

## Electricity Generation Coupled with Wastewater Treatment Using a Microbial Fuel Cell Composed of a Modified Cathode with a Ceramic Membrane and Cellulose Acetate Film

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**A noncompartmented microbial fuel cell (NCMFC) composed of a Mn(IV)-carbon plate and a Fe(III)-carbon plate was used for electricity generation from organic wastewater without consumption of external energy. The Fe(III)-carbon plate, coated with a porous ceramic membrane and a semipermeable cellulose acetate film, was used as a cathode, which substituted for the catholyte and cathode. The Mn(IV)-carbon plate was used as an anode without a membrane or film coating. A solar cell connected to the NCMFC activated electricity generation and bacterial consumption of organic matter contained in the wastewater. More than 99% of the organic matter was biochemically oxidized during wastewater flow through the four NCMFC units. A predominant bacterium isolated from the anode surface in both the conventional and the solar cell-linked NCMFC was found to be more than 99% similar to a Mn(II)-oxidizing bacterium and *Burkholderia* sp., based on 16S rDNA sequence analysis. The isolate reacted electrochemically with the Mn(IV)-modified anode and produced electricity in the NCMFC. After 90 days of incubation, a bacterial species that was enriched on the Mn(IV)-modified anode surface in all of the NCMFC units was found to be very similar to the initially isolated predominant species by comparing 16S rDNA sequences.**

**Keywords:** Noncompartmented microbial fuel cell, Mn(IV)-modified anode, Fe(III)-modified cathode, solar cell, wastewater treatment

In a two-compartment microbial fuel cell (MFC), the anode (anolyte) is separated from the cathode (catholyte) by a semi-permeable membrane, such as cation-selective nylon or cellulose polymer, to maintain the redox potential difference

between the anode and cathode [4, 32]. Catholyte aeration is absolutely required to maintain the oxidation potential of the cathode [2, 9, 18]. Ferricyanide solution has been used as the catholyte, which is reduced to ferrocyanide in coupling with the electrochemical oxidation of the cathode and is regenerated by catholyte aeration [33].

An electron mediator is essential for electricity generation by bacterial cells but may not be required by exoelectrogenic species that can directly transfer electrons from bacterial cells to an electrode [40]. In particular, Mn(IV)-reducing bacteria can actively transfer electrons to an electrode when a Mn(IV)-modified electrode is used as the anode [26]. A Fe(III)-modified electrode may substitute for ferricyanide because the redox potential difference between Fe(III)/Fe(II) (+0.78 V) and O<sub>2</sub>/H<sub>2</sub>O (+0.82 V) is not very high [3]. The combination of a Mn(IV)-modified anode and a Fe(III)-modified cathode may be ideal for composing an MFC without an electron mediator and oxidant, but not one from which catholyte aeration can be excluded [4, 29, 32]. In order to compose an MFC without catholyte aeration, the cathode has to function as a catalyst for the electrochemical reaction of protons, electrons, and oxygen [10], and as a redox barrier to conserve the potential difference between the anode and cathode. Graphite felt, to which neutral red (an electron mediator) is immobilized to catalyze electron transfer from bacterial cells to the electrode [30, 36], generally has been used as both the anode and cathode, but it is difficult to modify membranes with this material because of its soft and weak structure.

In wastewater treatment systems, aeration has typically been used to activate the biochemical oxidation of organic compounds, sulfide, ammonia, and nitrite [7, 31, 35, 37], but it is a highly costly process. Wastewater treatment using an MFC may be a less energy-dependent but less efficient process than an aeration-dependent system. Min *et al.* [19] reported the production of electricity from an MFC coupled with the treatment of swine wastewater, and Kim *et al.*

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[13] used a single-chamber MFC to remove odors from swine wastewater. MFCs have also been used for ammonium oxidation and biological denitrification, in which the reducing power generated by exoelectrogenic bacteria may be converted to metabolic energy by denitrifying bacteria [5, 6]. A low-cost and highly efficient process for wastewater treatment is in demand at this time since energy conservation and the use of green technology are required. Wastewater treatment using a serially connected noncompartmented microbial fuel cell (NCMFC) may satisfy these demands.

In this research, we tried to produce usable electricity from wastewater without consuming external energy. A three-layered cathode composed of a cellulose acetate film, a ceramic membrane, and a porous graphite plate was developed to create an NCMFC capable of operating without aeration. The NCMFC was designed to be a cascade-type system by serially connecting NCMFC units, by which the efficiency of electricity production and wastewater treatment can be easily controlled.

