Electricity generation from sweet potato-shochu waste using microbial fuel cells

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Electricity generation and treatment of sweet potato-shochu waste, acidic and organic-rich slurry, was examined using cassette-electrode microbial fuel cells (CE-MFCs). Among CE-MFCs with raw (73 g-chemical oxygen demand chromium COD_{Cr}/L) and different concentration of diluted sweet potato-shochu waste (0.5, 1, 5, 10, and 20 g-COD_{Cr}/L) without pH control, the maximum power density (1.2 W/m^3) and COD_{Cr} removal efficiency (67.4 ± 1.8%) were observed in the CE-MFCs with 10 g-COD_{Cr}/L shochu waste. The concentration of organic acid was decreased to below the quantification limits during the 9-day operation in the CE-MFC with 10 g-COD_{Cr}/L shochu waste. During the same period, the electrolyte pH was increased from 4.2 to 6.6. Microbial community analysis revealed that the genus *Clostridium* (75.4%) was predominant in the CE-MFCs with raw shochu waste, whereas *Bacteroides* (65.3%) and *Clostridium* (12.1%) were predominant in the CE-MFCs with 10 g-CODCr/L shochu waste.

Shochu is a traditional Japanese distilled spirit made from grain, such as rice, barley, and sweet potato. The amount of the shochu waste in sweet potato-shochu production is generally twice the volume of the produced sweet potato-shochu, which reaches approximately 8×10^5 tons per year in Japan (1). Shochu waste is generally acidic, and contains large amounts of organic compounds, ranging from 60 to 900 g/L in chemical oxygen demand (COD) (2). The Japanese central government enacted a regulation against dumping shochu waste in the sea in 2002, based on the protocol for the prevention of marine pollution by sea dumping (3); hence, the development of appropriate procedures to treat or use shochu waste is needed. Shochu waste can be roughly divided into solid and liquid content. The solid content has been used as swine and cow feed (4). The liquid content of shochu waste has been treated by aerobic treatment systems such as activated sludge treatment (5) which requires much energy and high operational costs. Methane fermentation is one option to effectively produce energy from the liquid content of shochu waste (1). However, there are several disadvantages, such as the production of hydrogen sulfide (H_2S) gas as a by-product during anaerobic digestion and the costly construction of anaerobic digestion facilities (6).

Microbial fuel cells (MFCs) are a bioreactor that can directly convert the chemical energy of organic compounds to electrical energy through the catalytic reaction of microorganisms (7). The advantages of MFCs compared with aerobic treatment and methane production are that (i) MFCs do not require energy converters for electricity generation (8), (ii) the theoretical energy recovery rate of MFCs may be higher than that of methane production because it is not limited by the Carnot cycle (9), (iii) the concern about greenhouse and toxic gas release may be avoidable (10), (iv) the release of odors from MFCs is negligible (11), (v) unlike aerobic processes, MFCs have the potential to reduce solid production (10). For these reasons, MFCs have been regarded as a promising technology for energy recovery from renewable organic waste (12).

A number of studies have reported attempts to generate electricity using MFCs with various kinds of organic substrates, such as winery wastewater (13), acidic food waste leachate (14) and wine lees (15). Among the factors influence the output, pH of electrolytes is a crucial for effective electricity generation in MFCs (16). Some studies indicated that neutral or a relatively high pH is ideal for increasing MFC performance. He et al. (17) investigated the effect of electrolyte pH change on the performance of an air-cathode MFC with feed solution (mainly composed of sodium acetate and phosphate buffer solution) between pH levels of 5 and 10, and concluded that the optimal pH for the air-cathode MFC was between 8 and 10. Yuan et al. (18) observed the morphology of biofilm using scanning electron microscopy (SEM) to investigate the effect of pH change on bacterial growth on the anode electrodes of two-chamber MFCs. The authors also have experimentally demonstrated that alkaline pH condition was favorable for enhancing the electrochemical interaction between bacteria and anode electrodes in the two-chamber MFCs. In addition, Kim et al. (19) reported that low pH distillery wastewater (pH < 4) was effectively used as the substrate for electricity generation by single-chamber MFCs consisting of two separator-electrode assemblies. The studies described above showed that the optimal pH condition

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for effective MFC operation depends on the type of substrate and MFC configuration. However, the electricity-producing mechanisms and its associated microorganisms under acidic condition are not well-understood yet.

MFC is a promising technology and its applicability for varieties of organic waste has been demonstrated. However, it has not been reported that MFC can be used for treatment of shochu waste. The objective of this study was to establish a practical platform for shochu waste treatment combined with electricity generation using MFCs. In this study, we constructed cassette electrode-MFCs (CE-MFCs) using sweet potato-shochu waste without any pH control and determined the optimal concentration for effective electricity generation and COD removal. Moreover, the microbial community analysis suggested that the key players for electricity generation and COD removal in the MFC were genus *Clostridium* and *Bacteroidetes*. To the best of our knowledge, this study is the first example in which applicability of MFC for sweet potato-shochu waste without pH control as the substrate was demonstrated.

MATERIALS AND METHODS

Shochu waste The shochu waste used in this study was produced during fermentation of sweet potato (Kogane-sengan) in the distillation process of shochu at the Watanabe Distillery Co., Ltd. in Miyazaki City, Japan. After the distillation process, the sweet potato-shochu waste was immediately collected from a waste storage tank and stored at $-20\,^\circ$C until use. The COD_{Cr} concentration and pH of the collected sweet potato-shochu waste were approximately 73 g/L and pH 4.1. The analytical methods of COD_{Cr} and pH measurement are described below.

