

Enrichment, Performance, and Microbial Diversity of a Thermophilic Mediatorless Microbial Fuel Cell

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A thermophilic mediatorless microbial fuel cell (ML-MFC) was developed for continuous electricity production while treating artificial wastewater concurrently. A maximum power density of 1030 ± 340 mW/m² was generated continuously at 55 °C with an anode retention time of 27 min (11 mL h⁻¹) and continuous pumping of air-saturated phosphate buffer into the cathode compartment at the retention time of 0.7 min (450 mL h⁻¹). Meanwhile, about 80% of the electrons available from acetate oxidation were recovered as current. Denaturing gradient gel electrophoresis (DGGE) and direct 16S-rRNA gene analysis revealed that the bacterial diversity in this ML-MFC system was lower than the inoculum. Direct 16S rDNA analysis showed that the dominant bacteria representing 57.8% of total population in anode compartment was phylogenetically very closely related to an uncultured clone, clone E4. Two sheets of graphite used as the anode showed different dominant bacterial population. For the first time, it is shown that thermophilic electrochemically active bacteria can be enriched to concurrently generate electricity and treat artificial wastewater in a thermophilic ML-MFC.

Introduction

Agricultural processing is an important industry in tropical countries with huge agricultural lands such as Malaysia, Indonesia, Thailand, etc. Among the important plantations in these countries are oil palm and sago plantations. The processing of these agricultural crops involves huge volumes of wastewater at elevated temperatures. These wastewaters are generally considered nontoxic but high in biological oxygen demand (BOD), chemical oxygen demand (COD), and organic matters which may cause environmental pollution. For example palm oil mill effluent (POME) contains high amounts of COD (50 000 mg/L), BOD (25 000 mg/L),

and total solids (40 500 mg/L) (1). However, due to its high content of organic matter, it is also an ideal candidate to generate renewable bioenergy. In general biological processes, a thermophilic process was found to be superior to processes conducted at ambient temperatures (2). Additionally, the elimination of possible pathogenic microbes at elevated temperatures was considered advantageous (3). Given these advantages, it was thought possible to use the above-mentioned wastewaters directly in mediatorless microbial fuel cells (ML-MFCs) to generate electricity.

The recent advancement in the field of microbial fuel cells (MFCs) provides promising technology to obtain energy and treat high organic content wastewater at the same time. The organic contaminants in wastewater are oxidized by electrochemically active bacteria or microbial consortium, and the resulting electrons are transferred directly or through natural mediators to the electrode. Electricity generation from various wastewaters such as domestic, food processing agricultural and hospital wastewater using MFC has been demonstrated (4–11).

Earlier versions of microbial fuel cells used electrochemically inactive microbes, and therefore the electrons could not be transferred from the microbial electron transport chain directly to the MFC electrode. Thus, electrochemical mediators such as thionine, methyl viologen, humic acid, ferricyanide, and others were employed to enable the shuttle of electrons from the microbial cells to the electrodes (12–15). These mediators in their oxidized form penetrate the microbial cell and become reduced during the microbial metabolism. The reduced mediators then diffuse out of the cell and travel to the anode where they are then oxidized. However, most of these mediators are expensive and toxic water-soluble phenolic compounds, causing their usage to be cost ineffective and not environmentally friendly.

Mediatorless microbial fuel cells have now been reported that do not require mediators to facilitate electron transfer to MFC electrodes (16). These (ML-MFC) systems utilize electrochemically active bacterium or microbial consortium as biocatalysts to convert chemical energy into electrical energy (7, 17–21). Organic materials are oxidized by microbes, and the resulting electrons are then transferred directly or indirectly to the electrode.

To our best knowledge, there is no report on the thermophilic mediatorless microbial fuel cells using electrochemically active microbial consortium. However, there is a report that investigates the use of a single thermophilic microorganism to generate electricity in a MFC with a mediator at elevated temperatures (22).