## MATERIALS AND METHODS

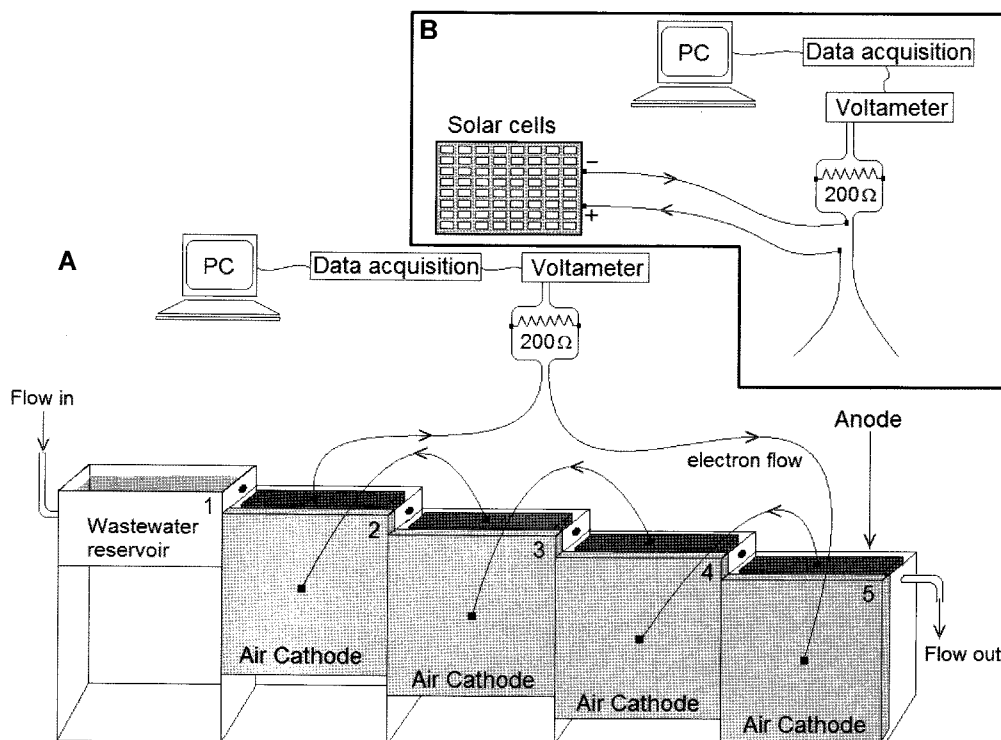
### Noncompartmented Microbial Fuel Cell

An NCMFC was designed for the purpose of eliminating the need for aeration and external energy consumption, as shown in Fig. 1. The external (porous graphite plate) and internal (cellulose acetate

membrane) cathode surfaces were exposed to atmosphere and anolyte, respectively. Wastewater was designed to flow spontaneously from the reservoir (reactor 1) to the fourth NCMFC unit (reactor 5) through the first, second, and third NCMFC units, owing to the height difference. NCMFC units were electrically connected by a closed circuit directly linked to  $200\ \Omega$  of external resistance. A solar cell was directly connected to the NCMFC by serial circuit, as shown Fig. 1B, and the system was named "solar cell-linked NCMFC" to differentiate it from a conventional NCMFC (Fig. 1A). The maximal electrical power of the solar cell was  $1.5\pm 0.1\ \text{V}$  and  $2.4\pm 0.1\ \text{mA}$ . Artificial wastewater was composed of 1 g/l crude yeast extract (yeast extract-S; Jenico, Korea), 2 g/l sodium acetate, 1 g/l soluble starch, 1 g/l dibasic potassium phosphate, and 2 ml/l trace mineral stock solution. The trace mineral stock solution contained 0.01 g/l  $\text{MnSO}_4$ , 0.01 g/l  $\text{MgSO}_4$ , 0.01 g/l  $\text{CaCl}_2$ , 0.002 g/l  $\text{NiCl}_2$ , 0.002 g/l  $\text{CoCl}_2$ , 0.002 g/l  $\text{SeSO}_4$ , 0.002 g/l  $\text{WSO}_4$ , 0.002 g/l  $\text{ZnSO}_4$ , 0.002 g/l  $\text{Al}_2(\text{SO}_4)_3$ , 0.0001 g/l  $\text{TiCl}_3$ , 0.002 g/l  $\text{MoSO}_4$ , and 10 mM EDTA. The artificial wastewater was not autoclaved but was stored at  $4^\circ\text{C}$  in a cold chamber while being supplied to the wastewater reservoir at a flow rate of  $1,000\pm 50\ \text{ml}$  per day. The volume of the reservoir and the NCMFC units was adjusted to 500 ml and 1,000 ml, respectively. The hydraulic retention time of wastewater in the reservoir and in each NCMFC unit was 12 and 24 h, respectively.

