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Comparison of electrochemical and microbiological characterization of microbial fuel cells equipped with SPEEK and Nafion membrane electrode assemblies

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Microbial fuel cells equipped with SPEEK-MEA (SPEEK-MFC) and Nafion-MEA (Nafion-MFC) were constructed with organic waste as electron donor and lake sediment as inoculum and were then evaluated comprehensively by electrochemical and microbial analyses. The proton conductivity of SPEEK was several hundreds-fold lower than that of Nafion 117, whereas the oxygen mass and diffusion transfer coefficients of SPEEK were 10-fold lower than those of Nafion 117. It was difficult to predict which was better membrane for MFC based on the feature of membrane. Analyses of polarization curves indicated that the potential of electricity production was similar in both MFCs, as the SPEEK-MFC produced 50–80% of the practical current density generated by the Nafion-MFC. Chronopotentiometry analyses indicated that the Nafion-MEA kept the performance longer than the SPEEK-MEA for long period, whereas performance of both anodes improved on time. Multidimensional scaling analyses based on DGGE profiles revealed the anolytic and biofilm communities of the SPEEK-MFC had developed differently from those of the Nafion-MFC. Clone library analyses indicated that *Geobacter* spp. represented 6.3% of the biofilm bacterial community in the Nafion-MFC but not detected in the SPEEK-MFC. Interestingly, the clone closely related to *Acetobacterium malicum* strain HAAP-1, belonging to the homoacetogens, became dominant in both anolytic and biofilm communities of the SPEEK-MFC. It was suggested that the lower proton conductivity of SPEEK-MEA allowed the bacteria closely related to strain HAAP-1 to be dominant specifically in SPEEK-MFC. These results indicated that Nafion-MFC ranked with SPEEK-MFC and that MEAs had strong selective pressure for electricity-producing bacterial community.

[**Key words:** Microbial fuel cell; Membrane electrode assembly; Bacterial community structure; SPEEK]

Microbial fuel cells (MFCs) are capable of generating electric power from organic matter using microbial activity, and are expected to be a novel green-energy producing system (1,2). Although current density produced from MFCs has been improved, the current density is still too low for practical applications. Therefore, it is significantly important to increase the current density to decrease internal resistance of MFCs. To date, mainly the configuration of device (3–5), electrodes (6,7) and exoelectrogens (8–10) have been investigated to improve electricity production of MFCs. It is also known that thinner proton exchange membranes and a short distance between the membrane and cathode electrode decrease internal resistance thereby improving current output (11–13).

In several membranes tested in chemical fuel cells, it was reported that sulfonated poly(ether–ether ketone) (SPEEK) had better performance than Nafions as a polymer electrolyte in fuel cells (14–17). Interestingly, SPEEK and Nafion 117 have different properties; the oxygen mass transfer coefficient and oxygen diffusion coefficient of SPEEK are one order of magnitude lower than those of Nafion 117 (15), whereas the proton conductivity of SPEEK is two order of magnitude lower than that of Nafion 117 (18,19). From only these membrane features, it is difficult to predict which membrane contributes to produce more current from an MFC. Ayyaru and Dharmalingam (15) reported that the maximum power density of SPEEK-MFC was two-fold higher than that of Nafion-MFC inoculated with *Escherichia coli* or waste water for 12 days.

However, the performance of MFC is decided by both features of membrane and another factor, i.e., microbial community adapted to an MFC. It had better investigate the MFC performance under long run for practical application of MFC. Therefore, we tried to evaluate the MFC performance comprehensively to investigate the effects of proton exchange membrane on MFC performance for long period. To address these issues, a membrane electrode assembly (MEA) was made by directly combining a proton exchange membrane with a cathode electrode with the intent of lowering internal resistance and MFCs equipped with either SPEEK or Nafion MEA were characterized electrochemically and microbial ecologically for over 5 months.

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MATERIALS AND METHODS

Membrane electrode assembly used in this study Two kinds of MEAs, SPEEK and Nafion, were made using a hot press technique. The SPEEK was prepared from commercially available poly(ether–ether ketone) (PEEK) (450 P standard viscosity grade, Victrex) according to the previous report (20) with modification: for sulfonation of PEEK, 5 g of PEEK was initially dissolved in 100 g of concentrated H_2SO_4 with vigorous stirring at 50°C in a nitrogen atmosphere for 6 h. The polymer solution was then poured into a large excess of ice-cold water under continuous mechanical agitation to obtain the SPEEK polymer precipitates. The polymer precipitate thus obtained was washed several times with distilled water until a neutral pH was achieved then the dried at 120°C. One gram of dried SPEEK and 20 g of *N*,*N*-dimethylacetamide (DMAc, 99.0 vol.% Wako Pure Chemical Industries, Ltd.) were mixed vigorously and dried to obtain a SPEEK membrane. The SPEEK membrane was soaked into 5 wt.% of H_2SO_4 solution at 60°C overnight and was then washed several times with distilled water and was then dried. Nafion 117 membrane (Aldrich) used in this study was boiled in 3% H_2O_2 for 1 h and was then washed in several times with distilled water and was boiled in distilled water for 1 h. Finally, the Nafion 117 was boiled in 1 M H_2SO_4 and was boiled in distilled water for 1 h. The 0.2 mL of Nafion solution (5 wt.% in lower aliphatic alcohols and water, 15–20, Aldrich) as a binder was plated on the carbon paper electrode electroplated with platinum (TGP-H-060, Chemix Co., Ltd., Japan; 0.5 mg Pt cm^2) and was pressed with SPEEK or Nafion 117 membranes under 2 MPa for 1 min at 120°C or 100°C to produce the final MEAs used in this study (AH-2003, As One Co., Ltd., Osaka, Japan), respectively (denoted SPEEK-MEA or Nafion-MEA).

