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Conversion of activated-sludge reactors to microbial fuel cells for wastewater treatment coupled to electricity generation

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Wastewater can be treated in microbial fuel cells (MFCs) with the aid of microbes that oxidize organic compounds using anodes as electron acceptors. Previous studies have suggested the utility of cassette-electrode (CE) MFCs for wastewater treatment, in which rice paddy-field soil was used as the inoculum. The present study attempted to convert an activated-sludge (AS) reactor to CE-MFC and use aerobic sludge in the tank as the source of microbes. We used laboratory-scale (1 L in capacity) reactors that were initially operated in an AS mode to treat synthetic wastewater, containing starch, yeast extract, peptone, plant oil, and detergents. After the organics removal became stable, the aeration was terminated, and CEs were inserted to initiate an MFC-mode operation. It was demonstrated that the MFC-mode operation treated the wastewater at similar efficiencies to those observed in the AS-mode operation with COD-removal efficiencies of 75–80%, maximum power densities of 150–200 mW m^{-2} and Coulombic efficiencies of 20–30%. These values were similar to those of CE-MFC inoculated with the soil. Anode microbial communities were analyzed by pyrotag sequencing of 16S rRNA gene PCR amplicons. Comparative analyses revealed that anode communities enriched from the aerobic sludge were largely different from those from the soil, suggesting that similar reactor performances can be supported by different community structures. The study demonstrates that it is possible to construct wastewater-treatment MFCs by inserting CEs into water-treatment tanks.

[**Key words:** Electricity generation; Activated sludge; Microbial fuel cell; Pyrotag sequencing; Microbial community]

Activated-sludge (AS) processes exploit aerobic microbes to remove organic and inorganic pollutants from wastewater (1). Although these processes have been successfully and widely used for the treatment of domestic and industrial wastewater, engineers and administrators are concerned about their intense energy consumption (2) and high sludge-disposal costs (3). In particular, aeration and agitation need large energy inputs, sharing ~50% of the total energy used in these processes. Energy saving in wastewater treatment is one of the major subjects in current environmental engineering and administrations (4).

Microbial fuel cells (MFCs), which use living microbes as electrode catalysts, have recently attracted wide attention as green-energy processes that generate electricity from a variety of organic and inorganic compounds (5,6). In particular, MFCs are expected to be applied to the recovery of energy from biomass wastes and wastewater (7). In MFCs, pollutants are biodegraded with anodes (rather than oxygen) as electron acceptors for microbial respiration, enabling aeration-free wastewater treatment. In addition, as much as electricity is generated, the energy conserved by microbes is reduced, allowing us to consider that waste sludge can be reduced. MFCs are therefore expected as energy- and cost-saving options for wastewater treatment (8,9), whereas, for their practical application, further researches should be done to improve treatment efficiencies, develop reliable operational schemes, and reduce material and operational costs (10).

A recent study has demonstrated that cassette-electrode (CE) MFCs (CE-MFCs) are applicable to aeration-less wastewater treatment, in which water runs through CEs in a slalom fashion (11). A similar wastewater-treatment MFC system (termed submerged exchangeable MFC) has also been reported elsewhere (12). A CE is composed of a flat cathode box (i.e., a flat electrode with two sides) sandwiched between two separator membranes and graphite-felt anodes, and a CE-MFC reactor is assembled from a series of exchangeable CEs inserted into an anaerobic digester (13,14). Since the use of such a CE configuration allows the design of a modular MFC reactor to be flexible in terms of size, shape, and number of units, CE-MFC is considered to be suitable for a large-scale application that also has the advantage of easy maintenance.

It should also be a merit of the CE configuration that an MFC system can be constructed by just inserting a desired number of CEs into a water tank. This is particularly the case for wastewater-treatment plants, since there have already been a number of water tanks and basins used for water managements. Our idea is to convert activated-sludge tanks to MFC reactors by removing aeration facilities and inserting CEs instead, so that the energy needed for wastewater treatment can be reduced. The primary purpose of the present study was to examine this idea in laboratory-scale reactors. In addition, since previous studies used either paddy-field soil or anaerobic sludge as an inoculum for wastewater-treatment CE-MFCs (11,12), the present study also examined the utility of

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aerobic sludge in the AS reactor as the source of microbes for electricity generation in CE-MFC.

