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

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

Kei Suzuki[1], Rubaba Owen[2], Joann MoK[1], Hiroki Mochihara[1], Takuya Hosokawa[1], Hiroko Kubota[1], Hisatoshi Sakamoto[3], Atsunori Matsuda[3], Yosuke Tashiro[1], and Hiroyuki Futamata[1,*]

Department of Applied Chemistry and Biochemical Engineering, Graduate School of Engineering, Shizuoka University, Hamamatsu, 432-8561, Japan[1], College of Health Sciences, University of KwaZulu Natal, Durban 4000, South Africa[2] and Department of Electrical and Electronic Information Engineering, Toyohashi University of Technology, Toyohashi, Aichi, 441-8580, Japan[3]

Running title: the performance of MFC equipped with SPEEK membrane

Authors mailing address:

Kei Suzuki: f5345018@ipc.shizuoka.ac.jp, Rubaba Owen: Rubaba@ukzn.ac.za, Joann MoK: joannmok@gmail.com, Hiroki Mochihara: f0330301@ipc.shizuoka.ac.jp, Takuya Hosokawa: hosokawa.takuya.14@shizuoka.ac.jp, Hiroko Kubota: kubota.hiroko.14@shizuoka.ac.jp, Hisatoshi Sakamoto: sakamoto.hisatoshi@kobelco.com, Atsunori Matsuda: matsuda@ee.tut.ac.jp, Yosuke Tashiro: tashiro.yosuke@shizuoka.ac.jp, Hiroyuki Futamata: thfutam@ipc.shizuoka.ac.jp

Key words:

Microbial fuel cell, Membrane electrode assembly, Bacterial community structure, SPEEK

*Corresponding author. Hiroyuki Futamata

E-mail: thfutam@ipc.shizuoka.ac.jp

Tel: +81-53-478-1178; Fax: +81-53-476-0095

Abstract

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 Nafion117, whereas the oxygen mass and diffusion transfer coefficients of SPEEK were ten-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.

Introduction

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 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 (15).

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.

MATERIALS AND METHODS

Membrane electrode assembly used in this study Two kinds of membrane electrode assemblies (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*-dimethyacetamide (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. Nafion117 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 Nafion117 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 alchohols 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 Nafion117 membranes under 2 MPa for 1 min at 120°C or 100°C to produce the final MEAs used in this study (AH-2003, AsOne 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 *Escherichia coli* 16S rRNA gene positions 8–27 [26] and 5′-AAGGAGGTGATCCAGCC-3′ (corresponding to *Escherichia 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^{TM} (Wako, Japan). PCR products were cloned into the vector pTA2 and introduced into competent DH5α cells using a TArget Clone^{TM} -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 *Escherichia 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:

$$\delta_{AB} = (\sum | n_A - n_B |) / [\sum (N_A + N_B)] \quad 0 \leq \delta_{AB} \leq 1 \qquad \text{(equation 1)},$$

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 were 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 minutes 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) 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 Co., Ltd., Tokyo, Japan) 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 Chemical analysis). 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, Colorimetic Method). The redox potential and pH were measured using an electrode (TPX-999Si, Toko Chemical Lab. 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, respectively (Fig. 1). An aperture was observed partially between the SPEEK membrane and cathode electrode in the SPEEK-MEA (Fig. 1A), whereas the Nafion117 membrane stuck almost completely to the cathode electrode (Fig. 1B).

Electricity producing properties of MFCs Electricity producing properties of the SPEEK-MFC and Nafion-MFC were somewhat similar (Fig. 2A). In stage I (from day 0 to day20), the current density of the Nafion-MFC was approximately 2-fold higher than that of the SPEEK-MFC. In stage II (day 21 to day 64), although the organic waste-decomposing solution was continuously fed into the MFCs, current production by both MFC types was very low, 2.7 ± 2.0 μW m^{-2} and 2.6 ± 2.0 μW m^{-2}, respectively. Since the current density was very low, 0.4 g of the lake sediment was inoculated again into both MFCs at day 52. The current density of the Nafion-MFC did not increase, whereas that of the SPEEK-MFC peaked at 0.47 ± 0.15 mW m^{-2} in

stage III (from day 65 to 85). Maximum current densities of the SPEEK- and Nafion-MFC increased gradually in stage IV (form 86 to 125) and these were stable at 0.47 ± 0.10 mW m^{-2} and 1.0 ± 0.14 mW m^{-2}, respectively in stage V (from day 127 to day 163). Polarization curve analyses showed that electrochemical properties of both MFCs were somewhat similar for all parameters (Table 1). Although initial internal resistance values of SPEEK- and Nafion-MFCs were 2900 Ω and 3580 Ω, these values decreased to 310 Ω and 440 Ω, respectively.

The COD removal efficiency was almost similar to each other and the changes of COD removal efficiencies were synchronized with those of current densities in both MFCs (Fig. 2B). The COD removal efficiencies of the SPEEK-MFC and the Nafion-MFC were approximately 64 ± 11% and 68 ± 11%, respectively with exception of stage II and IV. The COD removal efficiency of the control-MFC was similar to other MFCs exception of stage V. The coulombic efficiencies of the SPEEK-MFC and the Nafion-MFC were approximately 5.6 ± 4.2% and 7.0 ± 6.0%, respectively, with exception of day 88 and day 120 (Fig. 2C). The pH of the tank, the SPEEK-MFC, and the Nafion-MFC were almost stable at 7.2 ± 0.62, 8.5 ± 0.38, and 8.3 ± 0.34, respectively (Supplementary Fig. S3). After the organic waste was added in the tank, the pH temporary decreased but became stable at initial level.

