCs for recovering energy from biomass waste and wastewater (4). In MFCs, microbes degrade pollutants using anodes in place of oxygen as respiratory electron acceptors, thereby enabling aeration-free wastewater treatment. In addition, microbes retain less energy during the generation of electricity than that during aerobic processes, meaning that the amount of sludge discharged during wastewater treatment can be markedly reduced. MFCs are therefore expected to have applications in energy- and cost-efficient wastewater treatment processes (5–8).

MFCs have been examined for electricity generation from various compounds, including organic acids (9), carbohydrates (10,11), and proteins (12), and extensive efforts have been made to develop technologies that boost power output from MFCs (13–15). Electricity generation from short-chain fatty acids has also been examined (16). To our knowledge, however, there has been no report that has successfully used vegetable oils as substrates for electricity generation in MFCs. The aims of the present study were to evaluate MFCs for electricity generation from vegetable oils (e.g., soybean oil) and gain insight into how microbes convert vegetable oils into electricity.

MATERIALS AND METHODS

MFC setups Single-chamber MFCs (18 mL capacity) equipped with anodes (2.6 cm^2 projected area in each cell) and air cathodes (7 cm^2 in each cell) were used. An anode was made of graphite felt (GF, cat no. GF-80-3F, Sohgoh Carbon, Yokohama, Japan), and in some experiments, graphite felt coated with carbon nanotubes (CNTs) (17) was used as an anode material. An air cathode was made of carbon cloth (cat no. TCC-3250, Toho Tenax, Tokyo, Japan) and was coated with four polytetrafluoroethylene layers on one side and platinum catalysts (0.1 mg platinum cm^{-2}; TEC10E20TPM, Tanaka Kikinzoku Kogyo, Tokyo, Japan) on the other side (13). A filter paper (cat. no. 1004-240, GE Healthcare, Tokyo, Japan) was sandwiched between the anode and cathode to prevent them from making contact. The anode and cathode were electrically connected through an external resistor, R_{ext} (termed closed-circuit MFC, CC-MFC), and voltages across the resistor were monitored using a data logger (HA-1510, Graphtec, Yokohama, Japan).

Artificial oil-contaminated wastewater An artificial oil-contaminated wastewater contained soybean oil (20 g L^{-1}), $(NH_4)_2SO_4$ (2.22 g L^{-1}), KH_2PO_4

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(6.30 g L^{-1}), Na_2HPO_4 (7.10 g L^{-1}), $MgSO_4 \cdot 7H_2O$ (0.11 g L^{-1}), $FeSO_4 \cdot 7H_2O$ (0.01 g L^{-1}), $CaCl_2 \cdot 2H_2O$ (0.01 g L^{-1}), $MnCl_2 \cdot 4H_2O$ (0.01 g L^{-1}), and Difco yeast extract (0.11 g L^{-1}) (pH 7.0). The chemical oxygen-demand (COD_{cr}) of the artificial wastewater was approximately 40 g L^{-1}. In some experiments, the artificial wastewater was supplemented with Tween 20 (as an emulsifier) at a concentration of 1 g L^{-1}. Before use, the artificial wastewater was treated with a homogenizer (HV-0A-1-1.5s, Izumi Food Machinery, Hyogo, Japan) and sterilized by autoclaving.

Operation of MFCs The microbial inocula used in the present study were non-contaminated garden soil (NC-soil); oil-contaminated soil sampled from an edible oil factory in Tokyo (OC-soil); and activated sludge sampled from the wastewater treatment facility of an edible oil factory (WO-sludge). An MFC reactor was filled with 16 mL of the artificial oil-contaminated wastewater, and after being bubbled with pure nitrogen gas, it was inoculated with one of these inocula (0.9 g, wet weight) suspended in sterilized water. Unless otherwise stated, R_{ext} was set at 10,000 Ω. The MFC reactors were placed in an incubator (maintained at 30°C), and the cell voltage was automatically monitored using the logger. For comparing anode microbial communities, some MFC reactors whose anode and cathode were not connected (open circuit, OC-MFC) were also operated.

Measurements of MFC parameters COD_{cr} was measured using a COD meter (DR 2800, Hach, Loveland, CO, USA) and a COD 0–1500 ppm range kit (Hach). MFC performance was evaluated according to the method described elsewhere (2). Current (I, A) was calculated using the equation $I = E/R$, where E (V) is cell voltage, and R (ohm) is resistance, whereas current density (J, A cm^{-2}) was calculated based on the projected area of the anode (2.6 cm^2). Coulombic efficiency (ε) was calculated by dividing the total charge (coulombs) transferred to the anode by the theoretical maximum charge (the total coulombs produced by complete substrate oxidation to carbon dioxide). Polarization and power-density curves were drawn as described previously (2) using a potentiostat (HSV-100, Hokuto Denko, Tokyo, Japan). An internal resistance (R_{int}) and maximum power density (P_{max}) were obtained from these curves.