MATERIALS AND METHODS

Reactor configuration An AS reactor was composed of an aeration tank (approximately 1 L) and a settling tank (approximately 0.5 L), and settled sludge was returned to the aeration tank by gravity (Fig. 1A). The reactor had water inlet and outlet at upper parts, and it was set in a water bath (30°C).

CEs were constructed according to a previous report (11), except for separators being punctured polypropylene sheets (1 mm in thickness). A projection area of one anode or cathode was 8 cm × 9 cm, and the top side of CE opened to the air. Anodes were made of graphite felts (3 mm in thickness; Sohgoh Carbon, Yokohama, Japan), while cathodes were air cathodes (15) prepared as described previously (11). A CE-MFC reactor contained 6 CEs (12 sets of anode/cathode, a total anode or cathode area of 864 cm^2 in one CE-MFC) that were aligned to generate a slalom flow of wastewater (11) (Fig. 1B).

Operation and evaluation Chemicals used in this study were reagent grades purchased from Wako Pure Chemicals (Tokyo, Japan) unless otherwise specified. A synthetic wastewater contained (per liter) 200 mg of starch, 21 mg of Bacto peptone, 100 mg of Bacto yeast extract, 13 mg of Sunflower oil, 12 mg of Tween-20, 13 mg of urea, and inorganic ingredients as described previously (11); its pH was 7.0, and a total chemical oxygen demand (COD) concentration was approximately 500 mg L^{-1}. The synthetic wastewater was stored in a refrigerator (4°C) and infused into the reactor at a constant flow rate using a peristaltic pump (AC-2110, Atto Corp., Tokyo, Japan).

An operation in the AS mode was initiated by inoculating the aeration tank with 1 L of activated-sludge suspended solids obtained from a wastewater-treatment plant in a polymer-manufacturing factory (Shiga, Japan); its mixed-liquor suspended solid (MLSS) concentration was approximately 1500 mg L^{-1}. The synthetic wastewater was infused into the aeration tank at a hydraulic retention time (HRT) of 24 h, while air was supplied at approximately 3 L min^{-1}. A dissolved-oxygen concentration (DO) was measured using a Multiline FDO925 m (Wissenschaftlich-Technische Werkstätten, Weilheim, Germany). A COD concentration was measured using a COD reactor and a COD 0-1500 ppm range kit (Hach, Laveland, CO, USA). An MLSS concentration was measured according to Japan Industrial Standards method K0102, and it was maintained at approximately 2000 mg L^{-1} during the AS-mode operation. An COD-removal efficiency (CRE [%]) was calculated from the influent COD (COD_{in}) and effluent COD (COD_{ef}) as $CRE = [COD_{in}–COD_{ef}]/COD_{in}$.

Conversion of the AS reactor to CE-MFC was accomplished by terminating the aeration and inserting 6 CEs into the reactor (Fig. 1). Anodes and cathodes were connected in parallel via an external resister (R_{ext}), and a voltage across the resister was monitored using a data logger (HA-1510, Graphtec, Yokohama, Japan). MFC performances were evaluated according to methods described by Watanabe (6). Current (I) and power (P) were calculated from a measured voltage (E) at a set resistance (R_{ext}) using equations $I = E/R_{ext}$ and $P = IE$, respectively. A current density (J) was estimated by dividing I by the anode-projection area. Coulombic efficiency (ϵ_c) was calculated based on a COD removal ($COD_{in} - OD_{ef}$) and a measured current, using 1 g of COD = 0.125 mol of electron, and 1 $A = 5.39 \times 10^{23}$ electrons per day (6). Polarization and power–density curves were drawn using a potentiostat (HZ-5000, Hokuto Denko, Tokyo, Japan) as described previously (5), and the maximum power density (P_{max} [mW m^{-2}] based on the anode-projection area; the peak in a power–density curve), open-circuit potential (E_{oc} [V]), short-circuit current density (J_{sc} [mA m^{-2}], based on the anode-projection area) and internal resistance (R_{int}) were determined from these curves as described elsewhere (5). For comparison, rice paddy-field soil (10 g) obtained at the Egawa farm land of Noda Natural Symbiotic Farm Co. (Noda, Chiba, Japan) was also used as an inoculum for an MFC reactor whose configuration and operation were the same as those described above.