Chronopoteniometry analyses suggested that the potential limiting current densities of the anode in SPEEK- and Nafion-MFCs increased from 26 mA m^{-2} and 24 mA m^{-2} at day 127 to 51 mA m^{-2} and 57 mA m^{-2} at day 147, respectively (Fig. 3). The cathode potentials of the Nafion-MFC were stable at approximate 192±6.5 mV at day 127 and 190±6.0 mV at day 147, whereas the cathode potentials of SPEEK-MFC decreased from 198±44 mV at day 127 to 100±19 mV at day 147.

Changes in Organic acids concentration Organic acids in effluents from the tank and both MFCs were monitored (Fig. 4). Interestingly, although the same organic waste-decomposing solution from the tank was fed into the MFCs, components and concentration of organic acids in the effluents differed. Lactate was a main organic acid in the tank and both MFCs at stage I and the maximum concentration was approximately 35 mM. Propionate, butyrate, and acetate were intermingled at similar concentrations from late stage I to stage II in the tank and both MFCs. Acetate was a main organic acid in the tank from stage III to V and the maximum concentration was approximately 12 mM, while butyrate and propionate were almost half that of acetate. Acetate was also a main organic acid in SPEEK-MFC from stage III to V and the maximum concentration was approximately 20 mM. The concentration of butyrate and propionate were less than 5 mM. On the other hand, propionate, butyrate, and acetate were intermingled at stage III in Nafion-MFC, where these maximum concentrations were approximately less than 7 mM. Acetate was a main organic acid in the Nafion-MFC from late stage III and stage IV and the maximum concentration was approximately 20 mM and the concentration of butyrate and propionate were less than 5 mM as well in the SPEEK-MFC.

Bacterial population dynamics MDS analyses based on the DGGE profiles (Supplementary Fig. S4) were performed to investigate the effects of the SPEEK-MEA on the anolytic and biofilm bacterial community structure. All stress values were less than 0.20, indicating that these data were valuable statistically. The average of dissimilarity index values of the anolytic bacterial community structures in the tank, SPEEK-MFC, Nafion-MFC, and control-MFC were 0.70±0.17, 0.79±0.18, 0.77±0.15, and 0.78±0.15, respectively, suggested that their fluctuation of anolytic bacterial

communities were similar to each other. After day 92, these bacterial community structures in the tank, SPEEK-MFC, Nafion-MFC, and control-MFC had different dynamic equilibria, as the dissimilarity index values were 0.51±0.096, 0.58±0.14, 0.66±0.10, and 0.69±0.13, respectively (Fig. 5). These results suggested that anolytic bacterial communities developed more specifically. Although the dynamics of biofilm-communities of the MFCs did not necessarily synchronize with those of the anolytic communities (Fig. 5B-5D), dynamics of the biofilm and anolytic communities had similar positions macroscopically (Fig. 5E).

Bacterial community structure Clonal analyses targeting the 16S rRNA gene were performed to investigate the bacterial community structure in the lake sediment used as inoculum, the tank, SPEEK-MFC, Nafion-MFC, and control-MFC (Fig. 6). The results of sequence analyses are summarized in Supplemental table 1. These analyses revealed that the anolytic and biofilm community structures were significantly similar in the SPEEK-MFC, whereas these were slightly different from each other in the Nafion- and control MFC. The community structure of the inoculum sediment was diverse, in which α-, β-*proteobacteria* and *Firmicutes* (denoted the Fαβ-group) comprised about 30%. Conversely, the Fαβ-group dominated over 70% of total clones in the tank, anolyte and biofilm of all MFCs. Although the proportion of *Firmicutes* in the tank and sediment was 24% and 4.7%, respectively, that in the anolyte and biofilm of SPEEK-MFC was 98% and 96%, respectively. A clone closely related with *Acetobacterium malicum* strain HAAP-1 (98.4% identity) dominated 78% and 88% of *Firmicutes* in the anolyte and biofilm of the SPEEK-MFC, respectively. This clone was not detected in the sediment nor the tank. Although the proportion of *Firmicutes* in the anolyte and biofilm of the Nafion- and control-MFC was 56% and 21%, and 31%

and 40%, respectively, the clone closely related with *A. malicum* strain HAAP-1 was also not detected in these MFCs, instead these *Firmicutes* communites consisted of several genera. The proportion of δ-*proteobacteria* in the sediment was 24%, in which 6 clones (30%) belonged to the genus of *Geobacter*. *Geobacter* spp. shared only 6.3% of the biofilm bacterial community in the Nafion-MFC and was not detected in the SPEEK-MFC. All clones of δ-*proteobacteria* detected in the biofilm of the Nafion-MFC belonged to the genus of *Geobacter*, whereas all clones belonged to δ-*proteobacteria* detected in the control-MFC were closely related to sulfate-reducing bacterium.

DISCUSSION

We compared comprehensively the MFC performance equipped with the SPEEK-MEA and the Nafion-MEA by electrochemical and microbial analyses. SEM observation showed that there were not any cracks on the surface of the SPEEK- and Nafion-MEAs and not any pore (16) in both MEAs (Fig. 1). Serious problem is pointed out about MEAs; since the surface of carbon paper electroplated with platinum is significantly rough, many cracks occur in the thin layer of proton exchange membranes. These cracks allow oxygen to intrude into anode of MFC, resulting in a decrease of MFC performance (7,37). Our result suggested that the hot pressing technique was useful for attaching a thin membrane layer to a rough surface like carbon paper.