The study presented herein utilized artificial wastewater consisting of phosphate buffered basal medium (PBBM) incorporated with sodium acetate to enrich thermophilic electrochemically active bacteria in a thermophilic ML-MFC. This system was used to investigate the potential for electricity production using MFCs at elevated temperatures (55 °C), and the results are presented in this paper. Additionally, the bacterial community that resulted from the enrichment studies in these thermophilic ML-MFCs were analyzed using the genes of small ribosomal RNA.

Materials and Methods

Thermophilic Mediatorless Microbial Fuel Cells and Enrichment. The microbial fuel cell (MFC) was made of transparent polyacrylic plastic. Each MFC consisted of two compartments, anode and cathode, which were of equal volume and dimension (5 mL; 5.0 cm × 1.0 cm × 1.0 cm). Each compartment contained two pieces (4.5 cm × 1.0 cm

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× 0.5 cm) of graphite felt (GF series, Electrosynthesis, Amherst, NY) as electrodes, but the graphite felt used for the cathode electrode was coated with 0.3 mg/cm² of platinum powder (23). The projected surface of the electrode was used for the calculation in the present study. The anode and cathode compartments were separated by a Nafion 450 cation exchange membrane (DuPont, Wilmington, DE), and platinum wires (0.5 mm diameter) of 3-cm length were utilized as leads for both compartment electrodes. The internal resistance between the electrodes and the platinum wire was less than 3 Ω as measured by a multimeter (Model 2000, Keithley, MA). The wires were connected to an external resistance of 10 Ω during MFC operation, and a digital multimeter (Keithley Instruments, Cleveland, OH) was utilized to periodically measure the voltage drop across the load.

The anode compartment was fed continuously with effluent collected from a thermophilic anaerobic digester used to treat wastewater from a brewery. Twenty milliliters of effluent was flushed through the system, and then it was fed with Phosphate-Buffered Basal Medium (PBBM) containing sodium acetate via an up-flow pump-delivery system. Concurrently, the cathode compartment was continuously fed with air-saturated phosphate buffer (50 mM).

Peristaltic pumps (Watson-Marlow, Campel, U.K.) were used to feed MFCs at the desired flow-rate. The thermophilic condition was maintained by incubating the MFCs in a 55 °C waterbath which was covered with a stainless-steel lid throughout the experiment. The temperature in the water bath was monitored using a thermometer.

Phosphate Buffered Basal Medium (PBBM) with Acetate. Sodium acetate incorporated into PBBM was used as fuel throughout the study. PBBM was prepared as described previously (2). PBBM containing the fuel of various concentrations (100–400 mg L⁻¹ as COD) was adjusted to pH 7 before being autoclave at 121 °C for 15 min. The autoclaved fuels were cooled and then gassed with oxygen-free nitrogen for at least 2 h. The fuels were kept under anaerobic conditions by connecting to a nitrogen-containing gastight bag (SKC Inc., Eighty Four, PA) during feedings.

Analyses. The potential drop across an external 10-ohm resistor was measured using a digital multimeter (Keithley Instruments, Cleveland, OH) and recorded on a personal computer through a data acquisition system (Testpoint, Capital Equipment, Richmond, VA) every 5 min. The measured potential was converted to current according to the formula, current (ampere) = potential (volt)/resistance (ohm).

The standard method as described by Eaton and co-workers (25) was employed to measure COD using chromate as the oxidant. Polarization curves of the MFC were obtained by varying the applied external resistance and recording the steady-state potential after a minimum connection period of 1 h. The Coulombic efficiency (%) was calculated as $(C_A/C_{Th}) \times 100$, where C_A is the total coulombs obtained by integrating the current over time and C_{Th} is the theoretical Coulombic yield calculated from COD oxidation. All experiments were performed in triplicate, and the mean values are presented herein.