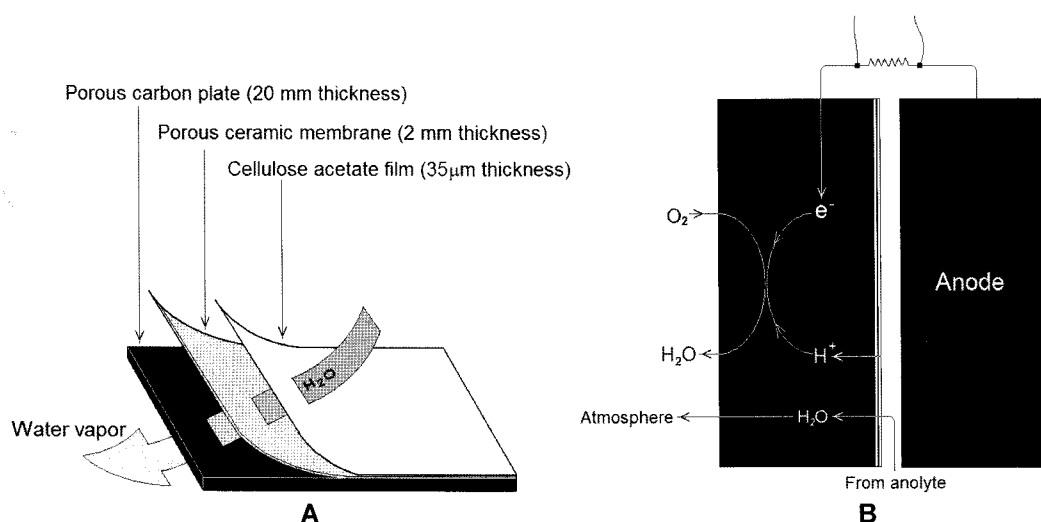
### Electrode Composition

A three-layered cathode was composed of a cellulose acetate film ( $35\ \mu\text{m}$  thickness; Electron Microscopy Science, U.S.A.), a porous ceramic membrane (2 mm thickness), and a modified porous carbon plate (20 mm thickness) with Fe(III) ion (the Fe(III)-carbon plate),



**Fig. 1.** Schematic diagram showing a conventional serial NCMFC composed of a four-step iso-system (A).

A solar cell was electrically connected to the NCMFC by series circuit (B), and the system was named "solar cell-linked NCMFC." Reactor 1, wastewater reservoir; reactors 2 through 5, serially connected NCMFC units.



**Fig. 2.** Schematic diagram showing an air cathode (A) and the cathodic reaction mechanism (B).

as shown in Fig. 2A. The Fe(III)-carbon plate was made from a paste composed of 50% (w/w) graphite powder, 44% (w/w) porcelain clay, 2% (w/w) Fe(III) ion, and 4% (w/w) kaolin powder. The paste was thoroughly mixed with a stirrer for 24 h, and then framed in a rectangular-shaped mold (250×200×20 mm) under 20 kg/cm<sup>2</sup> pressure, dried completely, and baked at 1,100°C for 6 h. A ceramic membrane was made by mixing 88% (w/w) porcelain clay and 12% (w/w) kaolin and baking at 1,100°C for 6 h. The cellulose acetate film was attached to the ceramic membrane using acetone vapor. The porous ceramic membrane and carbon plate were permeable to water, ions, and all kinds of water-soluble compounds; however, the cellulose acetate film was semipermeable, enabling the selective transfer of gases, protons, and water molecules, as shown in Fig. 2B. A porous carbon plate containing 2.0% (w/w) Mn(IV) ion [the Mn(IV)-carbon plate], which was used as the anode, was made following the same procedure as for the Fe(III)-carbon plate, but was not modified with the porous ceramic membrane and cellulose acetate film.

#### Scanning Electron Microscopy (SEM) Imaging

A piece of carbon electrode was completely dried at 110°C and used as a sample without any pretreatment. A scanning electron micrograph of the sample was prepared at the Korea Basic Science Institute (KBSI, Daegu, Korea).

#### COD Measurement

Chemical oxygen demand (COD) in the NCMFC unit was analyzed at an interval of 5–7 days based on the standard method [1].

#### Electricity Production in the NCMFC

Measurement of electricity production began immediately following the addition of bacteria to the NCMFC. One hundred ml each of bacterial communities obtained from anaerobic and aerobic wastewater treatment reactors (Jungrang Wastewater Treatment System, Seoul, Korea) was inoculated into each NCMFC unit (Fig. 1). Electricity generated by the serial NCMFC was measured with a voltmeter (Keithley 2700 Multimeter/Data Acquisition System, U.S.A.). The electric current was periodically measured to monitor the relationship between the experimentally measured current and the theoretical

current calculated from the electric voltage and external resistance. The data for electricity production were saved continuously in a PC at 1 h intervals.

#### Isolation and Identification of the Predominant Bacterium

After the NCMFC was in operation for more than 50 days, the bacterial community growing on the anode in each NCMFC unit was gathered by gently scraping the entire surface and then suspended in fresh medium (artificial wastewater) containing 10 g/l Mn(IV) ion in anaerobic culture tubes (Belco, U.S.A.). The suspended cells were serially diluted 10<sup>-1</sup> to 10<sup>-12</sup> with autoclaved medium and cultivated under anaerobic conditions. The bacteria found in the maximally diluted sample was confirmed to be a pure species and was then cultivated separately. 16S rDNA was amplified by direct PCR using universal primers (forward: 5'-GAGTTGGATCCTGGCTCAG-3'; and reverse: 5'-AAGGAGGGGATCCAGCC-3'). The PCR mixture (50 μl) contained 2.5 U *Taq* polymerase, 250 μM of each dNTP, 10 mM Tris-HCl (pH 9.0), 40 mM KCl, 100 ng template, 50 pM primer, and 1.5 mM MgCl<sub>2</sub>. Amplification was performed for 30 cycles of 1 min of denaturation at 95°C, 1 min of annealing at 55°C, and 2 min of extension at 72°C, using a PCR machine (T Gradient model; Biometra, Germany). PCR products were directly sequenced with an ABI Prism 3700 Genetic Analyzer by a professional company (Macrogen Inc., Republic of Korea) upon request. 16S rDNA sequences were compared with those in the GenBank database, and the species was identified on the basis of homology.