MFC operation CE-MFCs were constructed as previously described (20,21) with modification to the reactor size (Fig. S1). Graphite felt (Sogo Carbon) was cut into pieces (12 cm $\times$ 3.5 cm with 0.5-cm thickness), and those were used as anode electrodes (projected surface area of 99.5 cm^2). Air cathodes consisted of carbon cloth (TCC-3250, Toho Tenax; 16.8 cm $\times$ 2.5 cm) with four layers of PTFE and 0.69 mg-Pt cm^{-2} (projected surface area of 84 cm^2). Ten-time diluted sweet potato-shochu waste with a cassette-electrode composed of the carbon-felt anode electrode and the air cathode was inserted into a plastic cylinder (350 mL reactor volume), and sealed with a rubber stopper with square holes for open air configuration. Oxygen in the headspace gas of the CE-MFC was purged with N$_2$/CO$_2$ gas (80:20; v/v). The CE-MFC with ten-time diluted sweet potato-shochu waste (pH 4.0) was operated for more than 50 days in the trial operation.

After the trial operation, sweet potato-shochu waste diluted with ultrapure water to 0.5, 1, 5, 10 and 20 g-COD$_{Cr}$/L (pH were 4.5, 4.5, 4.3, 4.2, and 4.2, respectively) was used to determine the optimal concentration for effective electricity generation by CE-MFCs. A total of 300 mL of diluted or raw sweet potato-shochu waste (approximately 73 g-COD$_{Cr}$/L, pH 4.1) was poured into CE-MFCs. One milliliter of inocula were added to all the CE-MFCs in this study in order to stable establishment of microbial consortia. The inocula were prepared from anode biofilm of 4-month-operated CE-MFC with 5-times diluted sweet potato-shochu waste (approximately 15 g-COD$_{Cr}$/L). The anode biofilm were suspended in phosphate buffered saline (pH 7.4) and used as the inocula. The MFCs with different concentration of the sweet potato-shochu waste were operated as the same manner with the trial operation at 30 $^\circ$C for 92 days. During the operation, the electrolyte in each CE-MFC was continuously mixed with a magnetic stirrer at 600 rpm. All the CE-MFCs were operated in semi-batch mode, in which a half volume of the electrolyte in each CE-MFC was replaced with a fresh one when the power output of the CE-MFCs decreased lower than approximately 200 mV. Because the power output of the CE-MFC with 1 g-COD$_{Cr}$/L was continuously low from the beginning (0.0047 mW/m^3 on average), the operation of the CE-MFC was stopped at 60 days. Because the power output of CE-MFC with 0.5 g-COD$_{Cr}$/L was also continuously low from the beginning (0.041 mW/m^3), the electrolyte was replaced with 20 g-COD$_{Cr}$/L sweet potato-shochu waste after 24 days of operation. The power output data of CE-MFCs were automatically recorded every 30 s by a data logger (GL820; Graphtec, Tokyo, Japan). The external resistance in each CE-MFC was initially set at 470 Ω and subsequently changed it in order to maintain power output as high as possible. Current (I) and power density (P) were calculated as $I = V/R$ and $P = V^2/R$, respectively, where V is voltage measured by the data logger and R is the external resistance. Polarization curves were obtained by a potentiostat (G300 poteitiostat; Gamry Instruments, Warminister, PA, USA) at a scan rate of 1 mV/s from -1 V to $+0.05$ V as previously described (20).

Chemical analyses COD$_{Cr}$ concentration was measured by COD$_{Cr}$ High Range kit TNT 822 (Hach, Loveland, CO, USA) and a spectrophotometer (DR2800; Hach) according to the manufacturers' instructions. A portion (20-50 μL) of the electrolytes in the CE-MFCs was used for pH measurement by a pH meter (B-212 Twin Compact pH meter, Horiba, Kyoto, Japan). Organic acid concentration (acetic acid, propionic

acid, succinic acid, and citric acid) was analyzed by high-performance liquid chromatography (HPLC) (LC-20AD, UV/Vis detector SPD-20A, Shimadzu, Kyoto, Japan) fitted with a STR-ODS-II column (8 mm i.d. $\times$ 300 mm; Shimadzu). An eluent (100 mM perchloric acid) was fed as solvent at a flow rate of 0.8 ml/min at 40 $^\circ$C. The organic acid described above was identified by co-chromatography using the standard reagents purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Microbial community analysis Anode biofilm and electrolyte were collected from each of the CE-MFCs with raw sweet potato-shochu waste (73 g-COD$_{Cr}$/L) and 10 g-COD$_{Cr}$/L shochu waste. Sample collection of the CE-MFCs, especially for anode biofilms, could influence power output and change the operational conditions. Then, the sampling was done at the last period of operation (after 92 days of operation [October 15, 2015]). Total DNA was extracted by an Extrap Soil DNA Kit Plus ver. 2 (Nippon Steel & Sumikin Eco-Tech Co., Tokyo, Japan). 16S rRNA gene fragments (V4–V5 region) were amplified with universal primers U515F (5'-GTGYCAGCMGCCGCGGTA-3') and 926R (5'-CCGYCAATTCMTTTRAGTT-3') (22). Reaction mixtures for Polymerase chain reaction (PCR) contained 10$\times$ Ex Taq buffer (Takara Bio, Inc., Shiga, Japan), 0.2 mM dNTP Mixture (Takara Bio), 0.5 μM each primer, 0.05 U of Ex Taq polymerase (Takara Bio), and 1 ml template DNA in a 20-ml volume. PCR was performed with the following cycling condition: hot start at 95 $^\circ$C for 9 min, followed by 15–35 cycles consisting of denaturation (95 $^\circ$C for 15 s), annealing (55 $^\circ$C for 15 s), and extension (72 $^\circ$C for 30 s) and a final extension at 72 $^\circ$C for 2 min. The number of amplification cycles were 35 cycles for DNA extracted from raw sweet potato-shochu waste, 15 and 19 cycles for DNA extracted from the anode biofilm and the electrolyte of the CE-MFC with 73 g-COD$_{Cr}$/L sweet potato-shochu waste, 20 and 20 cycles for DNA extracted from the anode biofilm and the electrolyte of the CE-MFCs with 10 g-COD$_{Cr}$/L sweet potato-shochu waste, respectively. The libraries were sequenced using 2 $\times$ 250-bp paired-end sequencing on an Illumina MiSeq platform (Illumina, San Diego, CA, USA). The primer sequences were trimmed from the paired-end sequences. The trimmed sequences were merged using FLASH (23) with the default settings (e.g., a minimum of 10-bp overlap). The merged sequences were processed using QIIME followed by removal of chimeric sequences identified by UCHIME (24). Clustering was performed using the unweighted pair group method with arithmetic mean (UPGMA) in QIIME, and similar sequences were assigned into operational taxonomic units (OTUs) at 97% identity threshold. The representative sequences were aligned using the Greengenes database (25) at the same percent identity as the OTU picking threshold (97%).