Microbial Diversity Studies. The MFC was dismantled, and the graphite felts were removed from the anode compartment in a biosafety cabinet. A square piece (1 cm × 1 cm × 0.5 cm) of each graphite felt was cut with a sterile surgical blade before the MFC was put together and continued to operate. The square piece of graphite felt was processed to extract the total genomic DNA immediately. Total genomic DNA was isolated using the PowerSoil DNA kit as instructed by the manufacturer (MO BIO Laboratory, Carlsbad, CA). The purity and quality of the total genomic DNA were examined by agarose gel electrophoresis using 1.0% agarose

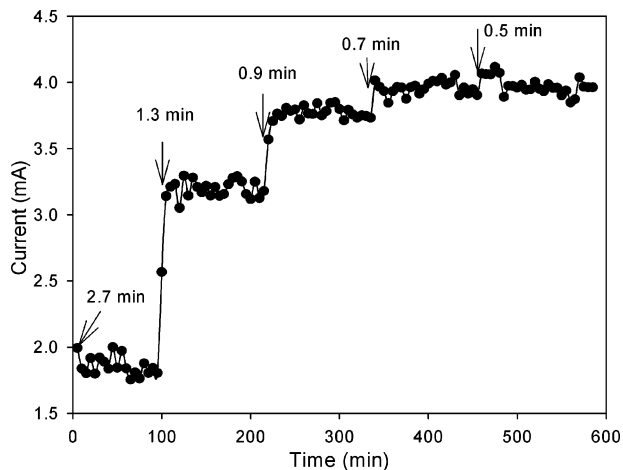


FIGURE 1. Effect of air-saturated phosphate buffer-retention time on current generation.

gel. Direct 16S rDNA sequence analysis and PCR-DGGE analysis were performed as previously described (18). The near complete 16S rRNA gene sequences have been deposited in the GenBank database and are available under accession numbers DQ424915-DQ424927.

Results

Enrichment of a Thermophilic Mediatorless Microbial Fuel Cell Using Acetate as Fuel.

The MFC anodes were continuously fed with 300 mg L⁻¹ (as COD) of acetate fuels at the retention time of 27 min (11 mL h⁻¹) after initial inoculation with digester effluent. Concurrently, the cathode was fed with air-saturated 50 mM phosphate buffer at the retention time of 0.9 min (338 mL h⁻¹). The recorded open-circuit potentials were around 0.9 V. The potential dropped immediately and started to increase slowly after a few days when the MFCs were operated closed-circuit with an external load of 10 Ω. A maximum closed-circuit potential of approximately 45 mV, which is equivalent to the current of 4.5 mA, was recorded as fast as 3 weeks after enrichment. After 2 months of operation, the anode effluents showed a COD removal percentage of 47 ± 9%, and the Coulombic efficiencies were 80 ± 3%.

Effects of Fuel-Feeding Rate. The effects of fuel-feeding rate on MFCs' performances were studied using 300 mg L⁻¹ (as COD) of acetate fuels. The current increased as the retention time reduced from 27 min (11 mL h⁻¹) to 13 min (23 mL h⁻¹), but no further current increases were observed when the flow rate decreased to 9 min (34 mL h⁻¹). Furthermore, at the retention time of 9 min, only 19 ± 3% of the COD value was removed compared to 43 ± 9%, at the retention time of 27 min (11 mL h⁻¹). Similarly, the Coulombic efficiency was also higher (90 ± 3%) at the lower feeding-rate relative to 70 ± 6% at the retention time of 9 min.

Effects of Air-Saturated Phosphate Buffer-Feeding Rate in Cathode Compartment on Current Generation.

The effects of cathode-feeding rate on MFCs' performances were studied using 300 mg L⁻¹ (as COD) of acetate fuels at the fixed anode retention time of 27 min (11 mL h⁻¹). As shown in Figure 1, the current increased rapidly as the cathode retention time reduced from 2.7 min (113 mL h⁻¹) to 0.7 min (450 mL h⁻¹). The current increased approximately 100% (from 2.0 ± 0.5 mA to 4.0 ± 1.0 mA). However no further increase in current was observed when the retention time decreased further to 0.5 min (563 mL h⁻¹).

