Korean J. Chem. Eng., **27**(3), 828-835 (2010)
DOI: 10.1007/s11814-010-0142-6

Characterization of an open biocathode microbial fuel cell for electricity generation and effluent polish

Guo-Wei Chen*,, Jae-Hwan Cha*, Soo-Jung Choi*, Tae-Ho Lee*, and Chang-Won Kim*,†**

*Department of Environmental Engineering, Pusan National University, Busan 609-735, Korea
**School of Civil Engineering, Hefei University of Technology, Hefei 230092, China
(*Received 3 September 2009 • accepted 12 October 2009*)

Abstract−The application of a biocathode in a microbial fuel cell (MFC) could be an alternative for the abiotic cathode MFCs that use noble metal catalysts and/or artificial mediators. An open biocathode MFC with oxygen reduction was investigated in this study and the roles of microbes in the cathode compartment were characterized. After 50-days operation, the MFC became stable and the power density of the MFC reached 2.55 W/m^3 at an influent flowrate of 0.20 mL/min. The concentration of chemical oxygen demand (COD) was significantly reduced from 372 mg/L (in the influent) to 22 mg/L (in the final effluent) at an influent flowrate of 0.20 mL/min. Microbial community analysis demonstrated that four major groups of the clones were identified, where 28 clone types were derived from the cathode microorganisms, which included *proteobacteria, Firmicutes*, *Bacteroidetes* and unclassified bacteria. Among these phylatypes, *Deltaproteobacteria* was the most abundant division with 25.0% of total clones, which plays important roles in the cathodic electron transfer process. The presence of symmetric peaks could be detected in the effluent of the cathode compartment, which confirmed that the possible electron mediators were excreted by cathodic bacteria involved in the electron transfer process.

Key words: Biocathode, Cathodic Microbe, Electrochemical Response, Microbial Fuel Cell

INTRODUCTION

Combining energy recovery and pollution control, the microbial fuel cell (MFC) is a promising technology for wastewater treatment for the future [1-5]. However, several key problems remain unresolved for the practical application of MFC, such as the need for expensive noble metal catalysts in the oxygen-reduced cathode, and the additional treatment facility required for polishing the unsatisfactory effluent quality, among many other problems [4,6,7].

To resolve these difficulties, researchers have been carrying out tests of various electrode materials and catalysts, or have added redox mediators to improve oxygen reduction efficiency. Various electrode materials have been investigated, such as carbon paper, carbon felt, carbon brush, carbon fiber, graphite of various types, Pt, Cu, Cu-Au, tungsten carbide, etc. [8-12]. While it is common to use Pt as a cathode catalyst, other polymer binders were tested for their roles as membranes, such as perfluorosulfonic acid (Nafion), poly tetrafluoroethylene (PTFE), as well as the noble-metal free electrocatalyst, cobalt tetramethylphenylporphyrin (CoTMPP), etc [13,14]. Furthermore, many researchers have also added alternative oxidants, that is, artificial electron redox mediators, such as ferricyanide, neutral red, thionin or methyl viologen, into the cathode compartment to assist cathodic reaction [8,9]. Unfortunately, due to the high cost and potential pollution, it is evident that none of these are realistic for practical application, even though these modifications could significantly improve cathode performance.

Compared to the above methods, the biocathode is a promising way to improve the cathode reaction performance without additional investment or potential pollution [15-18]. The presence of the seawater biofilm on the stainless steel surface has proven to be an efficient catalysis of oxygen reduction, and the biofilm-covered cathode is able to support a current density of up to 1.89 [19]. The current densities of up to a 2.2 A/m^2 cathode projected surface were obtained when oxygen was used as the ultimate electron acceptor on a biocathode that had been developed on graphite felt or woven carbon fibers [20]. Clauwaert et al. [21] developed an MFC in which microorganisms in the cathode performed a complete denitrification by using electrons supplied by microorganisms that were oxidizing acetate in the anode, and obtained an output of about 10 W/m^3. A two-chambered MFC with an autoheterotrophic denitrifying biofilm on the cathode that performed denitrification generated 9.4 mW/m^2 of anode surface with a substrate removal of 65% [22]. Moreover, the maximum current of 1.14 mA was obtained with the external resistance of 10 Ω in a similar system [23].