#### Electrochemical Analysis of an Isolate

In order to analyze electrochemical redox reactions between an electrode and a bacterium, cyclic voltammetry was conducted using a voltammetric potentiostat (BAS model CV50W, U.S.A.) linked to a data acquisition system. A Mn(IV)-modified electrode (5 mm diameter), a platinum wire, and an Ag/AgCl electrode were utilized as a working electrode, counter-electrode, and reference electrode, respectively. A plain carbon electrode was used as a working electrode for the control test. The reaction mixture was composed of 50 mM Tris-HCl buffer (pH 7.5) containing 5 mM NaCl, 30 mM glucose, and intact cells (OD<sub>660</sub>=5.0). Prior to use, the electrodes were cleaned with

ultrasonic cleaner, and the dissolved oxygen in the reaction mixture was purged by argon (99.99%) gassing. The scanning rate was 25 mV/s over a range of -500 to 500 mV. Data were recorded as variations in upper voltammograms (reduction reaction) and lower voltammograms (oxidation reaction).

#### Electricity Production by the Predominant Bacterium

A specially designed small-sized NCMFC (electrode area: 40 cm<sup>2</sup>; reactor volume: 50 ml) was used to test electricity generation by a pure culture of the predominant bacterium. Bacterial cells (OD<sub>660</sub>=10) obtained by centrifugation at 5,000 ×g and 4°C for 30 min were suspended in a reactant composed of 100 mM glucose and 25 mM phosphate buffer. The electric current was measured in the closed-circuit configuration.

#### Temperature Gradient Gel Electrophoresis (TGGE)

The bacterial community was sampled from the anode surface of the four NCMFC units (reactors 2 through 5 in Fig. 1) on days 10 and 90. DNA was extracted from the bacterial community with a DNA extraction kit (GeneEx, GeneAll Genomic Sx, Korea) according to the procedure specified by the manufacturer. The 16S rDNA variable region was amplified with the eubacterial V3 region forward primer 341f (5'-CCTACGGGAGGCAGCAG-3') and the universal V3 region reverse primer 518r (5'-ATTACCGCGGCTGCTGG-3'). A GC-clamp (5'-CGCCCGCCGCGCGGGCGGGCGGGGCGGGGGCACGGGGGGCTACGGGAGGCAGCAG-3') was attached to the 5' end of primer 341f [22]. The TGGE system (Dcode Universal Mutation Detection System; Bio-Rad, U.S.A.) was operated as specified by the manufacturer. Forty-five µl aliquots of PCR products were electrophoresed in gels containing 8% acrylamide, 6 M urea, and 20% formamide with a 1.5× TAE buffer system [34] at a constant voltage of 100 V for 12.5 h and then 40 V for 0.5 h, while applying a thermal gradient of 39 to 52°C. Before electrophoresis, the gel was equilibrated to the temperature gradient for 30 to 45 min. A specific TGGE band was excised and amplified with the same primers without the GC clamp and its sequence was compared with those in the GenBank database.

## RESULTS

#### Porous Carbon Plate

The SEM image of the carbon plate used as both the anode and cathode resembled a roll of tangled wire, as shown in Fig. 3. The inside pores may be adequate to provide a channel for the transfer of water molecules containing protons and oxygen. The wire-like carbon lattice may be sufficient to conduct electrons and catalyze reactions of electrons, protons, and oxygen.

#### The Predominant Bacterium

The bacterium isolated by the most probable number method was identified by 16S rDNA sequence analysis and was found to be 99% similar to various Mn-oxidizing bacteria and *Burkholderia* sp. found in the GenBank database. The sequence was deposited in GenBank and assigned as Accession No. FJ486119.

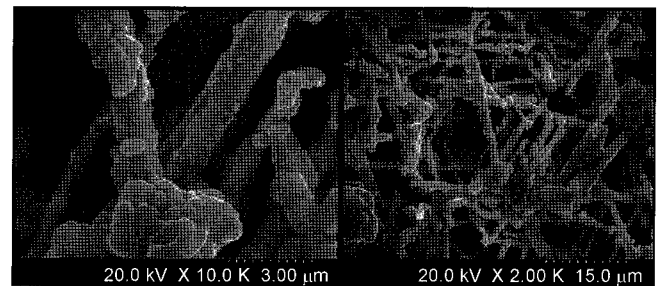


Fig. 3. SEM image of a porous carbon plate, which was made of porcelain clay, graphite powder, and kaolin powder, by baking at 1,100°C for 6 h.