Effects of Fuel Concentration. Fuels of various concentrations were employed to study the effects on MFC performance. Figure 2 shows the effects of fuel concentration on current generation, COD removal, and Coulombic ef-

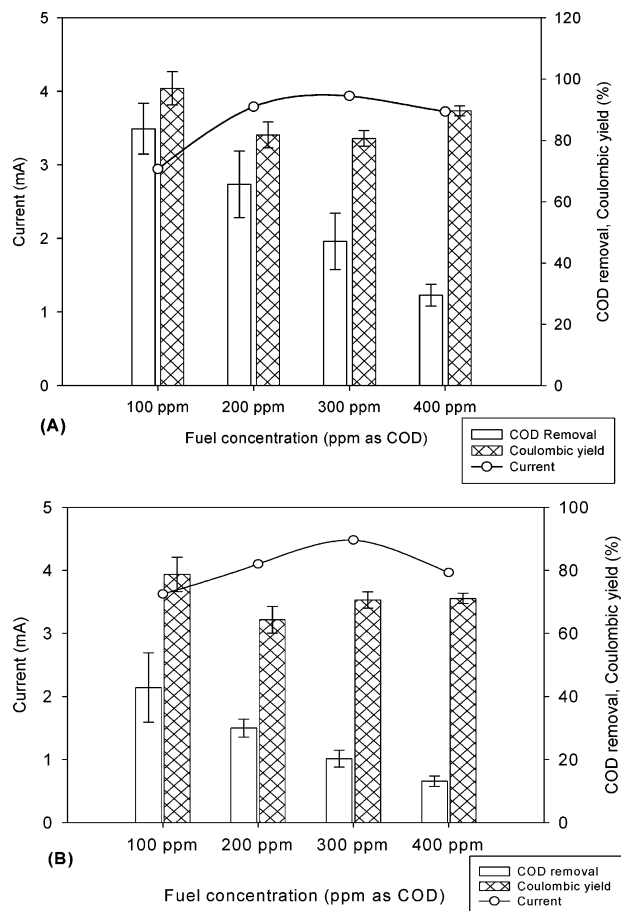


FIGURE 2. Effect of fuel concentration on current generation, COD removal, and Coulombic yield: (A) the fuel-feeding retention time, 27 min; air-saturated phosphate buffer-feeding rate, 0.7 min and (B) the fuel-feeding retention time, 9 min; air-saturated phosphate buffer-feeding rate, 0.7 min.

efficiency at the retention time of 27 min (11 mL h^{-1}). In general, higher fuel concentrations up to 300 mg L^{-1} resulted in higher current generation. However, at a fuel concentration of 400 mg L^{-1} the current generation slightly decreased. This higher fuel concentration also resulted in a lower percentage of COD removal. Regardless of fuel concentration used, all systems recorded a Coulombic efficiency of more than 80% with the highest efficiency ($96 \pm 5\%$) obtained from MFC fed with 100 mg L^{-1} of fuel.

MFCs operated with fuel containing 300 mg L^{-1} acetate as COD and a retention time of 27 min (11 mL h^{-1}) were used to measure power density. Polarization curves were obtained using the method described previously (Figure 3). The maximum current density, power density, and the COD loading rate were determined to be $9030 \pm 100 \text{ mA/m}^2$, $1030 \pm 340 \text{ mW/m}^2$, and $8.37 \text{ kg m}^{-3}\text{day}^{-1}$, respectively.