It has been reported that the proton conductivity of SPEEK and Nafion 117 used in this study are *ca.* 1.0×10^{-4} S cm^{-2} (20) and *ca.* 7.0×10^{-2} S cm^{-2} (18,38) at room temperature and 80% relative humidity, respectively. On the other hand, the oxygen mass transfer coefficient and the oxygen diffusion coefficient of SPEEK are 2.4×10^{-6}

cm s^{-1} and 4.8×10^{-8} cm^2 s^{-1}, respectively, and those of Nafion 117 are 1.6×10^{-5} cm s^{-1} and 3.0×10^{-7} cm^2 s^{-1}, respectively (15). Ayyaru et al. reported that the power density of SPEEK-MFCs is two-fold higher than that of Nafion-MFCs using *Escherichia coli* DH5-α for about 2 weeks (15). Lim et al. reported that membranes kinds of SPEEK lead better performance of MFC rather than Nafion membranes (16). These results suggest that the feature of oxygen transfer and diffusion of membrane is important for current generation in an MFC. However, this study showed that the Nafion-MFC exhibited similar or higher performance rather than that of the SPEEK-MFC (Table 1, Fig. 2A), suggesting that the effect of another factor, i.e., bacterial community, exerted on the performance of MFCs. Those membrane features are not always maintained for long period. Actually, CP analyses showed that the SPEEK-MEA did not kept the performance longer than the Nafion-MEA, suggesting that the resistance of the SPEEK-MEA was increased by biofilm on the SPEEK-MEA. It is reported that the biofouling reduces the performance of the membrane (16). These results suggest that the differences of feature of MEA would affect the bacterial community structure in these anode compartments.

As expectedly, MDS and clone library analyses revealed that bacterial community structures of the SPEEK- and Nafion-MFCs were significantly different from each other (Fig. 5 and 6). Although it has been reported that the kind of electron donor affects bacterial community structure in MFCs (39,40), the same organic-decomposing solution was fed into both MFCs in this study. Additionally, it has been demonstrated that the anode potential affects the community structure of biofilms on the surface of anode, resulting in different electricity-producing properties of the MFC (41-44). On day 147, CP analyses indicated that the anode potentials of the SPEEK- and Nafion-MFCs were approximately -183 mV (at about 20 mA m^{-2}) and -120 mV (at about 40 mA m^{-2}),

respectively (Fig. 3B). It has been reported that *G. sulfurreducens* becomes significantly limited below approximately -0.15 V of anode potential (45,46). This explains why clones closely related to *Geobacter* were not detected in the biofilm community in the SPEEK-MFC but were detected in the Nafion-MFC. *Geobacter* spp. have also been found to be enriched on the surface of the anode and contribute to higher electricity production performance (16,19,47,48). Therefore, it seems that the difference of *Geobacter* population densities could have caused the difference in current-producing activities between the SPEEK- and Nafion-MFCs.

As expectedly (21,49), the anolytic and biofilm communities from the Nafion-MFC were different from each other. Conversely, the anolytic and biofilm communities were more similar in the SPEEK-MFC. Interestingly, the clone closely related to *Acetobacterium malicum* HAAP-1 dominated both the anolyte and biofilm of the SPEEK-MFC. Since this clone was not detected in the sediment, tank, control-MFC nor the Nafion-MFC, this indicates that the bacterium closely related to *A. malicum* strain HAAP-1 was a significantly minor population and was enriched specifically in the SPEEK-MFC. *A. malicum* strain HAAP-1 is a homoacetogen and can grow well in a mineral medium on H_2 (50). Acetogens carry out the following reaction; $4H_2 + H^+ + 2HCO_3^- \rightarrow CH_3COO^- + 4H_2O$ ($\Delta G^{0'} = -105$ kJ) (51). Since it was suggested that the proton conductivity of the SPEEK-MEA was significantly decreased than that of the Nafion-MEA by biofilm on the SPEEK-MEA, hydrogen would have accumulated more in the SPEEK-MFC than the Nafion-MFC, allowing the bacterium closely related to *A. malicum* strain HAAP-1 to become dominant specifically in the SPEEK-MFC. However, it was not known the real reason why *A. malicum* strain HAAP-1 became dominant in the SPEEK-MFC, yet.

In conclusion, it was demonstrated that the composition of the MEA directly

influenced bacterial communities in MFCs, resulting in different electricity-producing properties. Unexpectedly, the Nafion-MFC was close or higher than the SPEEK-MFC in current producing ability, suggesting that the comprehensive analyses are useful for a practical evaluation of MFC for long period. Furthermore, it is suggested that the flexibility of bacterial community structure is important for electricity production. Therefore, it is important to investigate how to control extracellular electron transfer for producing highly current density as well as material research.

ACKNOWLEDGMENTS

This research was carried out as a part of grant KAKENHI (B) 26281038, Japan. It was also partially supported by ALCA project, Japan Science and Technology Agency and Tokai Industrial Technology Foundation (H260304No1).