#### COD Variation

To simulate the operating conditions of an actual wastewater treatment system, the wastewater was not sterilized, the reactor was not closed, and the temperature and pH were not controlled. Accordingly, various heterotrophic bacteria could have grown in the reservoir containing the artificial wastewater; however, oxygen-dependent catabolism may have been limited in the absence of aeration or an oxidant. The COD was about 1,500±150 mg/l in the reservoir of the serial NCMFC operated under open-circuit conditions, 1,300±100 mg/l in the first NCMFC unit, 1,100±80 mg/l in the second unit, 1,000±50 mg/l in the third unit, and 900±50 mg/l in the fourth unit (data not shown). Under open-circuit conditions, both the anode and cathode function simply as the matrix for biofilm formation [23], which may inhibit bacterial metabolism and growth by blocking oxygen diffusion [14, 15]. In the closed-circuit system, the anode is electrically linked to the cathode, and anodic potential is therefore balanced with cathodic potential. The anodic potential induced by the aerobically oxidized cathode may be limited below the oxygen potential (+0.82 V)

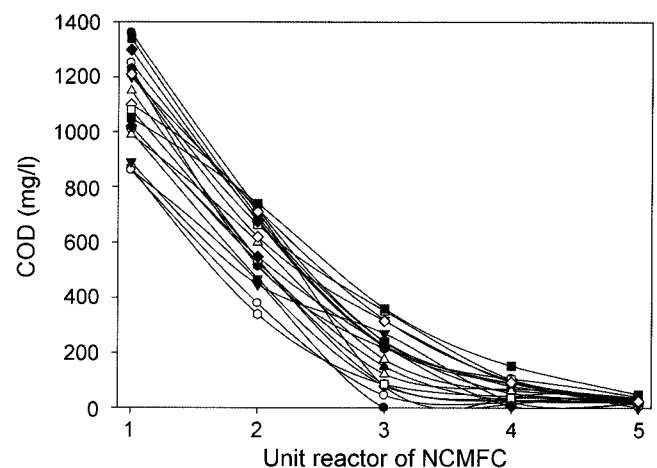
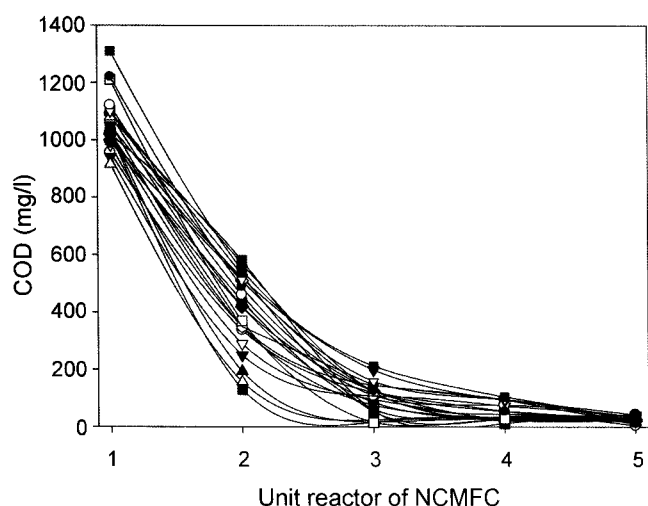


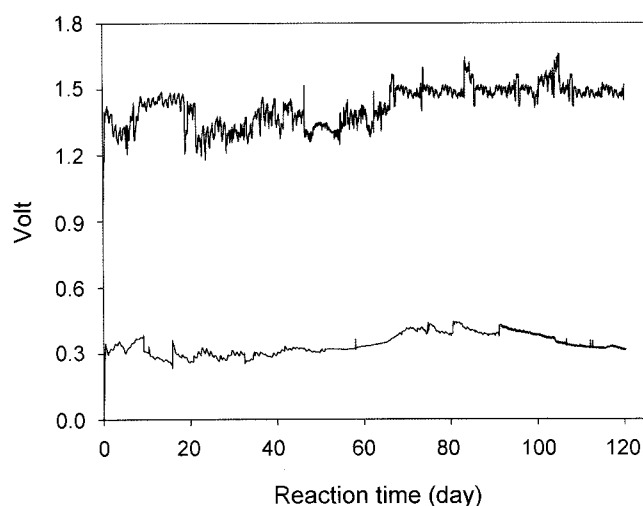
Fig. 4. COD variation in a serially connected conventional NCMFC composed of four units.

Wastewater flowed spontaneously from the reservoir (1) to the fourth unit (5) through the first (3), second (4), and third NCMFC units.



**Fig. 5.** COD variation in a serially connected solar cell-linked NCMFC composed of four units.

Wastewater flowed spontaneously from the reservoir (1) to the fourth unit (5) through the first (3), second (4) and third NCMFC units.



**Fig. 6.** Electricity generation by a conventional NCMFC (lower graph) and a solar cell-linked NCMFC (upper graph), which were operated under closed-circuit configuration with 200  $\Omega$  resistance.

because the oxygen balance is below 0.2 in air. However, the anodic potential induced by the solar cell may be maintained at 1.5 V owing to the constant solar cell potential. In practice, the COD in the first and second unit reactors was more actively decreased in the solar cell-linked NCMFC than in the conventional NCMFC, as shown in Figs. 4 and 5.