DGGE. Genomic DNA was extracted from the anaerobic digester effluent (used as MFC inoculum), and also each sheet of graphite felt was used as the MFC anode. DNA extracted from the graphite felts showed fewer bands than the anaerobic digester effluent used to initiate the enrichment process (Figure 4). On the basis of migration distance on DGGE gel, each sheet of graphite felt used as the anode showed a different bacteria community. Almost all intense bands found on sheet A (next to the membrane) migrated a short distance and therefore had a lower G+C content. Meanwhile, most of the nested PCR bands of sheet B (away from the membrane) migrated a long distance, suggesting a higher G+C content. Additionally, the bands in sheet A showed higher intensity compared to those of sheet B when

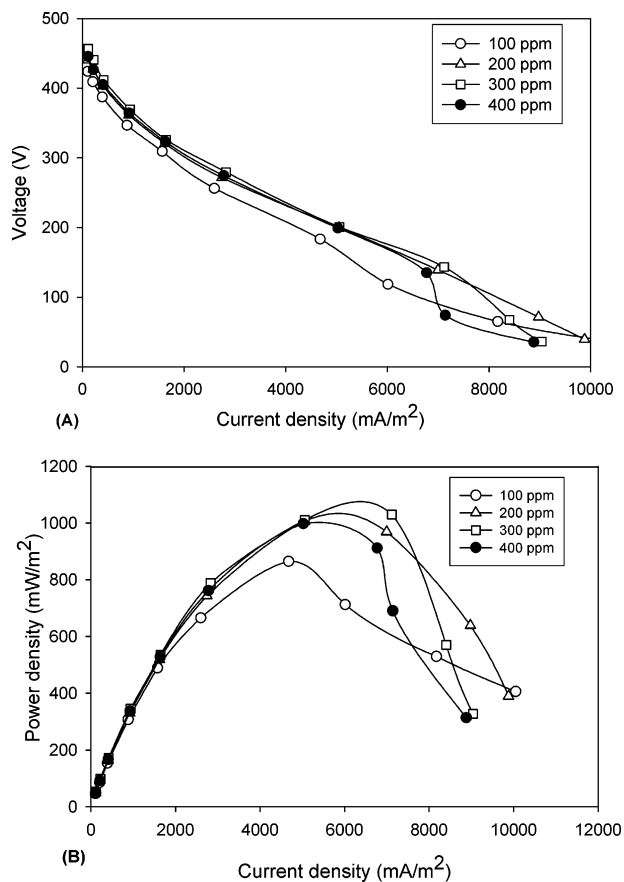


FIGURE 3. Effects of fuel concentration on the thermophilic MFC: (A) polarization curve and (B) power density versus current density. The fuel-feeding retention time was fixed at 27 min, and the air-saturated phosphate buffer-retention time was 0.7 min.

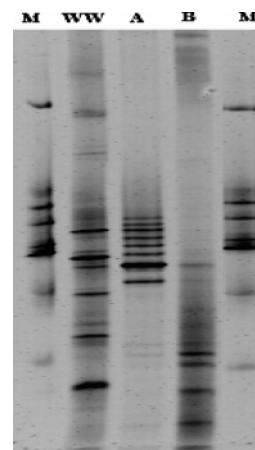


FIGURE 4. Comparison of bacterial diversity in two anode electrode graphite felts of the thermophilic MFC enriched with acetate: M, 100 bp DNA ladder (TaKaRa, Japan); WW, wastewater from distillery; A, electrode attached to cation membrane; and B, electrode far from cation membrane.

a similar quantity of PCR products was loaded to the DGGE gel. However, there were some PCR products that migrated the same distances, suggesting that some common bacterial populations were present in both sheets.

Bacterial Diversity of Sheet A. *Escherichia coli* transformed with amplicon of sheet A was plated on LB agar plates containing 4 mg mL^{-1} X-gal, 0.5 mM IPTG , and $50 \mu\text{g mL}^{-1}$ ampicillin, and a total of 164 white transformants were selected. Recombinant plasmids isolated from these transformants were subjected to RFLP analysis. The results

TABLE 1. Identification of Recombinant Clones Based on 16S rDNA Sequence Analysis Identity of Cloned 16S rRNA Gene^a