References

1. **Logan, B. E. and Regan, J. M.:** Microbial fuel cells-challenges and applications, Environ. Sci. Technol., **40**, 5172-5180 (2006).
2. **Rabaey, K. and Verstraete, W.:** Microbial fuel cells: novel biotechnology for energy generation, Trends Biotechnol., **23**, 291-298 (2005).
3. **Park, D. H and Zeikus, J. G.:** Improved fuel cell and electrode designs for producing electricity from microbial degradation, Biotechnol. Bioeng., **81**, 348-355 (2003).
4. **He, Z., Minteer, S. D., Angenent, L. T.:** Electricity generation from artificial wastewater using an upflow microbial fuel cell, Environ. Sci. Technol., **39**, 5262-5267 (2005).
5. **Shimoyama, T., Komukai, S., Yamazawa, A., Ueno, Y., Logan, B. E., Watanabe, K.:** Electricity generation from model organic wastewater in a cassette-electrode microbial fuel cell, Appl. Microbiol. Biotechnol., **80**, 325-330 (2008).
6. **Du, Y., Feng, Y., Qu, Y., Ren, N., Liu, H.:** Electricity generation and pollutant degradation using a novel biocathode coupled photoelectrochemical cell, Environ. Sci. Technol., **48**, 7634-7641 (2014).
7. **Rubaba, O., Araki, Y., Yamamoto, S., Suzuki, K., Sakamoto, H., Matsuda, A., Futamata, H.:** Electricity producing property and bacterial community structure in microbial fuel cell equipped with membrane electrode assembly, J. Biosci. Bioeng., **116**, 106-113 (2013).
8. **Ishii, S., Watanabe, K., Yabuki, S., Logan, B. E., Sekiguchi, Y.:** Comparison of electrode reduction activities of *Geobacter sulfurreducens* and an enriched consortium in an Air-cathode microbial fuel cell, Appl. Environ. Microbiol., **74**,

7348-7355 (2008).

9. **Reguera, G., McCarthy, K. D., Mehta, T., Nicoll, J. S., Tuominen, M. T., Lovley, D. R.:** Extracellular electron transfer via microbial nanowires, Nature **435**, 1098-1101 (2005).

10. **Bretschger, O., Obraztsova, A., Sturm, C. A., Chang, I. S., Gorby, Y. A., Reed, S. B., Culley, D. E., Reardon, C. L., Barua, S., Romine, M. F., and other 8 authors:** Current production and metal oxide reduction by *Shewanella oneidensis* MR-1 wild type and mutants, Appl. Environ. Microbiol., **73**, 7003-7012 (2007).

11. **Liu, H., Grot, S., Logan B. E.:** Electrochemically assisted microbial production of hydrogen from acetate, Environ. Sci. Technol., **39,** 4317-4320 (2005).

12. **Cheng, S., Liu, H., Logan, B. E.:** Increased power generation in a continuous flow MFC with advective flow through the porous anode and reduced electrode spacing, Environ. Sci. Technol., **40,** 2426-2432 (2006).

13. **Liu, H., Cheng, S., Huang, L., Logan, B. E.:** Scale-up of membrane-free single-chamber microbial fuel cells, J. Power Sources, **179,** 274-289 (2008).

14. **Kaliaguine, S., Mikhailenko, S. D., Wang, K. P., Xing, P., Robertson, G., Guiver, M.:** Properties of SPEEK based PEMs for fuel cell application, Catal. Today., **82**, 213-222 (2003).

15. **Ayyaru, S., and Dharmalingam, S.:** Development of MFC using sulphonated polyether ether ketone (SPEEK) membrane for electricity generation from waste water, Bioresource Technology, **102,** 11167-11171 (2011).

16. **Lim, S. S., Daud, W. R. W., Jahim, J. M., Ghasemi, M., Chong, P. S., Ismail, M.:** Sulfonated poly(ether ether ketone)/poly(ether sulfone) composite membranes as an alternative proton exchange membrane in microbial fuel cells, Int. J. Hydrogen Energy, **37,** 11409-11424 (2012).

1 **17. Oh, S. E., Kim, J. R., Joo, J.-H., Logan, B. E.:** Effects of applied voltages and
2 dissolved oxygen on sustained power generation by microbial fuel cells, Water Sci.
3 Technol., **60,** 1311-1317 (2009).
4 **18. Sone, Y., Ekdunge, P., Simonsson, D.:** Proton conductivity of Nafion 117 as
5 measured by a four-electrode AC impedance method, J. Electrochem. Soc., **143**,
6 1254-1259 (1996).
7 **19. Bond, D.R. and Lovley, D.R.:** Electricity production by *Geobacter sulfurreducens*
8 attached to electrodes, Appl. Environ. Microbiol. **69,** 1548-1555 (2003).
9 **20. Oh, S. Y., Yoshida, T., Kawamura, G., Muto, H,, Sakai, M., Matsuda, A.:**
10 Composite electrolytes composed of Cs-substituted phosphotungstic acid and
11 sulfonated poly(ether-ether ketone) for fuel cell systems, Materials. Sci. and
12 Engineer. B, **173**, 260-266 (2010).
13 **21. Yamamoto, S., Suzuki, K., Araki, Y., Mochihara, H., Hosokawa, T., Kubota, H.,**
14 **Chiba, Y., Rubaba, O., Tashiro, Y., Futamata, H.:** Dynamics of different
15 bacterial communities are capable of generating sustainable electricity from
16 microbial fuel cells with organic waste, Microbes. Environ., **29**, 145-153 (2014).
17 **22. Löffler, F. E., Sanfold, R. A., Tiedje, J. M,:** Initial characterization of a reductive
18 dehalogenase from *Desulfitobacterium chlororespirans* Co23., Appl. Environ.
19 Microbiol., **62**, 3809-3818 (1996).
20 **23. Biebl, H. and Pfennig, N.:** Growth yields of green sulfur bacteria in mixed culture
21 with sulfur and sulfate reducing bacteria, Arch. Microbiol., **117**, 9-16. (1978).
22 **24. Hiraishi, A., Yonemitsu, Y., Matsushita, M., Shin, Y. K., Kuraishi, H.,**
23 **Kawahara, K.:** Characterization of *Porphyrobacter sanguineus* sp. nov., an
24 aerobic bacteriochlorophyII-containing bacterium capable of degrading biphenyl
25 and dibenzofuran, Arch. Microbiol., **178**, 45-52 (2002).