### Electricity Production

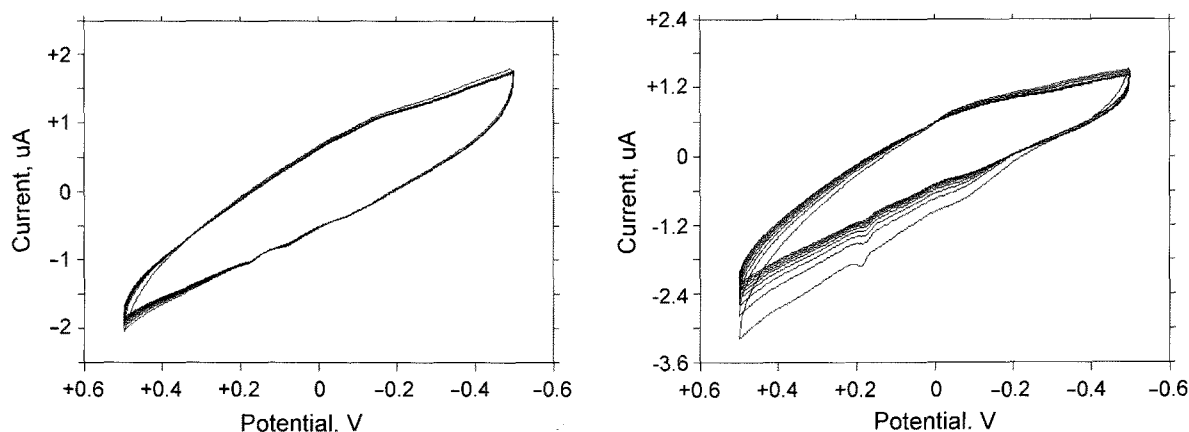
The electric power of the solar cells may induce anode oxidation and cathode reduction in the NCMFC, thereby potentially activating bacterial catabolism and catalyzing the reaction of protons, electrons, and oxygen [8, 38]. As shown in Fig. 6, the electricity generated by the conventional NCMFC, in which the electric energy had to be continuously consumed through the resistance, was  $0.35 \pm 0.05$  V under closed circuit with 200  $\Omega$  of external resistance. Current generation was periodically monitored using an amperemeter serially connected to the external resistance between the anode and cathode. The experimentally monitored current was consistent with the calculated value with an error of less than 5%. The current calculated from the voltage and resistance was about 1.75 mA, which corresponds to about 0.6 mW (2.16 Wh) per 0.1 m<sup>2</sup> anode (about 6 mW per 1 m<sup>2</sup> anode) for 1 h. The electricity generated by the solar cell-linked NCMFC was  $1.52 \pm 0.1$  V under closed circuit with 200  $\Omega$  of external resistance. The current calculated from the voltage and resistance was about 7.5 mA, which corresponds to about 11.25 mW (40.5 Wh) per 0.1 m<sup>2</sup> anode (about 113 mW per 1 m<sup>2</sup> anode).

Experimentally, about 1,000 mg/l (31.3 mM) COD was consumed in 24 h, corresponding to the amount that is theoretically required for the metabolic oxidation of 5 mM

glucose coupled with regeneration of 40 mM NADH and 10 mM FADH<sub>2</sub>. A total of 50 mM of reducing power (XH<sub>2</sub>) corresponds to 100 mmol of e<sup>-</sup>, which corresponds to 9,649 (96,487 × 0.1) coulombs. The experimental potential difference between the Mn(IV)-anode and the Fe(III)-cathode measured in a previous study was 0.343 V (0.693 – 0.35) [26]. Mn(IV) can be reduced to Mn(II) when coupled with biochemical metabolism and reoxidized to Mn(IV) when coupled with the reduction of Fe(III) to Fe(II), which is reoxidized to Fe(III) by atmospheric oxygen. The theoretical electric energy based on COD consumption over 24 h can be calculated as follows: 9,649 coulomb × 0.343 V = 3,310 J, which corresponds to 38.3 mW (3,310 J ÷ 24 h ÷ 3,600 s = 38.3 mW or mJ/s). Accordingly, energy efficiency was 0.6/38.3 (1.57%) in the conventional NCMFC and 11.25/38.3 (29.4%) in the solar cell-linked NCMFC.

### Electrochemical Analysis of the Isolate

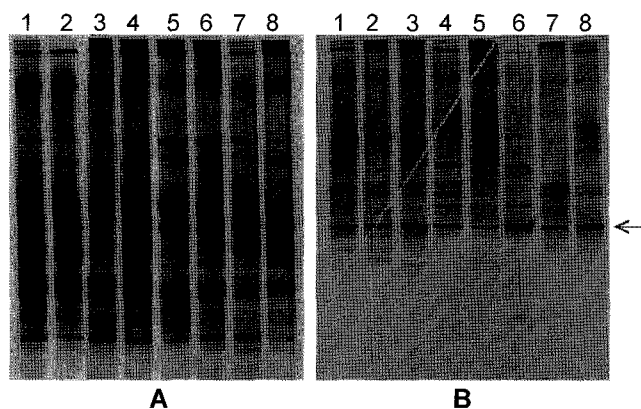
During cyclic voltammetry using the Mn(IV)-modified electrode as the working electrode, the lower voltammograms (Fig. 7, right) were gradually moved downward in a wide range from 500 to -200 mV (*vs.* Ag/AgCl); however, voltammograms obtained using the plain carbon electrode (Fig. 7, left) were not. These results show that electrons may have been unidirectionally transferred from bacterial cells to the Mn(IV)-modified electrode in coupling with the metabolic oxidation of glucose, in which glucose functioned as an electron donor, the bacterial cells functioned as a catalyst, and the anode functioned as an electron acceptor. In addition, the lower peak around 200 mV (*vs.* Ag/AgCl) may represent a specific potential for the redox reaction between the electrode and bacterial cells but not for electron transfer from bacterial cells to the electrode.