RFLP pattern	# clone			closest identification	homology (%)	accession no.	source
	mix	A	B				
I	115 (56.3%)	143 (87.2%)		uncultured bacterium clone E4 <i>Deferribacter desulfuricans</i>	99 88	AY526503 AB086060	thermophilic anaerobic sludge deep-sea hydrothermal vent
II	4	1		uncultured bacterium clone E4	99	AY526503	thermophilic anaerobic sludge
III	7	6	4	<i>Thermodesulfovibrio yellowstonii</i>	99	AB231858	thermophilic granular sludges
IV	30 (15.1%)	8	144 (84.2%)	<i>Coprothermobacter</i> sp.	98	AB162803	thermophilic anaerobic digestion sludge
V	3	1	2	<i>Coprothermobacter</i> sp.	99	AB162803	thermophilic anaerobic digestion sludge
VI	4	2	6	<i>Coprothermobacter</i> sp.	99	AB162803	thermophilic anaerobic digestion sludge
VII	2		4	<i>Coprothermobacter</i> sp.	94	AB162803	thermophilic anaerobic digestion sludge
VIII	14		4	Uncultured bacterium clone E4	97	AY603000	thermophilic anaerobic sludge
IX	3		3	<i>Coprothermobacter</i> sp.	98	AB162803	thermophilic anaerobic digestion sludge
X	4	1	3	<i>Coprothermobacter</i> sp.	99	AB162803	thermophilic anaerobic digestion sludge
XI	8		1	uncultured bacterium clone E4	99	AY526503	thermophilic anaerobic sludge
XII	3	1		uncultured bacterium clone E4	99	AY526503	thermophilic anaerobic sludge
XIII	2	1		<i>Coprothermobacter</i> sp.	89	AB162803	thermophilic anaerobic digestion sludge
total	199	164	171				

^a % = percentage of similarity between cloned 16S rRNA gene and the closest relative in the NCBI database.

TABLE 2. Comparison of MFC Performances

biocatalyst	operation condition	operation mode	anode mediator	cathode mediator	power density (mW/m ²)	sources
enriched microbial consortium	thermophilic	continuous	none	none	1030 ± 340	this work
enriched microbial consortium	mesophilic	continuous	none	none	560	21
<i>Shewanella oneidensis</i>	mesophilic	continuous	yes	yes	3000	32
enriched microbial consortium	mesophilic	batch	none	none	8.3	7
<i>Shewanella putrefaciens</i>	mesophilic	batch	none	none	0.32	17
<i>Geobacter sulfurreducens</i>	mesophilic	batch	none	yes	16	29
<i>Rhodferax ferrireducens</i>	mesophilic	batch	none	yes	8.2	4
enriched microbial consortium	mesophilic	batch	none	none	480	31
enriched microbial consortium	mesophilic	batch	none	none	1330	28
enriched microbial consortium	mesophilic	batch	none	yes	3600	20
enriched microbial consortium	mesophilic	batch	yes	yes	788	30

clustered these 164 clones into 9 patterns (Table 1). Pattern I was dominant (87.2%; 143 clones) in sheet A. The cloned 16S rRNA gene sequences of this pattern was closely related to an uncultured clone, E4 (GenBank accession number AY526503; 99% homology) and distantly related to *Deferribacter desulfuricans* (GenBank accession number AB086060; 88%). The second major pattern was pattern IV, representing only 4.9% of the total clones in sheet A. The 16S rRNA gene sequence of pattern IV is closely related to *Coprothermobacter* sp. (GenBank accession number AB162803).

Bacterial Diversity of Sheet B. Of the total 171 white transformants obtained from sheet B, 9 patterns were observed after 16S-RFLP analysis (Table 1). However, some of the patterns observed in sheet A were not in sheet B and vice versa. The main pattern in this sheet was pattern IV (84.2%; 144 clones), followed by pattern VI (3.5%; 6 clones). Both 16S rRNA gene sequences of patterns I and VI were closely related to *Coprothermobacter* sp. (GenBank accession number AB162803). Pattern I, which was the major pattern in sheet A, was not observed in sheet B.