25. **Futamata, H., Yoshida, N., Kurogi, T., Kaiya, S., Hiraishi, A.:** Reductive dechlorination of chloroethenes by *Dehalococcoides*-containing cultures enriched from a polychlorinated-dioxin-contaminated microcosm, ISME J., **1**, 471-479 (2007).
26. **Brosius, J., Dull, T. J., Sleeter, D. D., Noller, H. F.:** Gene organization and primary structure of a ribosomal RNA operon from *Escherichia coli*., J. Mol. Biol., **148**, 107-127 (1981).
27. **Sambrook, J., Fritsch, E. F., Maniatis, T.:** Molecular cloning: A laboratory manual, 2nd ed, Cold Spring Harbor Laboratory Press, New York, USA (1989).
28. **Muyzer, G., de Waal, E. C., Uitterlinden, A. G.:** Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA, Appl. Environ. Microbiol., **59**, 695-700 (1993).
29. **Clarke, K. R.:** Non-parametric multivariate analyses of changes in community structure, Austral. Ecol., **18**, 117-143 (1993).
30. **Faith, D. P., Minchin, P. R., Belbin, L.:** Compositional dissimilarity as a robust measure of ecological distance, Plant. Ecol., **69**, 57-68 (1987).
31. **Bray, J. R. and Curtis, T. J.:** An ordination of the upland forest communities of southern Wisconsin, Ecol. Monogr., **27**, 325-349 (1957).
32. **Michie, G. M.:** Use of the Bray-Curtis similarity measure in cluster analysis of foraminiferal date, Math. Geosci., **14**, 661-667 (1982).
33. **Shiro, S., Yamamoto, A., Umehara, Y., Hayashi, M., Yoshida, N., Nishiwaki, A., Yamakawa, T., Saeki, Y.:** Effect of *Rj* genotype and cultivation temperature on the community structure of soybean-nodulating bradyrhizobia, Appl. Envrion. Microbiol., **78**, 1243-1250 (2012).

1 **34. Liu, H. and Logan, B. E.:** Electricity generation using an air-cathode single
2 chamber microbial fuel cell in the presence and absence of a proton exchange
3 membrane, Environ. Sci. Technol., **38**, 4040-4046 (2004).
4 **35. Shimoyama, T., Yamazawa, A., Ueno, Y., Watanabe, K.:** Phylogenetic analyses
5 of bacterial communities developed in a cassette-electrode microbial fuel cell,
6 Microbe. Environ., **24**, 188-192 (2009).
7 **36. Rabaey, K., Lissens, G., Siciliano, S. D., Verstaete, W.:** A microbial fuel cell
8 capable of converting glucose to electricity at high rate and efficiency, Biotechnol.
9 Lett., **25**, 1531-1535 (2003).
10 **37. Liu, H., and Logan, B. E.:** Electricity generation using a air-cathode single
11 chamber microbial fuel cell in the presence and absence of a proton exchange
12 membrane, Environ. Sci. Technol., **38,** 4040-4046 (2004).
13 **38. Ochi, S., Kamishima, O., Mizusaki, J., Kawamura, J.:** Investigation of proton
14 diffusion in Nafion ® 117 membrane by electrical conductivity and NMR, Solid.
15 State. Ionics., **180**, 580-584 (2009).
16 **39. Kiely, P. D., Rader, G., Regan, J. M., Logan, B. E.:** Long-term cathode
17 performance and the microbial communities that develop in microbial fuel cells fed
18 different fermentation endproducts, Bioresour. Technol., **102**, 361-366 (2011).
19 **40. Jung, S. and Regan, J. M.:** Comparison of anode bacterial communities and
20 performance in microbial fuel cells with different electron donors, Appl. Microbiol.
21 Biotechnol., **77**, 393-402 (2007).
22 **41. Aelterman, P., Freguia, S., Keller, J., Verstraete, W., Rabaey, K.:** The anode
23 potential regulates bacterial activity in microbial fuel cells, Appl. Microbiol.
24 Biotechnol., **78**, 409-418 (2008).
25 **42. Jung, S. and Regan, J. M.:** Influence of external resistance on electrogenesis,

methanogenesis, and anode prokaryotic communities in microbial fuel cells, Appl. Environ. Microbiol., **77**, 564-571 (2011).

43. Rismani-Yazdi, H., Christy, A. D., Carver, S. M., Yu, Z., Dehority, B. A., Tuovinen, O. H.: Effect of external resistance on bacterial diversity and metabolism in cellulose-fed microbial fuel cells, Bioresour. Technol., **102**, 278-283 (2011).

44. Torres, C. I., Karajmalnik-Brown, R., Parameswaran, P., Marucus, A. K., Wanger, G., Gorby, Y. A., Rittmann, B. E.: Selecting anode-respiring bacteria based on anode potential: phylogenetic, electrochemical, and microscopic characterization, Environ. Sci. Technol., **43**, 9519-9524 (2009).

45. Torres, C. I., Marcus, A. K., Parameswaran, P., Rittmann, B. E.: Kinetic experiments for evaluating the Nernst-Monod model for anode-respiring bacteria (ARB) in a biofilm anode, Environ. Sci. Technol., **42**, 6593-6597 (2008).