**Fig. 7.** Cyclic voltammograms obtained from intact cells of the isolate during reaction with 30 mM glucose on a plain carbon electrode (left) and a Mn(IV)-modified carbon electrode (right) used as a working electrode.

### Bacterial Community TGGE Patterns

TGGE was performed to monitor variation in the bacterial community growing on the Mn(IV)-modified anode surface before and after NCMFC reaction conditions were stabilized. As shown in Fig. 8, the bacterial community at an early reaction time (day 10, panel A) was relatively diverse based on the appearance of multiple bands; however, a common band (marked with an arrow) remained at a later time when electricity production had been stabilized (day 90, panel B), suggesting that certain exoelectrogenic bacteria dominated the Mn(IV)-modified anode surface, whereas some other types of bacteria were excluded from the community. The partial 16S rDNA sequence of the arrow-marked TGGE band, which was deposited in the GenBank database under Accession No. FJ486118, was found to be 100% similar to that of Mn-oxidizing bacteria and various *Burkholderia* sp.



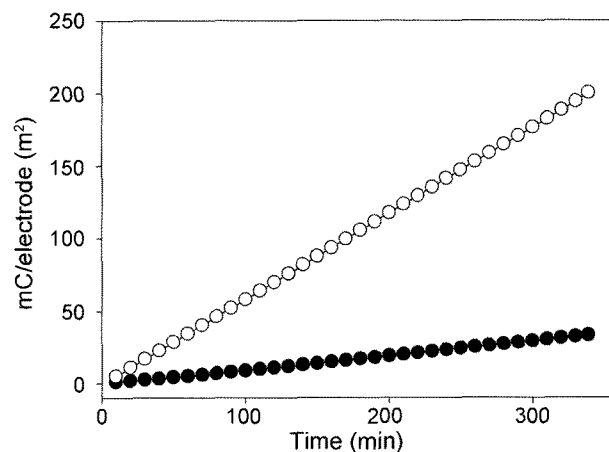
**Fig. 8.** TGGE profiles based on the variable region of the 16S rRNA gene from the bacterial community growing on the anode surface on day 10 (A) and day 90 (B) in conventional NCMFC units 2 through 5 (lanes 1 to 4, respectively) and in solar cell-linked NCMFC units 2 through 5 (lanes 5 to 8, respectively) (see Fig. 1).

### Electricity Production by the Isolate

Generation of electricity by a pure culture of the isolate was much higher in the NCMFC composed of the Mn(IV)-modified anode and the Fe(III)-modified cathode than in the NCMFC containing the plain carbon anode, as shown in Fig. 9. This result provides a practical clue that the isolate may be an exoelectrogenic strain, whose reducing power may be transferred to the anode in coupling with the Mn(IV)/Mn(II) redox reaction.

### DISCUSSION

Generally, electric energy in an MFC is generated by the proton gradient between the anolyte and catholyte and the electron-driving force between the anode and cathode. Both are generated by coupling the oxidation–reduction (redox) reaction of the anode with bacterial metabolism. In order to conserve the proton gradient and the electron-



**Fig. 9.** Electricity production in an NCMFC composed of a Mn(IV)-modified anode (○) and a plain anode (●) by the resting cells of the predominant species isolated from the anode surface.

driving force, the catholyte has to be separated from the anolyte by a redox barrier [38] and selectively aerated. Catholyte aeration is a costly process that is dependent on an external source of electricity. Accordingly, a substitute MFC without catholyte aeration is absolutely required for the production of useful electricity. A system developed for harvesting electricity from marine or river sediments [16, 17, 28] makes it possible to produce useful electric energy without aeration. However, this system depends on a redox potential difference between the anaerobic sediment and the upper aerobic layer of seawater, making the proton gradient difficult to conserve and resulting in an efficiency that may be lower than in a reactor-type MFC [24, 27].

Since Park and Zeikus [25, 26] developed a modified electrode with Fe(III) or Mn(IV) ion capable of functioning as an oxidant or an electron mediator and a single-compartment fuel cell capable of operating without aeration [26], various modified MFCs have been studied [11, 20, 21]. A cathode composed of a porous carbon plate (Fig. 3), a porous ceramic membrane, and a semipermeable cellulose acetate film may be sufficient to conserve the proton gradient and the electron-driving force because water molecules inside the pores of the cathode may function as a proton carrier (catholyte), the cathode exposed to air may function as an electron acceptor, and the cellulose acetate film may function as a redox barrier (Fig. 2).