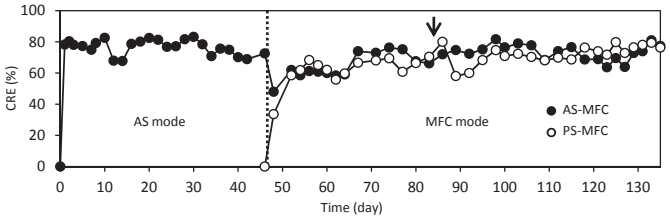

FIG. 2. Changes in CREs during the wastewater-treatment experiment using AS, AS-MFC, and PS-MFC. The dashed line indicates the time when the reactor mode was changed from AS to MFC, while the arrow indicates the time when excess sludge and biofilms were removed.

Microbial-community analyses An amount of proteins adhering to an anode was determined using a BCA protein assay kit (Pierce, Rockford, IL, USA) as described previously (16). In the present study, piece of anode graphite felt (0.5 cm × 0.5 cm) was used for the measurement.

DNA was extracted from an anode graphite-felt piece (0.5 cm × 0.5 cm) using FAST DNA spin kit for soil (Q-Bio, Carlsbad, CA, USA) according to the manufacturer's instruction and finally dissolved in 50 µl of the DES solution supplied in the kit. For sequence analyses, PCR amplification of 16S rRNA gene fragments (the V1–V3 region) was performed using primers ad-tag-8F (5′-CGTATCGCCTCCCTCGCGCCATCAG- XXXXXXGAGTTTGATCMTGGCTCAG-3′) and ad-533R (5′-CTATGCGCCTTGC- CAGCCCGCTCAGTTACCGCKRCTGCTGRCAC) (17), in which the underlined sequences were adapters for pyrosequencing and XXXXXX was an arbitrary tag sequence for sample identification (18). PCR conditions were as described elsewhere (11). An amplicon was purified using a QIAquick PCR purification kit (Qiagen K. K., Tokyo, Japan), and amplicons from different samples were mixed at a same concentration (1 ng μl^{-1} each). The mixed amplicons were subjected to pyrosequencing using a genome sequencer FLX system (Roche Applied Science, Tokyo, Japan) at the Dragon Genomics Center (Mie, Japan), and phylogenetic analyses were conducted using a DDBJ 16S rRNA database (February 21, 2012), a BLASTN program (19) and an RDP classifier (20).

Nucleotide sequences determined in the present study are deposited into the NCBI short reads archive database (accession number: DRA001024).

RESULTS AND DISCUSSION

COD removal The synthetic wastewater (total COD of approximately 500 mg L^{-1}, in which inorganic ingredients accounted for 12%) was infused into the reactor (Fig. 1) at HRT of 24 h, and CRE was monitored. As shown in Fig. 2, in the initial AS-mode operation, CRE rapidly reached at approximately 80% and was stable thereafter. During this period, DO was maintained above 2 mg L^{-1}. On day 45, the reactor configuration was changed to

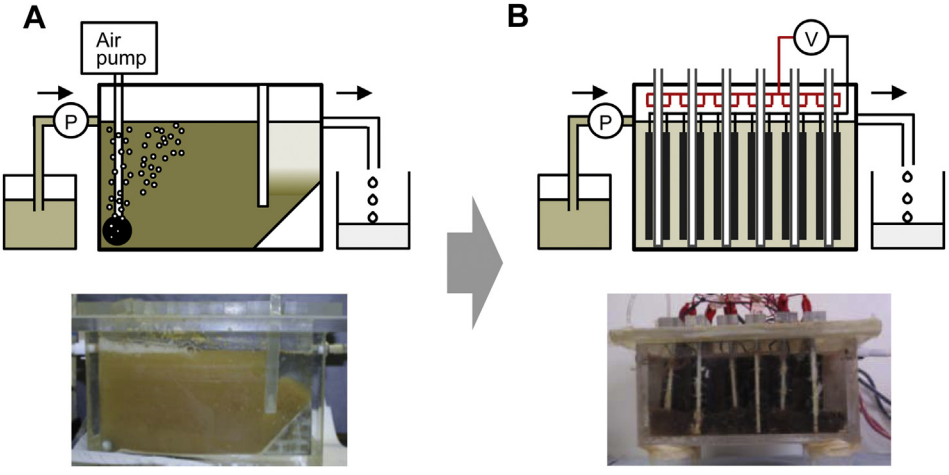

FIG. 1. Schematics and photos for laboratory-scale AS (A) and CE-MFC (B) reactors. The same tank was used for these reactors.