46. Srikanth, S., Marsili, E., Flickinger, M. C., Bond, D. R.: Electrochemical characterization of *Geobacter sulfurreducens* cells immobilized on graphite paper electrodes, Biotechnol. Bioeng., **99**, 1065-1073 (2008).

47. Dunaj, S. J., Vallino, J. J., Hines, M. E., Gay, M., Kobylijanec, C., Rooney-Varga, J. N.: Relationships between soil organic matter, nutrients, bacterial community structure, and the performance of microbial fuel cells, Environ. Sci. Technol., **46**, 1914-1922 (2012).

48. Miyahara, M., Hashimoto, K., Watanabe, K.: Use of cassette-electrode microbial fuel cell for wastewater treatment, J. Biosci. Bioeng., **11**, 176-181 (2013).

49. Ishii, S., Suzuki, S., Norden-Krichmar, T. M., Nealson, K. H., Sekiguchi, Y., Gorby, Y. A., Bretschger, O.: Functionally stable and phylogenetically diverse microbial enrichments from microbial fuel cells during wastewater treatment, PLos

1 One **7**, e30495 (2012).
2 **50. Adrian, N. R. and Arnett, C. M.:** Anaerobic biodegradation of Hexahydro-
3 1,3,5-trinitro- 1,3,5- triazine (RDX) by *Acetobacterium malicum* strain HAAP-1
4 isolated from a methanogenic mixed culture, Curr. Microbiol., **48**, 332-340 (2004).
5 **51. Nakamura, K., and Kamagata, Y.:** Recent topics on methanogenic syntrophs, J.
6 Environ. Biotechnol., **5,** 81-89 (2006). (in Japanese).

Figure Ledgends

Figure 1. SEM images of MEAs. (A) Cross-sectional SEM image of SPEEK-MEA. (I) SPPEK membrane, (II) cathode electrode. Bar means scale of 200 μm. (B) Cross-sectional SEM image of Nafion-MEA. (III) Nafion117 membrane, (IV) cathode electrode. Bar means scale of 200 μm.

Figure 2. (A) Electricity production from SPEEK-MFC (closed diamonds) and Nafion-MFC (gray diamonds) with 10 Ω of external resistance. The running era was separated into six stages; stage I (day 0-20), stage II (day 21-64), stage III (day 65-85), stage IV (day 86-126), and stage V (day 127 -163). The arrow means when the lake sediment was added again in the anode of MFCs. The SPEEK-MFC (black line) and the Nafion-MFC (gray line). (B) COD removal efficiency of the SPEEK-MFC (filled black diamonds) , the Nafion-MFC (Filled gray diamond), and control-MFC. (opened diamonds) (C) Coulombic efficiency of the SPEEK-MFC (black line) and the Nafion-MFC (gray line).

Figure 3. Results of chronopoteniometry analyses at (A) day 127and (B) day 147. Closed diamonds and gray squares indicate the data of anode potentials in SPEEK-MFC and Nafion-MFC, respectively. Open diamonds and open aquares indicate the data of cathode potentials in SPEEK-MFC and Nafion-MFC, respectively.

Figure 4. Monitoring of concentrations of organic acids in effluents from the organic waste-decomposing tank and MFCs. (A); the organic waste-decomposing tank, (B); SPEEK-MFC, (C); Nafion-MFC, (D); control-MFC. Red line; lactate, blue line;

butyrate, green line; propionate, black line; formate, orange line; acetate.

Figure 5. Multidimensional scaling (MDS) analyses based on DGGE profiles. (A); the organic waste-decomposing tank, (B); SPEEK-MFC, (C); Nafion-MFC, (D); control-MFC, (E); all plots were shown under the same scales. The number beside the plots means the sampling date and "B" beside the number means the biofilm sample.

Figure 6. Phylogenetic distribution of 16S rRNA gene clones from lake sediment at day 163, the organic-decomposing tank (the tank), anolytic and biofilm samples in SPEEK-MFC and Nafion-MFC at day 167. S; sediment sample, T; the tanke, SP-A; anolytic sample in SPEEK-MFC, SP-B; biofilm sample in SPEEK-MFC, Na-A; anolytic sample in Nafion-MFC, Na-B; biofilm sample in Nafion-MFC, CA; anolytic sample in control-MFC, CB; biofilm sample in control-MFC. The number above each bar indicates the total number of sequenced clones.

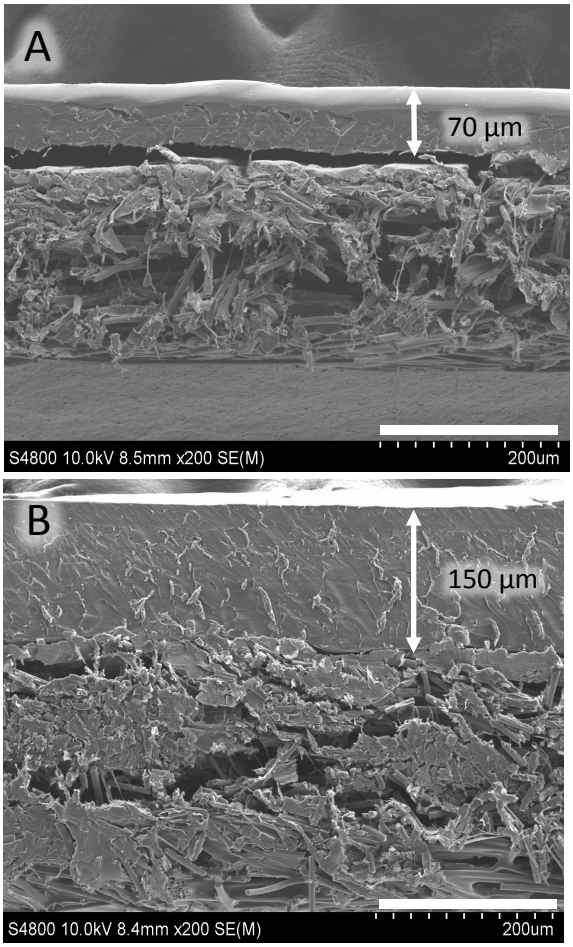

Figure 1. Suzuki et al.