Nucleotide accession no. Amplicon sequences of the 16S rRNA genes of the OTU mentioned above were deposited in NCBI BioProject PRJDB7036. The nucleotide sequences from raw sweet potato shochu waste, the anode biofilm of the CE-MFCs with raw sweet potato-shochu waste, the electrolyte of the CE-MFCs with raw sweet potato-shochu waste, anode biofilm of the CE-MFCs with 10 g-chemical oxygen demand chromium (COD$_{Cr}$)/L sweet potato-shochu waste, and the electrolyte of the CE-MFCs with 10 g-COD$_{Cr}$/L sweet potato-shochu waste are available through accession numbers LC408665–LC408956, LC407426–LC407879, LC408161–LC408664, LC407104–LC407425 and LC407880–LC408160, respectively.

RESULTS AND DISCUSSION

Optimal sweet potato-shochu waste concentration for electricity generation and COD$_{Cr}$ removal In a trial operation, the CE-MFC with ten-time diluted sweet potato-shochu waste (pH 4.1, 0.5 L reactor volume) exhibited power output after 4 days of operation, and its maximum power density was 0.44 W/m^3 at 8 days (Fig. S2). The result showed that sweet potato-shochu waste can be used as electrolyte for electricity generation by MFCs. In general, the optimal pH for effective electricity generation by MFCs is neutral or relatively high pH (26). However, the CE-MFC with sweet potato-shochu waste used in this study exhibited power output without pH control, although shochu waste, including sweet potato-shochu waste, is an acidic, organic acid-rich slurry.

The performance of the CE-MFCs with serially diluted sweet potato-shochu waste (0.5, 1, 5, 10, and 20 g-COD$_{Cr}$/L) and raw sweet potato-shochu waste (73 g-COD$_{Cr}$/L) was examined to determine the optimal concentration for effective electricity generation and COD$_{Cr}$ removal by CE-MFCs. The power output of the CE-MFCs was observed in all experimental conditions without pH control, as well as the trial operation (Fig. 1). The average power densities for the initial 30 days were 0.0002, 0.19, 0.30, 0.22 and 0.08 W/m^3 in the CE-MFCs with 1, 5, 10, 20, and 73 g-COD$_{Cr}$/L sweet potato-shochu waste, respectively (the electrolyte in the CE-MFC with

0.5 g-COD$_{Cr}$/L was replaced by 20 g-COD$_{Cr}$/L sweet potato-shochu waste after 24 days of operation [average power density was 0.041 mW/m^3]). The maximum power density was 0.018 (day 18), 0.015 (day 18), 1.1 (day 23), 1.2 (day 14), 0.64 (day 44) and 0.27 W/m^3 (day 32), in the CE-MFCs with 0.5, 1, 5, 10, 20 and 73 g-COD$_{Cr}$/L sweet potato-shochu waste, respectively (Fig. S3A, B). These results indicated that using 10 g-COD$_{Cr}$/L sweet potato-shochu waste as an electrolyte of CE-MFC may be a better choice than using other concentrations of sweet potato-shochu waste in terms of electricity generation (Fig. S3C).

The power output of CE-MFCs with 0.5 and 1 g-COD$_{Cr}$/L was continuously low, probably because of inadequate organic compound concentration. After replacing the 0.5 g-COD$_{Cr}$/L sweet potato-shochu waste with 20 g-COD$_{Cr}$/L sweet potato-shochu waste, its maximum power density was substantially increased to 0.64 W/m^3 on day 44, but still lower than the maximum power density in the CE-MFC with 10 g-COD$_{Cr}$/L sweet potato-shochu waste. From all the results described above, more than 5 g-COD$_{Cr}$/L of sweet potato-shochu waste, at least, is necessary to effectively generate electricity, whereas too many organic compounds in the electrolytes of MFCs might not provide effective electricity generation. The CE-MFC with 73 g-COD$_{Cr}$/L (raw) sweet potato-shochu waste showed a sharp decrease in power density. Özkaya et al. (27) investigated electricity generation in a two-chamber Ti–TiO$_2$ electrode-MFC continuously fed with young landfill leachate at varying strengths of wastewater (1–50 COD-g/L) and hydraulic retention times (HRT) (0.25–2 days). In that study, the electricity generation was increased according to the increase in the strength of leachate wastewater and the decrease in HRT up to an organic