MFC configuration and operation Mediator-less air-cathode MFCs were constructed to evaluate novel MEAs by electrochemical and microbiological analyses. The SPEEK-MEA or Nafion-MEA on one side was used as the cathode electrode, providing a total projected cathode surface area (on one side) of 4.0 cm^2. A total of 135 pieces of cubic (125 mm^3) graphite felts (Sohgoh-C Co., Ltd., Yokohama, Japan) were packed into the anode chamber (36 mL in capacity) and the total projected anode surface area was 0.02025 m^2 (21). Twenty of these pieces were directly connected to platinum wires (0.3 mm; AlfaAesar). Sediment (0.4 g) from the brackish lake Sanaru (Hamamatsu, Japan) was used as the initial inoculum with 20 mM sodium lactate as the initial carbon and energy source in BE medium (7) and the electrodes were then connected with an external resistance (10 Ω). The BE medium contained 0.5 g of KH_2PO_4, 0.20 g of $MgSO_4{\cdot}7H_2O$, 0.15 g of $CaCl_2{\cdot}2H_2O$, 0.5 g of NH_4Cl, 2.5 g of $NaHCO_3$, 1.0 mL of Se/W solution (22), 1.0 mL of trace elements solution SL8 (23), and 1.0 mL of vitamin solution PV1 (24) per liter. As a control, an MFC was run under an open circuit condition (control MFC) that was also constructed with the same materials. Organic waste was collected from the cafeteria and 10 g or 20 g of this (Supplementary Fig. S1) was placed directly in a bottle (organic waste-decomposing tank) that contained 1 L of $NaHCO_3$ solution (2.5 g L^{-1}) to control the pH. Sea sand was put on the bottom of this organic waste-decomposing tank (denoted as the tank) as a filter bed. After 14 days incubation, the filtered digested solution was continuously fed into MFCs at a feeding rate of 36 mL d^{-1} (i.e., the hydraulic residence time was 1.0 day). It was confirmed that organic acids were completely consumed in the anode before feeding the filtered digested solution from the tank. MFC voltage (V) was recorded every 5 min across a 10 Ω resistance (R) by a data logger connected to a personal computer.

Bacterial community analyses Anolytic culture (1.0 mL) was directly sampled from the anode compartment of MFCs and cells were collected by centrifugation for 5 min at 4°C and 20,000 ×*g*. Pieces of anode were cut off and kept at −20°C until DNA extraction. DNA was extracted according to the conventional method described by Futamata et al. (25). Bacterial community structure was analyzed by clone library analysis targeting 16S rRNA gene and multidimensional scaling (MDS) analysis based on denaturing gradient gel electrophoresis (DGGE) profile. The sediment of lake Sanaru used as an inoculum was analyzed as the sample at day 0. DNA fragments of 16S rRNA genes were amplified by using primers 5′-AGAGTTTGATCCTGGCTCAG-3′ (corresponding to *E. coli* 16S rRNA gene positions 8–27 (26) and 5′-AAGGAGGTGATCCAGCC-3′ (corresponding to *E. coli* 16S rRNA gene positions 1525–1542). Amplification was performed with a thermal cycler PC320 (ASTEC, Osaka, Japan) by using 50 μL mixture containing 0.5 U of KOD FX DNA polymerase (Toyobo Co. Ltd., Osaka, Japan), buffer solution attached with the PCR kit, each deoxynucleoside triphosphate at a concentration of 400 μM, 15 pmol of each primer, and 50 ng of template DNA. The PCR conditions were 2 min for activation of the polymerase at 94°C and then 25 cycles of 1 min at 94°C, 1 min at 53°C, and 1 min at 72°C, and finally 10 min of extension at 72°C. The PCR products were checked by electrophoresis on 1.5% (w/v) agarose gel in TAE buffer (27) and stained with GelRed (Wako, Japan). PCR products were cloned into the vector pTA2 and introduced into competent DH5α cells using a TArget Clone-Plus kit according to the manufacturer's recommendations. Clones were isolated by screening for blue/white phenotypes and incubated in TB medium supplemented with kanamycin (50 mg L^{-1}). Plasmid DNA was extracted using a Wizard Minipreps DNA Purification System (Promega, Madison, WI, USA) according to the manufacturer's directions. The DNA was digested with *Eco*RI and electrophoresed, thereby confirming whether an insert was of expected size. Bacterial community structures were also analyzed by DGGE analysis targeting 16S rRNA genes. The variable region V3 of bacterial 16S rRNA genes (corresponding to positions 341–534 in the *E. coli* sequence) was amplified using primers P2 and P3 (containing a 40 bp GC clamp (28)) and a thermal cycler PC320 as described previously (20). A Dcode DGGE system (Bio-Rad Laboratories, Inc., CA., USA) was used for electrophoresis as recommended by the manufacture. A total of 10 μL of a PCR-amplified mixture was subjected to electrophoresis in a 10% (w/vol) polyacrylamide gel at 200 V for 3.5 h at 60°C. Gel gradients used for separation, which were applied in parallel to the electrophoresis direction, were 35%–55%. After electrophoresis, the gel was stained with SYBR Green I (FMC Bioproducts) for 30 min as recommended by the manufacture. The intensity of bands in DGGE gels was measured using a Gel Doc XR+ (Bio-Rad). MDS analysis was performed with these bands intensities. Because DGGE analysis does not necessarily completely reproduce the same result, all intensities and locations of DGGE bands used in MDS analysis were compensated by comparing intensities and locations of common samples in different DGGE gels. MDS analysis based on the Bray–Curtis index was used to analyze the dynamics of bacterial community structure because this index is recognized as one of the most useful methods for evaluating the differences among populations (29,30). The following equation was used for the calculation of the Bray–Curtis index:

$$\delta_{AB} = \left(\sum|n_A - n_B|\right) \Big/ \left[\sum(N_A + N_B)\right] 0 \le \delta_{AB} \le 1 \quad (1)$$

where δ_{AB} means dissimilarity index between communities A and B, n_A and n_B mean the intensity of DGGE band in clusters of A and B, and N_A and N_B means the total intensity of DGGE bands in A and B, respectively (30–32). MDS analysis and the cluster analysis were conducted using the R software program v2.12.1 (The R Project for Statistical Computing: http://www.r-project.org/; University of Tsukuba, Japan: http://cran.md.tsukuba.ac.jp) (33). Commands used in R software program v2.12.1 are shown in Supplementary Fig. S2. The 3D graph was constructed using RINEARN Graph 3D v.5.2.0 software.

Scanning electron microscopy observation The morphologies of the MEAs were characterized by field emission scanning electron microscope (FE-SEM, Hitachi, S-4800, operating at 10 kV).

Electrochemical analyses Voltage across the external resistor (10 Ω) was automatically monitored every 5 min using a data logger (GL200A, Graphtec, Tokyo, Japan) connected to a personal computer. In order to evaluate the cell performance, a polarization curve was measured using a potentiostat (HAV-110, Hokuto Denko Co., Ltd., Tokyo, Japan) at 2 mV min^{-1} of a slope range in an approximate interval. Cell-performance indices (open-circuit voltage [V_{OC}], short-circuit current density per projected surface area of anode-electrode [I_{SC}], maximum power density per projected surface area of anode-electrode [P_{max}], and internal resistance [R_{int}]) were calculated from the slopes of polarization curves. In some tests, an Ag/AgCl reference electrode (0.199 V versus standard hydrogen electrode [SHE], HX-R6, Hokuto Denko) was placed into the anode compartments to determine individual electrode potentials. Coulombic efficiency was obtained by calculating the ratio of total recovered coulombs by integrating the current over time to the theoretical amount of coulombs that can be produced from organic waste (see the Chemical analysis section). Detailed information can be found in a previous report (34). Chronopotentiometry (CP) was performed at appropriate intervals of current using the potentiostat. The potential limiting current density of the anode was estimated as the current density at the crossing point of anode and cathode potential lines.

Chemical analyses Liquid samples including small particles were collected from the effluent solution of the tank for measurement of redox potential, pH, and COD_{cr} by using the colorimetric standard method (5220D. Closed Reflux, Colorimetric Method). The redox potential and pH were measured using an electrode (TPX-999Si, Toko Chemical Laboratories Co., Ltd., Tokyo, Japan). In MFCs, COD_{cr} has primarily been used (35) to monitor the microbial metabolism as the number of electrons released from organics corresponded to COD_{cr} removal (1 g of COD_{cr} is equivalent to 125 mmol of electron (36)). These liquid samples were also filtered (Millipore LG [pore size; 0.2 μm, diameter; 13 mm], Millipore Corporation, Billerica, MA, USA) for organic acids quantification by an HPLC equipped with a Shodex RSpak KC-811 column (300 × 8.0 mm) (Showa Denko Co. Ltd., Kanagawa, Japan) and UV detector. Column oven was set at 50°C, samples were eluted with 0.1% H_3PO_4 solution at 1.0 mL min^{-1} of flow rate and elutes were monitored at 210 nm. Formate, pyruvate, lactate, butyrate and acetate were identified according to the retention time and the concentration was determined by comparing the peak area with that of its respective standard sample.

Nucleotide sequence accession numbers The nucleotide sequences reported in this paper have been deposited in the GenBank database under accession numbers LC070236 to LC070657.

RESULTS

SEM observation of MEAs SEM observation revealed that there were no cracks in the membranes and thickness of the SPEEK-MEA and Nafion-MEA were approximately 70 μm and 150 μm,