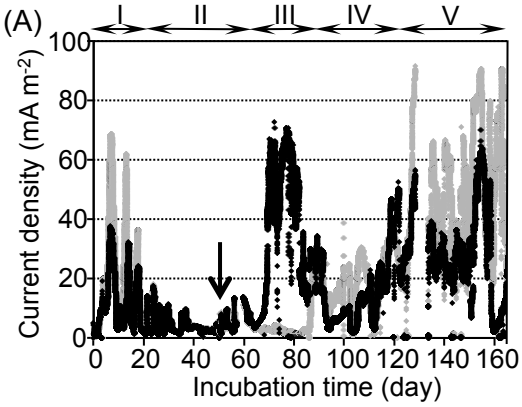

Figure 2. Suzuki et al.

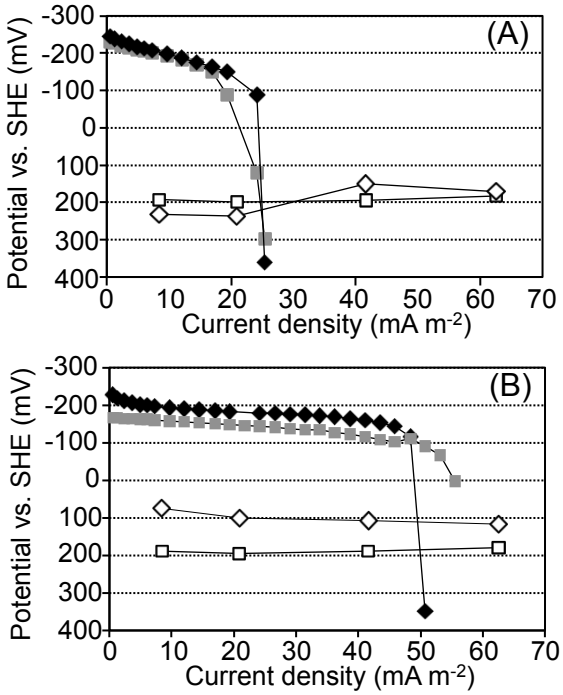

Figure 3. Suzuki et al.

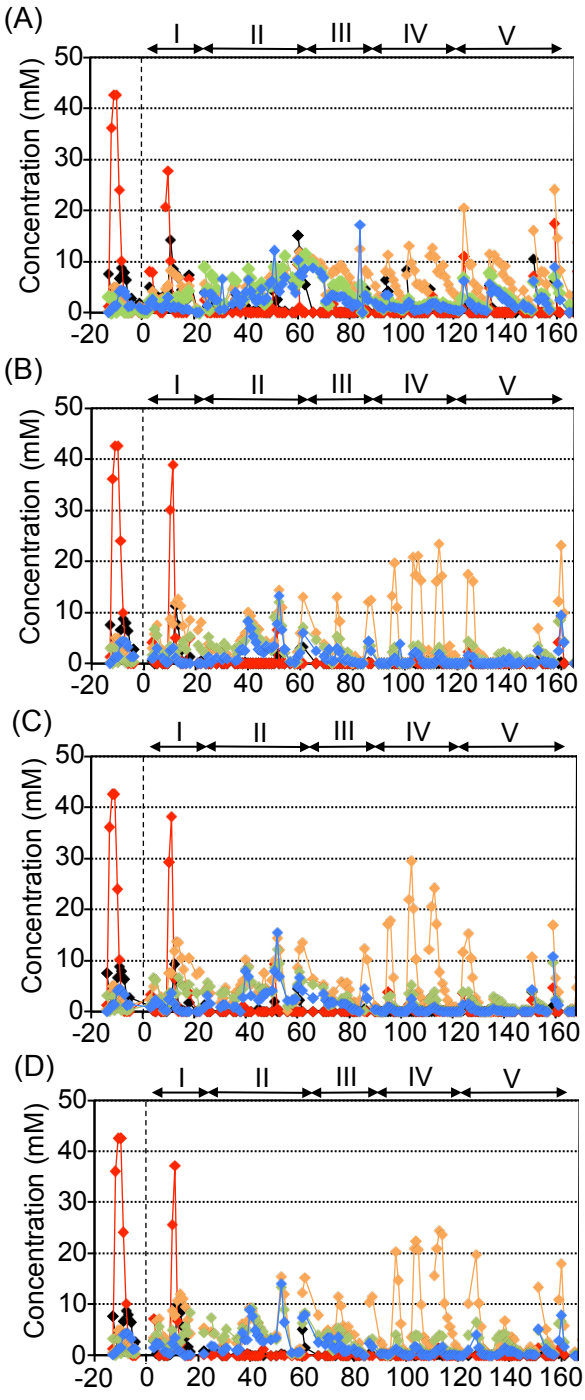

Figure 4. Suzuki et al.