Microbial community analyses DNA was extracted from the OC-soil (0.5 g), and GF pieces in CC-MFC and OC-MFC (5 mm × 5 mm in total size) using a FAST DNA Spin Kit for Soil (Q-Bio, Carlsbad, CA, USA) according to the manufacturer's instructions. PCR amplification of 16S rRNA gene fragments was performed using U27f (5′-AGAGTTTGATCMTGGCTCAG-3′, nucleotide position 27 to 46 in the *Escherichia coli* sequence) as a forward primer and U1492r (5′-GGYTACCTTGTTACGACTT-3′, nucleotide position 1492 to 1510) as a reverse primer (18). A PCR buffer (50 μL) contained 1.25 U of DNA polymerase (KOD dash, Toyobo, Tokyo), 0.2 mM dNTPs, 0.2 μM of each primer and an appropriate amount of template DNA. The amplification conditions were as follows: an initial step at 94°C for 3 min; 30 cycles consisting of 94°C for 30 s, 50°C for 30 s, and 74°C for 30 s; and a final elongation step at 74°C for 3 min. Amplified fragments were purified with a QIAquick PCR Purification Kit (Qiagen, Tokyo, Japan), ligated into the pGEM-T vector (Promega, Tokyo, Japan), and cloned into competent cells (*E. coli* JM109, Takara, Shiga, Japan) as described elsewhere (18). Clones containing appropriate sizes of the insertion were selected by electrophoresis analysis, and their nucleotide sequences were determined as described previously (19). Nucleotide sequences obtained from clone libraries (OC-soil, CC-MFC, and OC-MFC) were aligned to each other using the MEGA program ver. 5.1 (20), and assigned to phylotypes (classified as a unique clone or group of clones with sequence similarity of >0.98). Database searches were conducted using the BLAST program (21) and the GenBank database.

The nucleotide sequences determined in the present study were deposited in the DDBJ, EMBL, and NCBI nucleotide sequence databases under accession numbers AB911123 to AB911139.

RESULTS

Effects of emulsifier As the initial step, we examined electricity generation in MFCs that were filled with the artificial oil-contaminated wastewater and inoculated with NC-soil. This soil sample was used, because previous studies have successfully used similar soils as inocula for MFCs treating organic substrates (8,15,19). However, the current density (J) did not increase during the 25-day experiment (Fig. 1). We believed that this was due to the low solubility of the oils in water and examined whether supplementation of the artificial wastewater with the emulsifier could increase electrical output from the MFCs (Fig. 1). We found that, in MFC supplemented with the emulsifier, J started to increase from day 10 and reached 6 μA cm^{-2} on day 20. This demonstrated the utility of an emulsifier for generating electricity from vegetable oils in an MFC.

Effects of inocula We also examined the effects of microbial inocula on electricity generation from vegetable oils (the emulsifier was not added). We operated the MFCs that were filled with the artificial oil-contaminated wastewater and inoculated them with either NC-soil, OC-soil, or WO-sludge. We believed that microbial sources that are acclimatized to oily materials would be beneficial to an MFC for treating recalcitrant oil components. We operated these MFCs for approximately 20 days and found that the MFC inoculated with OC-soil generated the highest J, followed by the MFC inoculated with WO-sludge (Fig. 2).

Effects of anode modification Previous studies have developed methods for modifying anode surfaces for enhancing the power output from MFCs (17,22,23). Among these, direct modification of the graphite anodes with CNTs is attractive, since a modified anode is stable for a long time even in the presence of anaerobic microbial communities (17). Furthermore, we considered that the hydrophobic surfaces of the CNTs could preferentially trap large amounts of oils from wastewater and promote the microbial attack. In the present study, we examined the utility of CNT-modified graphite felt (CNT-GF) as an anode material for MFCs treating vegetable oil, and we compared its power output with that of an MFC equipped with the non-modified GF anode. In this trial, according to the results of the aforementioned experiments, the artificial oil-contaminated wastewater was supplemented with the emulsifier, and OC-soil was used as the inoculum. We found that the electrical output from the MFC equipped with the CNT-GF anode was much greater than that from the MFC with the nonmodified GF anode (Fig. 3); this was clearly shown after R_{ext} was changed from 10,000 to 1000 Ω, and then further decreased to 100 Ω. At R_{ext} of 100 Ω, J of CNT-GF MFC reached 500 μA cm^{-2} (Fig. 3).

Polarization analyses were conducted for the oil-fueled MFC with CNT-GF (Fig. 4), and the performance data are summarized in

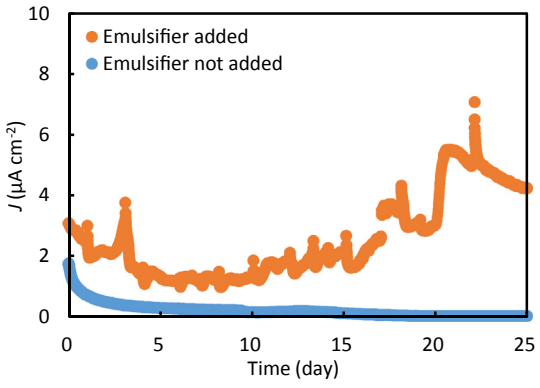

FIG. 1. Effects of the emulsifier on electricity generation in the oil-fueled MFCs.

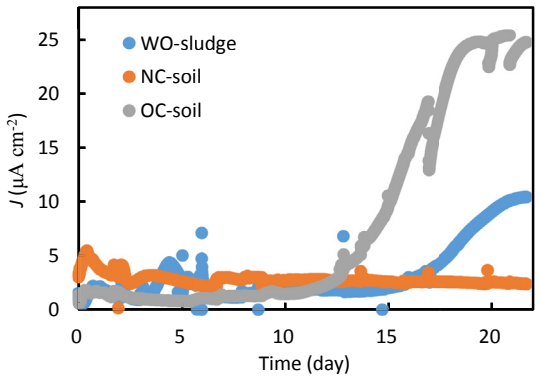

FIG. 2. Effects of microbial inocula on electricity generation in the oil-fueled MFCs.

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