loading rate of 100 g-COD/L/day. In addition, a further increase in organic loading rate to 200 g-COD/L/day caused a sharp decrease in electricity generation, implying that the electrons derived from the oxidation of organic compounds flowed into a non-electricity-generating process, such as methane production, under a high organic loading rate condition. Similarly, power outputs of the CE-MFCs with more than 20 g-COD$_{Cr}$/L sweet potato-shochu waste were lower than those of CE-MFCs with 10 g-COD$_{Cr}$/L sweet potato-shochu waste in this study, indicating that the electrons were diverted to non-electricity-generating processes in the CE-MFCs with more than 20 g-COD$_{Cr}$/L sweet potato-shochu waste. Moreover, as mentioned below, pH was kept lower in the CE-MFC with 73 g-COD$_{Cr}$/L sweet potato-shochu waste than that with 10 g-COD$_{Cr}$/L sweet potato-shochu waste, implying the low pH also influenced the power output in the MFCs with sweet potato-shochu waste as discussed below. Power outputs of all the examined MFCs were gradually decreased overall (Fig. 1). So far, why the power output decreased is not clear but the cathode electrodes were covered with fibers probably derived from sweet potato when the CE-electrodes were disassembled in order to anode biofilm collection. The fibers maybe inhibited cathode reaction on the surface of the cathode electrodes by preventing proton from accessing to the cathode surface.

The COD$_{Cr}$ removal efficiency was calculated as; [the initial COD$_{Cr}$ concentration – before the first semi-batch COD$_{Cr}$ concentration]/the initial COD$_{Cr}$ concentration × 100 (%). COD$_{Cr}$ of a CE-MFC with 10 g-CODCr/L shochu waste under open-circuit condition was increased to 17.6 g/L (removal efficiency was −75.5%) after 10 days operation probably because the solid materials, such as
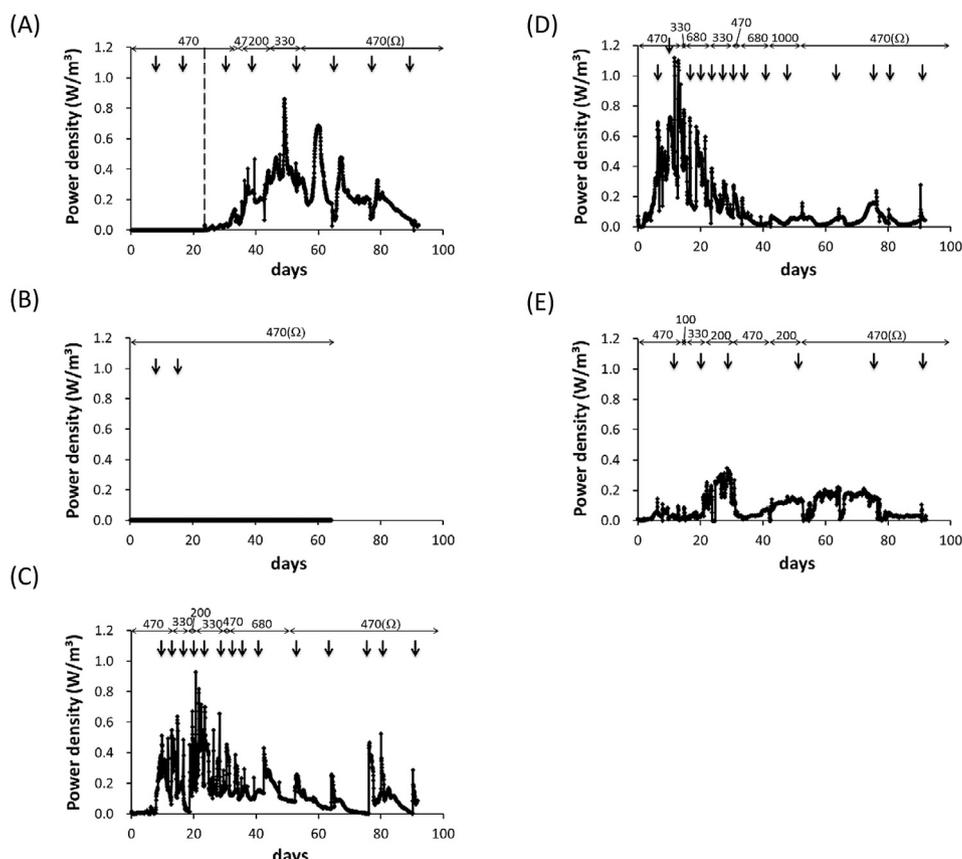