The effluent of the anode compartment in a continuous process cannot usually be discharged due to unsatisfactory quality [4,6,24]. An additional treatment facility is therefore needed for polishing the effluent from the MFC. In an attempt to solve this problem, the anodic effluent was introduced into the cathode compartment as a catholyte in this study. Electricity generation as well as excellent effluent quality can be expected in this configuration.

The application of a biocathode is a promising approach in MFCs, and a number of studies have contributed to the progress of its performance as mentioned above [15,16,22,23]. Moreover, improvement of effluent quality from the MFC has been of great interest. However, only limited information is available on the characterization of the cathode in such a sequential flowchart, particularly in

†To whom correspondence should be addressed.
E-mail: cwkim@pusan.ac.kr

terms of the roles of microbes presented in the cathode compartment. To address this, an open biocathode fed by anodic effluent in a microbial fuel cell was established in this study, and more information was investigated about the microbial functions of this approach.

MATERIALS AND METHODS

1. Construction and Operation of MFC

Fig. 1 shows a schematic diagram of the open biocathode microbial fuel cell used in this study [25]. The reactor was made of plexiglass. The anodic compartment was located at the bottom part, while the cathodic was placed on the upper side of the anodic compartment. The two compartments were separated with an Ultrex cation exchange membrane (125 mm×145 mm, Membranes International Inc.). The cathodic compartment was open to the ambient circumstances. The volume of both the anode and cathode compartments was 319 mL (120 mm×140 mm×19 mm). Plain granular graphite with a porosity of 0.55 served as the electrode in each compartment. The two compartments were connected by an external resistor of 30 Ω through graphite rods. Anaerobic and aerobic sludge from a pilot wastewater treatment plant (Pusan National University, Busan, South Korea) was seeded into the anode and the cathode compartments, respectively. To improve the effluent quality from the anodic compartment, it is an alternative to introduce anodic effluent into the cathodic compartment, which served as not only a catholyte but also a carbon source for the cathodic microbes [6]. After flowing through the cathodic compartment, the effluent was then polished.

Synthetic wastewater was used in this experiment, which was composed of sodium acetate (500 mg/L) as the sole carbon source, as well as other nutrient solution (KH_2PO_4 6.2 g/L, K_2HPO_4 10.0 g/L, NH_4Cl, 1.0 g/L, $NaHCO_3$ 2.0 g/L, NaCl 0.5 g/L, $NaSO_4$ 0.5 g/L, and $MgSO_4 \cdot 7H_2O$ 0.2 g/L). In addition, another microelement solution of 1.0 mL/L was added, which contained (unit in mg/L): $CaCl_2 \cdot 6H_2O$ 130; $FeCl_3$ 1,000; H_3BO_3 6; $ZnCl_2$ 70; $CuCl_2$ 2; $MnSO_4 \cdot H_2O$ 100; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ 206; $AlCl_3$ 50; $CoCl_2$ 238 and $NiCl_2$ 24. For microorganism enrichment, the flowrate of 0.10 mL min^{-1} was adopted. Oxygen served as the terminal electron acceptor in the cathodic compartment by passive ventilation. The MFC was operated at an ambient temperature of 22 °C using an air conditioner. When the MFC became stable, various flowrates ranging from 0.10 to 0.3 mL min^{-1} were tested to optimize the performance.