Total Anode Bacterial Diversity. One hundred ninety-nine white transformants were obtained for 16S-RFLP analysis from the amplicon of DNA extracted from both anode sheets (Table 2). From the RFLP analysis of these transformants 13 patterns were obtained. The major pattern, pattern

I (57.8%, 115 clones), was closely related to an uncultured clone, E4 (GenBank accession number AY526503). This pattern was also the major pattern observed in the bacterial diversity of sheet A. The second major pattern (15.1%, 30 clones), pattern IV (closely related to *Coprothermobacter* sp.; GenBank accession number AB162803), was the major pattern obtained from sheet B.

Discussion

Electricity was generated continuously from artificial wastewater incorporated with acetate by thermophilic ML-MFC in this study. The polarization curve method showed that both power density and current density increased with the increase in fuel concentration up to 300 mg L⁻¹ (Figure 3). However, at a fuel concentration of 400 mg L⁻¹, the power density decreased and was lower than that of 200 mg L⁻¹. The maximum power density (1030 ± 340 mW/m²) was recorded when the MFC was fed with 300 mg L⁻¹ of acetate fuel, suggesting possibly saturation of microbial activity at this concentration. This also demonstrated that fuel concentrations up to 300 mg L⁻¹ enable better power density in thermophilic MFCs.

It is well documented that high concentrations of short-chain fatty acids have bacteriostatic and/or bactericidal effects (26, 27). This may explain the decline of power density

when the MFC was fed with a high concentration of acetate. The high concentration of acetate might be toxic to the electrochemically active bacteria, which results in lower power densities. The optimal conditions that generated a maximum power density were a fuel concentration of 300 mg L⁻¹, an anode retention time of 27 min (11 mL h⁻¹), and a cathode air-saturated phosphate buffer-retention time of 0.7 min (450 mL h⁻¹). However, only about 50% of COD was removed, and approximately 80% of electrons was recovered as current from acetate oxidation. This result is similar to a mesophilic MFC fed with acetate, which showed a K_s value of 141 mg L⁻¹ (28). It should be noted that the COD removal efficiency is low probably due to the short fuel retention time. This fact is not a concern at this point because this exploratory study was conducted to show that a thermophilic ML-MFC is possible. The fuel removal efficiency can be improved by increasing the retention time.

The maximum power density demonstrated in the present study was comparably higher than those recently reported (4, 7, 17, 21, 29–31) except for two systems (20, 32) as shown in Table 2. The maximum power density obtained was also comparable to that reported by Liu et al. (28). However, it should be noted that our system did not employ any cathode mediator (20, 32). It is also not necessary to increase the solution ionic strength to obtain comparable power density (28). Additionally, the Coulombic efficiency in this system was higher than that reported by Liu and co-workers (80 ± 3% vs 61%). In this exploratory study, air-saturated buffer solution was pumped through the cathode compartment. Although a considerable amount of energy is needed to saturate the buffer with air and to pump it, we are not too concerned as a single chamber MFC with an air-breathable cathode can eliminate these problems without scarifying the performance (28). On the other hand, the use of mediators to obtain higher power density is difficult to substitute.

DGGE analysis showed that bacterial populations on the anode of thermophilic MFC after 3 months of enrichment were different from those of the anaerobic digester effluent which was used to inoculate the MFC. The bacterial populations were less diverse after the enrichment than those of the inoculum as shown in the DGGE gel. This suggests that the electrochemically active bacteria most likely have been enriched during the operation. DGGE gel showed that both sheets of graphite felt used as the anode consisted of different bacterial communities. The reason for the different bacterial communities in these two closely packed sheets is not clear. This is the first report confirming the enrichment of electrochemically active thermophilic communities using a mediatorless MFCs.