FIG. 1. Power density of the cassette-electrode microbial fuel cells (CE-MFCs) with different concentrations of sweet potato-shochu waste; (A) 0.5 and 20 g-chemical oxygen demand chromium (COD$_{Cr}$)/L, (B) 1 g-COD$_{Cr}$/L, (C) 5 g-COD$_{Cr}$/L, (D) 10 g-COD$_{Cr}$/L, (E) 73 g-COD$_{Cr}$/L (raw sweet potato-shochu waste). Arrows indicate the time points of semi-batch treatment, in which the electrolyte in each CE-MFC was replaced with a fresh one. Double-headed arrows and the numbers above them indicate time periods and external resistance. The dotted line in panel A indicates the time when the concentration of the electrolyte in the CE-MFC with 0.5 g-COD$_{Cr}$/L was replaced by 20 g-COD$_{Cr}$/L sweet potato-shochu waste.
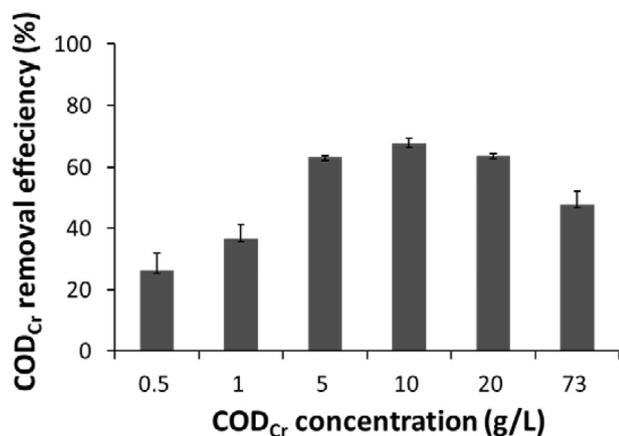
FIG. 2. Chemical oxygen demand chromium (COD_{Cr}) removal efficiency of the cassette-electrode microbial fuel cells (CE-MFCs) with 0.5, 1, 5, 10, 20 COD_{Cr}/L (diluted sweet potato-shochu waste) and 73 g-COD_{Cr}/L (raw sweet potato-shochu waste). Error bars indicate standard error from triplicate measurement.

cellulosic fibers, were degraded and became detectable compounds by COD measurement. The COD_{Cr} removal efficiency of the CE-MFCs with 0.5, 1, 5, 10, 20 and 73 g-COD_{Cr}/L were $26.2 \pm 5.8\%$ (day 0−8), $36.5 \pm 4.8\%$ (days 0−8), $63.1 \pm 0.4\%$ (day 0−9), $67.4 \pm 1.8\%$ (day 0−9), $63.4 \pm 0.7\%$ (day 24−34) and $47.7 \pm 4.4\%$ (day 0−14), respectively (Fig. 2). These results clearly demonstrated that MFC under closed-circuit conditions enhanced the COD_{Cr} removal, comparing with the COD_{Cr} increase of CE-MFC under open-circuit conditions. Among the examined concentrations, the highest COD_{Cr} removal efficiency was achieved by the CE-MFC with 10 g-COD_{Cr}/L. Taken together with the results described above, it is possible that using 10 g-COD_{Cr}/L sweet potato-shochu waste as electrolytes of CE-MFCs is the best choice in terms of electricity generation and COD_{Cr} removal.

Coulombic efficiency was relatively low (1.7%) in the CE-MFC with 10 g-COD_{Cr}/L sweet potato-shochu waste, comparing with other articles using brewery wastewater (Table 1), probably because abundant organic compounds in the shochu waste could be utilized for biomass growth rather than current generation under acidic condition where effective current-producing bacteria cannot grow. Other possible factors influencing Coulombic efficiency are existence of alternative electron acceptor, such as oxygen, nitrate and sulfate, and methanogenesis, which have not been measured in this study.

The relationship between the change in pH levels and organic acid concentrations in MFCs Shochu waste contains more than 90% water (1). Watanabe et al. (5) reported that shochu waste supernatant (which was obtained by centrifuging sweet potato-shochu waste at 7000 rpm for 10 min) contains 3.5 g/L of organic acid. The abundance of organic acid in shochu waste leads to a low pH value of around 4. In this study, to better understand the relationship between the change in pH and organic acid concentration in MFCs, the pH and the concentration of acetic acid, propionic acid, succinic acid, and citric acid were measured in the CE-MFC with 10 g-COD_{Cr}/L sweet potato-shochu waste (Fig. 3). Each organic acid concentration was measured on days 0, 9, 14, and 20, when a half volume of the electrolyte in CE-MFC with 10 g-COD_{Cr}/L was replaced with a fresh one as a semi-batch mode. The initial concentration of acetic acid, citric acid, and succinic acid were 880 mg/L, 390 mg/L and 67.0 mg/L, respectively, whereas the propionic acid concentration was below the quantification limit (<50 mg/L). the semi-batch), all the measured organic acid concentrations were decreased to below the quantification limits After 9 days of operation (just before the first semi-batch

treatment), the measured concentrations of acetic acid, propionic acid, succinic acid, and citric acid were decreased to below the quantification limits, 100 mg/L, 50 mg/L, 50 mg/L and 50 mg/L, respectively. At the same time, the pH was increased to 6.6 in the CE-MFC with 10 g-COD_{Cr}/L sweet potato-shochu waste (the initial pH of 10 g-COD_{Cr}/L sweet potato-shochu waste was 4.2). A similar phenomenon (the rise in pH of electrolytes in MFCs) was observed in a previous study using other kinds of organic acid. Li et al. (14) has experimentally demonstrated that the oxidation of volatile fatty acid led to the anolyte pH increase from approximately 4.9 to 6.2 (obtained using ImageJ software) after 12 days of operation in the control-MFC (food waste leachate only) with acidic food waste leachate.

In this study, the pH of the electrolytes in the CE-MFCs with 0.5, 1, 5, and 73 g/L was also measured for 20 days (Fig. 4). The initial pH of the electrolytes in the CE-MFCs with 0.5 and 1 g-COD_{Cr}/L sweet potato-shochu waste were pH 4.5 and 4.4, respectively, but after 8 days of operation (just before the first semi-batch), the pH values were increased to 5.3 and 5.8, respectively. The pH of the electrolytes in the CE-MFCs with 5 and 10 g-COD_{Cr}/L sweet potato-shochu waste was quickly increased compared with other experimental conditions, and the results were consistent with their higher maximum power density and COD_{Cr} removals. In the CE-MFCs with 73 g-COD_{Cr}/L (raw) sweet potato-shochu waste, the pH was gradually increased, but still acidic (approximately pH 5.0) even after 10 days of operation.