2. Electrochemical Monitor and Analysis

The external resistor (R) was fixed at 30 Ω, and the current (I) (mA) can be calculated as follows: I=V/R=Q/t, where V is the voltage (mV), Q is the charge (C), and t is the time (s). The power output of the cells P (W) was calculated as follows: P=I×V. Internal resistance (R_{int}) was determined by the slope of the polarization curves

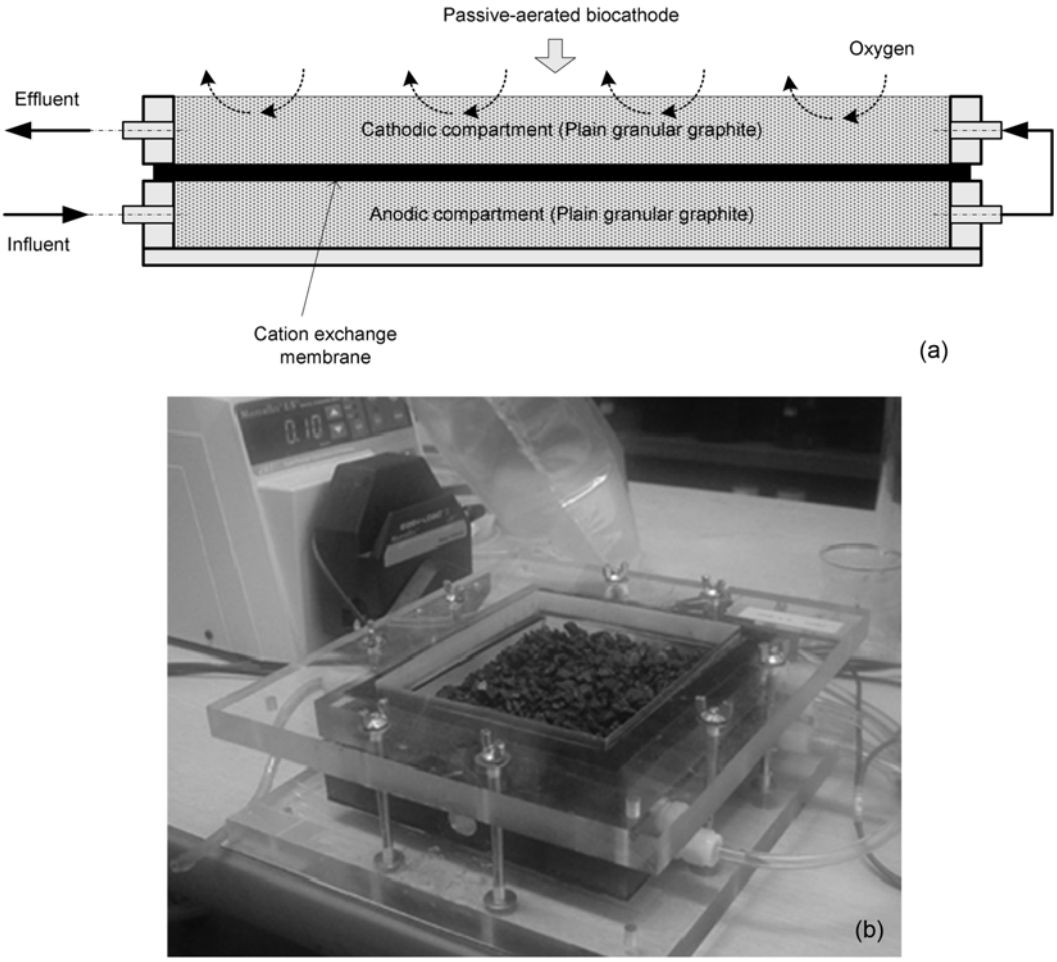

Fig. 1. Schematic (a) and laboratory-scale prototype (b) of the open biocathode in a two-chambered microbial fuel cell.

[13].