The electric potential between anode and cathode in the conventional NCMFC under closed-circuit conditions with 200  $\Omega$  of external resistance was  $0.35 \pm 0.05$  V, which is much lower than  $2.0 \pm 0.2$  V, the potential measured under open-circuit conditions (data not shown). On the other hand, the potential in the solar cell-linked NCMFC under closed-circuit conditions with 200  $\Omega$  of external resistance was  $1.46 \pm 0.1$  V, which is lower than  $2.87 \pm 0.1$  V, the potential measured under open-circuit conditions (data not shown). The difference in electric potential generated under open- and closed-circuit conditions is caused by an imbalance between the electric energy generated by the NCMFC and consumed by the external resistance. These results indicate that generation of electric energy (voltage  $\times$  current) in the conventional NCMFC depends on the reducing power and electron-driving force generated by bacterial metabolism; however, generation of electric energy in the solar cell-linked NCMFC depends on the reducing power generated by bacterial metabolism and the electron-driving force of the solar cell. The electric voltage is proportional to the electron-driving force from anode to cathode, and the electric current is proportional to the reducing power generated by bacterial cells. The solar cells induce the electron-driving force from anode to cathode, by which the oxidation reaction coupled with bacterial catabolism may be activated in the anode and the reduction reaction of oxygen with protons and electrons may be activated in the cathode [10, 39].

During operation of the conventional and solar cell-linked NCMFCs, an electrode-active bacterium was enriched on the surface of the Mn(IV)-modified anode and was found to be the same species in both systems based on 16S rDNA sequencing (GenBank Accession No. FJ486119). Cyclic voltammetry results suggest that this species unidirectionally donated electrons to the Mn(IV)-modified electrode (Fig. 7, right). The fact that electricity was selectively generated by a pure culture of the isolated bacterium in the NCMFC with the Mn(IV)-modified anode suggests the possibility that the isolate may be a Mn(IV)-electrode-specific exoelectrogenic species.

TGGE results indicate that a bacterial species was enriched in all units of the NCMFC after cultivation for more than 90 days (arrow-marked band, Fig. 8). The 16S rDNA sequence of this species (GenBank Accession No. FJ486118) was found to be very similar to the predominant species initially isolated from the Mn(IV)-modified anode in the conventional and solar cell-linked NCMFCs. Under anaerobic conditions, the Mn(IV)-modified anode may be a unique electron acceptor capable of substituting for oxygen, and a specific bacterial species capable of respiring under the anodic oxidation potential may be spontaneously enriched, thereby coupling electricity generation with wastewater treatment. Electricity generation may be limited in the third and fourth NCMFC units owing to limiting organic carbon, which was almost completely consumed in the first and second NCMFC units. This phenomenon may limit electricity generation and efficiency when treating wastewater using the NCMFC. The efficiency of electricity generation was 1.57% in the conventional NCMFC but was 29.4% in the solar cell-linked NCMFC. The electron-driving force generated by the solar cell can activate bacterial catabolism coupled with the Fe(III)/Fe(II) and Mn(IV)/Mn(II) redox reactions, thereby increasing electric voltage. The electric current cannot be generated by the solar cell but can be generated by the metabolic oxidation of organic compounds, which may be a limiting factor for electricity generation by MFCs. Accordingly, the solar cell-linked MFC may be a solution to improve wastewater treatment efficiency coupled with electricity generation.

TGGE band patterns showed that the diversity of the bacterial community growing on the anode surface was decreased after 90 days of incubation time, especially in the solar cell-linked NCMFC. The oxidation potential generated on the anode surface by the electron-driving force of the solar cell may have activated exoelectrogenic bacteria and weakened the competitive ability of other bacterial species, perhaps contributing to the difference in electric energy efficiency between the conventional and the solar cell-linked NCMFCs.

MFCs have been applied to systems for wastewater treatment, odor removal, and microbial denitrification. Kim *et al.* [13] produced 228 mW/m<sup>2</sup> of electric energy at

an electric potential of 0.4 V from 165 ml in a single-chambered MFC with 1,000  $\Omega$  of external resistance, consuming about 6,946 mg/l of COD. Jia *et al.* [12] reported that 1.7 mW/m<sup>2</sup> of electric energy was produced by an MFC using denitrifying bacteria as an electron acceptor with a coulombic efficiency of 7%. Zuo *et al.* [40] used a 10-ml U-tube-type two-compartment MFC with an external resistance of 250 to 5,000  $\Omega$  to isolate an exoelectrogenic bacterium, producing a maximal 89 mW/m<sup>2</sup>, which was 17% of the electric energy production. Park and Zeikus [24] reported that 787.5 mW/m<sup>2</sup> of electric energy was produced by a two-compartment MFC equipped with an Mn(IV)-anode and Fe(III)-cathode, using anaerobic sewage sludge. Although some of these systems may be useful, it is difficult to estimate and compare differences in their efficiency of electricity generation and wastewater treatment because reactor volumes (10 to 1,000 ml) and reaction times (24 h to 120 days) were too variable, and some external resistances were also too low (100) or too high (5,000) to directly compare electric currents calculated from the relationship between potential and resistance.

In conclusion, the electricity generated from the solar cell-linked NCMFC may be useful because an electric voltage of 1.5 V and a current of 7.5 mA may be sufficient to recharge a battery, and electricity production can be easily amplified by increasing the electrode area and the number of unit reactors in the NCMFC. Electricity production was stabilized after 10 days of incubation and was stably maintained during incubation for more than 120 days. Electric energy generated from fossil fuels has to be consumed for the operation of conventional MFCs, which is a limiting factor for useful energy production. The solar cell-linked NCMFC may not be a complete solution for useful electricity production by MFCs; however, it gives us a possible solution for the design and development of a new concept in MFCs.

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