In general, the anode electrolyte of MFC is acidified during operation because protons are produced by the oxidation of organic compounds through bacterial metabolism (28). Oliveira et al. (29) described that continuous operation of an MFC leads to the acidification of the anode electrolyte because of slow and incomplete proton diffusion and migration through an ion-exchange membrane. In this study, the pH of the electrolytes in the CE-MFCs was increased in all the experimental conditions. All the measured organic acid concentrations with 10 g-COD/L were decreased to below detection limits after 9 days of operation. According to the composition of shochu waste, organic acids are the primary acid source. Therefore, the reaction rate of organic acid consumption seems to be much higher than the proton generation rate at the anode. Acidic conditions are favorable to the cathode reaction because it is a proton-consuming reaction. From this point of view, the anode reaction seems to be rate limiting in the MFC using shochu waste.

The power output of the MFCs in this study were relatively lower than previous studies using brewery wastewater with different types of MFCs and synthetic wastewater with CE-MFCs (Table 1). In the previous studies in Table 1, pH values of the electrolyte were around neutral, whereas those of this study were around 4. However, power output of MFCs using low-pH distillery wastewater was much higher than that of this study (19). In that study, carbon nanotubes were used for cathode reaction by which the reaction was more effective coupled with sufficient supply of proton from the acidic electrolyte. In MFCs using low-pH electrolyte, efficient cathode reaction may be more important than other power limiting factors.

Microbial community structure in shochu waste MFCs The microbial community structure of five samples, (i) raw sweet potato-shochu waste, (ii) the anode biofilm of the CE-MFCs with 10 g-COD_{Cr}/L sweet potato-shochu waste, (iii) the electrolyte of the CE-MFCs with 10 g-COD_{Cr}/L sweet potato-shochu waste, (iv) the anode biofilm of the CE-MFCs with 73 g-COD_{Cr}/L (raw) sweet potato-shochu waste, and (v) the electrolyte of the CE-MFCs with 73 g-COD_{Cr}/L (raw) sweet potato-shochu waste, was analyzed to investigate the microorganisms associated with converting chemical energy to electrical energy in the CE-MFCs. The anode biofilm and the electrolyte samples were collected from the CE-

TABLE 1. Performance of MFCs with brewery waste and MFCs using cassette electrode system.

Substrate	Type of MFC	Anode/Cathode material	Reactor volume (L)	pH	mW/m^2	W/m^3	COD removal (%)/ operation time or HRT[a]	Coulombic efficiency (%)	Reference
Brewery wastewater	Single chamber	Carbon cloth/Air-cathode	0.028	6.5	205	5.1	87/4 days	27	44
Beer Brewery wastewater	Single chamber	Carbon fiber/Air-cathode	0.1	6.6	264	9.52	43/2.13 h HRT[a]	19.75	45
Brewery wastewater	10 L serpentine-type	Graphite felt/Air-cathode	10	6.69	97.2	6	86.4/10 days	7.6	46
Distillery wastewater	Single chamber	Graphite felt/Air-cathode	0.65	4	280	12.9	–	–	19
Distillery wastewater	Single chamber	Plain graphite plate/Air-cathode	0.5	6[b]	124	1.7	72.8/–	–	47
Distillery wastewater	Anaerobic fluidized bed	Carbon fiber paper/Carbon fiber paper	7.27	6.89–7.5[b]	124	0.27	89.95/22.5 h HRT	–	48
Distillery wastewater	Dual chamber	Plain graphite plate/Plain graphite plate	0.25	8	202	–	63.5/–	–	49
Bad wine	Two chamber	Carbon felt/Graphite	0.125	6.4–7.0[b]		3.82	41/72 h	45	50
Red wine lees	Single chamber	Graphite fiber brush/Air-cathode	0.028	7.0[b]	111	–	27/–	9	15
White wine lees	Single chamber	Graphite fiber brush/Air-cathode	0.028	6.92[b]	262	–	90/–	15	15
Winery wastewater	Single chamber cubic-shaped	Ammonia treated graphite brush/Air-cathode	0.028	7.2	–	31.7	65/1 day	18	51
Acidic food waste	Two chamber	Carbon felt/Carbon felt	0.0756	4.76	–	0.432	95/–	20	14
Shochu waste	Casette electrode	Graphite felt/Air-cathode	0.3	4.1	42.9	1.2	67.4/9 days	1.7	This study
Cattle manure	Casette electrode	Graphite felt/Air-cathode	0.55	8.6	765	16.3	56.7/10 days	26.4	20
Syntheic wastewater	Casette electrode	Graphite felt/Air-cathode	1	7–8[b]	150	–	80/6 h HRT	20	52
Syntheic wastewater	Casette electrode	Graphite felt/Air-cathode	1	7–8[b]	188	–	85/12 h HRT	30	53

[a] HRT, hydraulic retention time.
[b] The pH was adjusted.

MFCs after 92 days of operation. A previous study demonstrated that microbial communities were stable after 140 days in a cellulose-fed MFC (30). We then hypothesized that microbial community structure in the CE-MFCs would also be stable after the operation periods in this study. By the sequence analysis of samples i–v described above, 101,619, 50,043, 54,433, 106,451, and 152,907 high-quality raw sequence reads (the average length of each read was 420 bp) were obtained, respectively.