Sequencing of the cloned 16S rRNA genes revealed that all bacterial populations in the anode compartment belong to thermophilic bacteria, as expected. Combining the results of all graphite felt examined, there are only 13 different patterns of bacteria populations, based on the direct 16S rRNA gene analysis. Of all the 13 representative clones sequenced, 5 patterns showed the highest sequence homology to an uncultured clone E4 (97–99% homology), 7 patterns were related to genus *Coprothermobacter* (89–99% homology), and 1 was related to *Thermodesulfobivrio* (99%).

Pattern I, representing 57.8% of the cloned rDNA sequences obtained, are phylogenetically associated with an uncultured clone, E4 (99%). Clone E4 was initially identified as a member of a microbial community in a thermophilic lab-scale methanol-fed anaerobic digester. The dominant clone was also distantly related to *Deferribacter desulfuricans* SSM1 (88%). *Deferribacter desulfuricans* SSM1, a novel sulfur-, nitrate-, and arsenate-reducing thermophile, was initially isolated from a deep-sea hydrothermal vent. Interestingly, this pattern existed only in sheet A, which is near to the cation membrane but not in sheet B. The dominant clones

in sheet B were closely related to a *Coprothermobacter* sp. P1 (AB162803) of the *Thermoanaerobacteriales* order, originally isolated from a thermophilic anaerobic digester. However, this cloned 16S rRNA gene only represents a minority population in sheet A. The reason for this is not presently known. However, clones from all samples were phylogenetically related to thermophilic and fermentative clones in the GenBank database. The presence of these thermophilic microorganisms supports the idea that thermophilic electrochemically active bacteria exist.

The complete absences of *Proteobacteria* representatives among the total 534 clones screened in the present study was unexpected, considering the great diversity of *Proteobacteria* reported from MFCs operated at mesophilic conditions (7, 18, 28, 33, 34). Also, the bacterial diversity of the thermophilic MFC was much lower compared to the mesophilic counterpart. The direct 16S rRNA gene analysis confirmed the result of DGGE analysis, which showed that the bacterial communities on both electrodes were different.

The ultimate goal of the thermophilic ML-MFC is to remove organic pollutants in agricultural processing wastewater and convert them into electricity. We have successfully proven that in using the thermophilic ML-MFC, about 50% of the organic content in the artificial wastewater could be removed. At the same time, about 80% of the available electrons were recovered as current. However, due to the organic concentration limitation, dilution of the agricultural wastewater to appropriate concentrations may be one of the options to enable optimal treatment and electricity generation. Additionally, further studies have to be carried out at a larger scale to examine the feasibilities of this thermophilic ML-MFC system to treat real wastewater and to generate electricity concurrently. Also, since agroindustrial wastewater does not contain fatty acid concentrations as high as the acetate concentration added to the artificial wastewater in the present study, the wastewater might not be toxic to the microbial community in a MFC. Further works need to be done to develop a MFC system which does not have the limitation of organic content and thus can be utilized to generate electrical energy and treat wastewater concurrently in an agricultural processing plant. Alternatively, organic matter in the high strength wastewater can be fermented to produce hydrogen before fed to a MFC as suggested by Liu et al. (35).

In conclusion, we have successfully developed a thermophilic ML-MFC to generate electricity and treat artificial wastewater simultaneously. Using direct 16S rDNA and DGGE analysis, we were able to characterize the bacterial community in the anode compartment. To the best of the authors' knowledge, this study is the first to examine the feasibility of a thermophilic mediatorless MFC and also the microbial diversity of a thermophilic ML-MFC. Although there is still room to improve, our study demonstrates that high temperature artificial wastewater could be used as feedstock in ML-MFC for electricity generation. Additionally, the reduction of COD in the wastewater by the ML-MFC could be a promising option to reduce the COD contents before being discharged to the environment.

Acknowledgments

This work was supported by the Ministry of Science and Technology, Korea through the National Research Laboratory program. J.B.C. was supported by an APEC postdoctoral fellowship from the Korea Foundation of Science and Technology.

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Received for review June 6, 2006. Revised manuscript received August 9, 2006. Accepted August 11, 2006.

ES0613512