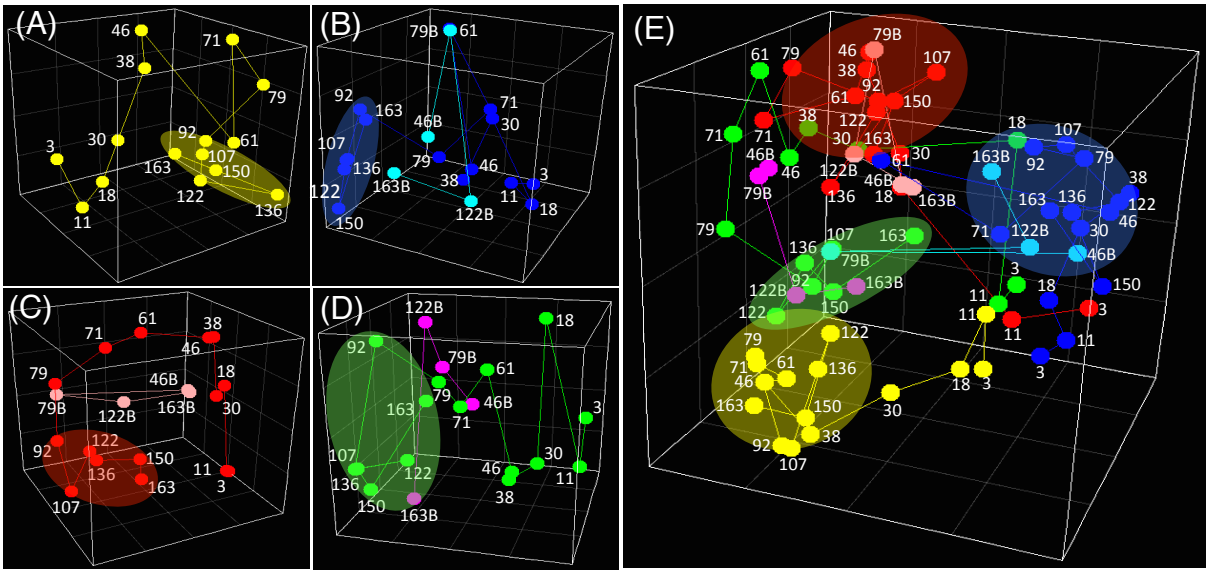

Figure 5. Suzuki et al.

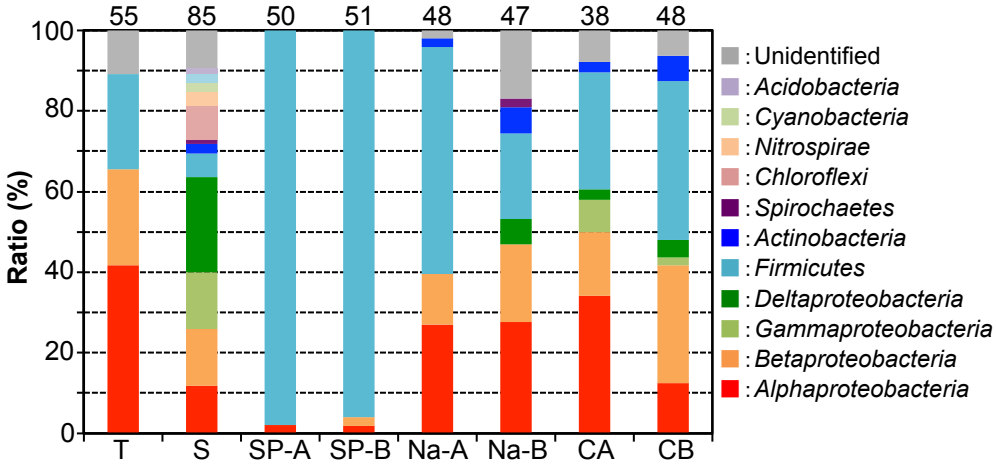

Figure 6. Suzuki et al.

TABLE 1. Electrochemical properties of SPEEK-MFC and Nafion-MFC.

Incubation time (d)	SPEEK-MFC				Nafion-MFC			
	V_{oc} (V)	I_{max} (mA m^{-2})	P_{max} (W m^{-3})	R_{in} (Ω)	V_{oc} (V)	I_{max} (mA m^{-2})	P_{max} (W m^{-3})	R_{in} (Ω)
3	0.59	10	0.73	2670	0.57	13	0.81	3490
79	0.52	64	2.8	270	NT[a]	NT[a]	NT[a]	NT[a]
127	0.58	25	1.5	940	0.50	32	2.5	310

[a]: Not tested.

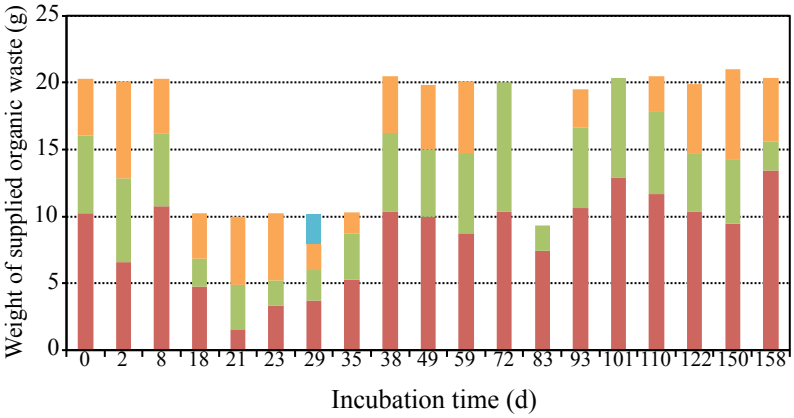

Figure S1.
The weight of organic waste supplied in the organic waste-decomposing tank. Red; rice, green; vegetable, orange; meat, and blue; fish.