According to homology search results, in the raw sweet potato-shochu waste sample (before used in MFC operation), the genus *Bacillus* was the most abundant bacteria (40.9%), followed by *Bacteroides* (8.4%) and *Methanobacterium* (4.5%) (Fig. 5A). Some microorganisms belonging to *Bacillus* are acidophilic thermophiles (31). It is not surprising that such thermophiles or thermotolerant microorganisms were predominant in the raw sweet potato-shochu waste sample because the distillation process of shochu is carried out at high temperature. Some of microorganisms belonging to *Bacillus* are acid-tolerant (32). Therefore, the acidic shochu waste might provide another advantageous selective pressure for the survival of the *Bacillus*. To the best of our knowledge, this is the first study of microbial structure of sweet potato-shochu waste by next-generation sequencing method, revealing the predominant microorganisms.

In both the anode biofilm and electrolyte samples collected from CE-MFC with 10 g-COD$_{Cr}$/L sweet potato-shochu waste, the most abundant genus were *Bacteroides* (Fig. 5B,C), implying that *Bacteroides* spp. bacteria were involved in electricity generation. In the anode biofilm sample, the second most abundant genus were *Clostridium* (12.1%), followed by *Ruminococcus* (4.1%), whereas the bacterial abundance in the electrolyte sample were opposite (8.6% for *Ruminococcus* and 7.8% for *Clostridium*). *Bacteroides* and *Clostridium* have often been detected in anode biofilms of MFCs (33,34). Meanwhile, in this study, only five of 50,043 raw sequence reads were identified as *Geobacteraceae*, which is one of the most important bacteria associated with electricity generation in MFCs (35). Previous studies described that the cooperative relationship between *Geobacter* and acetate-producing bacteria, such as the bacteria belonging to *Bacteroides* and *Clostridium*, provide an efficient electricity generation in MFCs by continuous organic compound degradation (*Bacteroides* and *Clostridium*) and direct conversion of chemical energy to electrical energy (*Geobacter*) (14,36). The *Geobacteraceae* are represented by the genus *Geobacter*

including *Geobacter sulfurreducens*, which is an electricity-generating bacteria in pure-culture MFCs with a higher power density than any other microorganism (37,38). However, in this study, bacteria other than *Geobacter* appear to play key roles in electricity generation in the CE-MFCs with sweet potato-shochu waste. In Sasaki et al. (39), the number of *Geobacteraceae* cells and the current density in CE-MFCs using synthetic wastewater were decreased according to the decrease in the pH of electrolytes. In this study, the low pH condition seems to lead to a very small number of *Geobacter* in the anode biofilm. Instead, *Clostridium* and/or *Bacteroides* may be the key players for electricity generation under acidic conditions.

In both the anode biofilm and electrolyte samples collected from the CE-MFC with 73 g-COD$_{Cr}$/L (raw) sweet potato-shochu waste, the most dominant genus were *Clostridium*, followed by
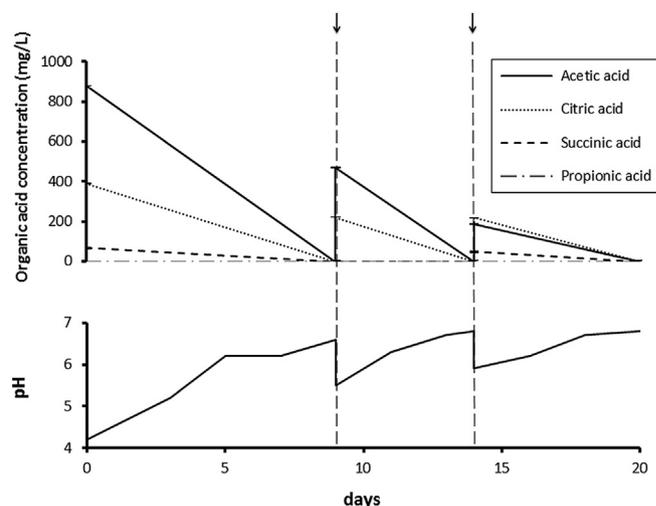

FIG. 3. Change in pH and organic acid concentration in the electrolyte of the cassette-electrode microbial fuel cell (CE-MFC) with 10 g-chemical oxygen demand chromium (COD$_{Cr}$)/L sweet potato-shochu waste. Arrows and dotted lines indicate the times of semi-batch treatment, in which the electrolyte in each CE-MFC was replaced with a fresh one. Error bars indicate standard error from triplicate measurement (organic acid concentration).
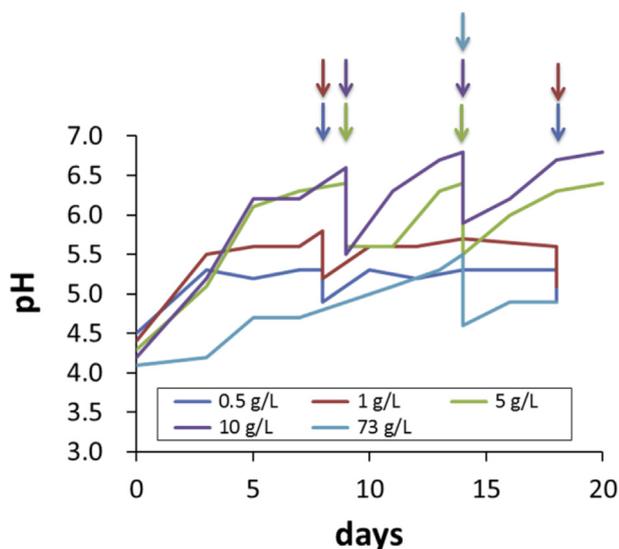
FIG. 4. pH change of the electrolytes in the cassette-electrode microbial fuel cells (CE-MFCs) with 0.5, 1, 5, and 10 chemical oxygen demand chromium (COD_{Cr})/L (diluted sweet potato-shochu waste) and 73 g-COD_{Cr}/L (raw sweet potato-shochu waste) during the initial 20 days of operation. Arrows indicate the times of semi-batch treatment, in which the electrolyte in each CE-MFC was replaced with a fresh one.

Rummeliibacillus (Fig. 5D,E). *Clostridium* was also detected in the raw sweet potato-shochu waste (1.1%), but the proportion of *Clostridium* was significantly increased during electricity generation. A previous study reported that *Clostridium acetobutylicum* and *C. thermohydrosulfuricum* used cellulosic waste for electricity generation in MFCs (40). Other literature has reported that *C. acetobutylicum* showed the ability to generate electricity in a graphite electrode-MFC using Clostridial growth medium containing glucose (41). Park et al. (42) have reported that *Clostridium* sp. EG3 isolated from a mediator-less microbial fuel cell using starch

processing wastewater has electrochemical and Fe (III)-reducing activities under neutral or relatively low pH conditions. Combined with the results of this study, *Clostridium* was suggested to be involved in electricity generation in MFCs using sweet potato-shochu waste.

Among the closest relatives to OTUs at species level in the samples analyzed in this study, an OTU identified in raw sweet potato-shochu waste showed similarity with *Bacillus flexus*, which has been known to be an electricity-generating microorganism (43) (Table S1). However, taxonomically any closest species to the identified OTUs in MFC samples were not known to be electricity-generating bacteria. This result implies that microorganisms involved in electricity generation in the MFCs using shochu waste might not be known electricity-generating bacterial species.

There were clear difference in the microbial structures between MFCs with 10 g- and 73 g-COD_{Cr}/L sweet potato-shochu waste, whereas the same inoculum was used. The raw and diluted sweet potato-shochu waste used for semi-batch treatment contained a variety of microorganisms alive and the microbial community structures were formed during the operation. Based on these facts, what defined the microbial community structure of the sweet potato-shochu waste seems to be the concentration of the organic compounds rather than inoculum in this study. However, the inocula used in this study were derived from trial experiments using around 10 g-COD_{Cr}/L sweet potato-shochu waste, and, thus, it might influence the optimal organic concentration.

To the best of our knowledge, this is the first study of electricity generation using sweet potato-shochu waste by MFCs. The microbial community analysis also firstly revealed that *Bacteroidetes* and *Clostridium* were the key players for treatment and/or electricity generation in the MFCs using sweet potato-shochu waste. Although the mechanisms of electron transfer to anode electrodes in MFCs under acidic conditions remain unclear, the results of microbial community analysis in this study suggested that the potential candidates as key players of electricity generation in MFCs under acidic conditions. Further studies of these microorganisms, such as
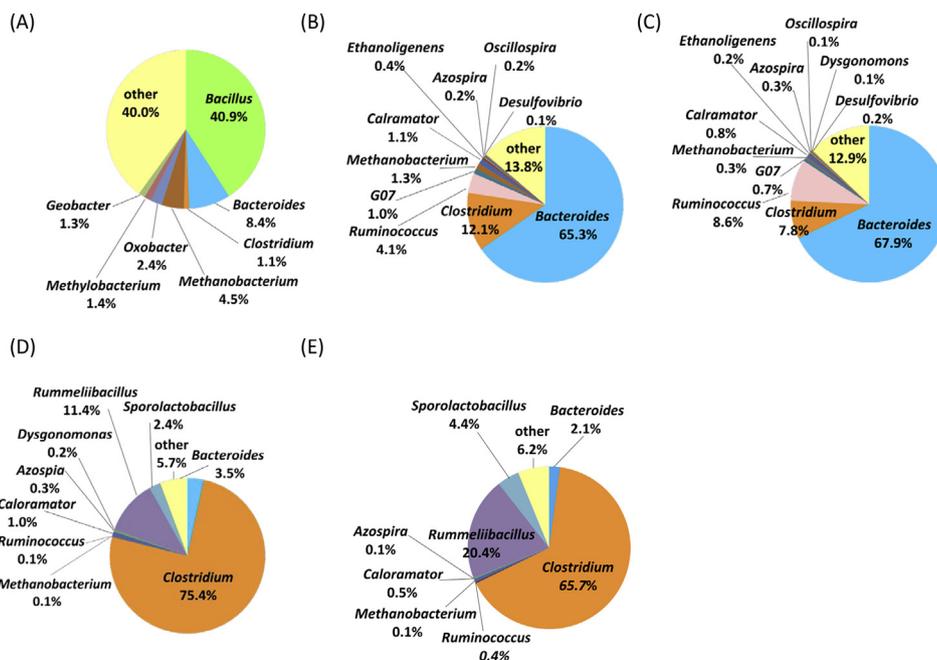

FIG. 5. Pie charts of the microbial community at the genus level; (A) raw sweet potato-shochu waste sample, (B) the anode biofilm sample of the cassette-electrode microbial fuel cells (CE-MFCs) with 10 g-chemical oxygen demand chromium (COD_{Cr})/L sweet potato-shochu waste, (C) the electrolyte sample of the CE-MFCs with 10 g-COD_{Cr}/L sweet potato-shochu waste, (D) the anode biofilm sample of the CE-MFCs with 73 g-COD_{Cr}/L (raw) sweet potato-shochu waste, and (E) the electrolyte sample of the CE-MFCs with 73 g-COD_{Cr}/L (raw) sweet potato-shochu waste.

isolation and characterization, will reveal the detailed mechanisms of electron transfer mechanisms under acidic conditions.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jbiosc.2018.12.015.

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