The cyclic and pulse differential voltammograms were carried out by a potentiostat (Model KST-P1, Kosentech, South Korea). A polarization curve was obtained from the linear sweep voltammetry [3,26]. The cyclic and pulse differential voltammograms were adopted to detect the function of microorganism in 25-mL samples by the classic three electrode method [27]. The cyclic voltammetry was generally performed in a CV test kit (Cell stand C3, BAS, Warwickshire, United Kingdom) by starting from −800 mV and going up to 800 mV and back at a scan rate of 50 mV/s. The pulse differential voltammogram was generally performed in a CV test kit in a range of −1,200 mV to 1,200 mV at a scan rate of 5 mV/s. All the data of the cyclic and pulse differential voltammograms were logged by a personal computer that was connected to the potentiostat.

The working electrode was a glassy carbon electrode (MF-2012; BAS, Warwickshire, United Kingdom) that was cleaned in deionized water prior to use. The counter electrode was a platinum counter electrode (MF-1032; BAS, Warwickshire, United Kingdom), and an Ag/AgCl electrode (MF-2052; BAS, Warwickshire, United Kingdom) was selected as the reference electrode. All three electrodes were inserted into the test vial, while avoiding any contact between the electrodes. In the classical convention, potentials become more negative along the (positive) x-axis, and reduction (cathodic) currents are positive. In our cyclic or pulse differential voltammograms, the convention of International Union of Pure and Applied Chemistry (IUPAC) was adopted and the opposite is applied, that is, the anodic current is positive and positive potentials are right-hand plotted.

3. DNA Extraction

After the 180-d stable operation, the sludge samples were collected from the cathode compartment of the microbial fuel cell. DNA was extracted from the MFC samples using the Powersoil™ DNA isolation kit (MoBio, USA). DNA extracts were examined by agarose (1% w/v) gel electrophoresis and were compared with the 1 Kb +DNALadder (SolGent, Korea).

4. PCR Amplification of 16S rDNA Gene

Amplification of 16S rDNA gene fragments of the *Eubacteria* from the extracted DNA was performed by using the following primer combinations in a polymerase chain reaction (PCR) with EUB 8F (5'-AGAGTTTGATCMTGGCTCAG-3') and EUB 1392R (5'-ACGGGCGGTGTGTACAAG-3'). Amplification reactions were performed with 2.5 μl of a 10× Taq buffer, 0.5 μl of 10 mM dNTP, 1 μl of each primer (10 pmol), 2 μl of DNA template and a 0.125 μl Taq DNA polymerase (SolGent, Korea) in a 25 μl PCR stock solution with deionized water (total amount was 25 μl). Reaction mixtures were held at 95 °C for 9 min followed by 30 cycles of 95 °C for 1 min, 53 °C for 1 min and 72 °C for 2 min per cycle, with a final extension step of 10 min at 72 °C [28]. The PCR products were purified using a PCR purification kit (Bioneer, Korea).

5. 16S rDNA Gene Cloning, Sequencing and Analysis

Cloning of the purified PCR products was carried out with the 2× Rapid Ligation buffer using the pGEM-T Easy Vectors (Promega, Madian, USA). Ligation reactions were performed with 3 μl of 2× rapid ligation buffer, 1 μl of pGEM-T Easy Vector, 1 μl of T4 DNA ligase and 3 μl of PCR product with deionized water to a final volume of 10 μl. Competent JM109 cells (Promega, Madian, USA) were transformed with the ligated DNA. All procedures were carried out according to the supplier's instructions. White colonies were selected at random and nucleic acids were extracted from a sector of *E. coli* colonies. Amplification of DNA inserts was performed using primers M13F (5'-AGTCACGACGTTGTA-3'), and M13R (5'-CAGGAAACAGCTATGAC-3'). PCR products of the correct size were selected randomly. Representatives from the different clone types were sequenced by SolGent (Korea), and then were also subjected to the National Centre for Biotechnology (NCBI) BLAST (http://www.ncbi.nlm.nih.gov/blast/) search to identify sequences with the highest similarity. Partial 16S rRNA sequences, their closest relatives and appropriate types of strain sequences were aligned using CLUSTAL W [28].

6. Chemical Analysis

An auto analyzer (Model AA3, Bran+Luebbe, German) was used to determine the concentration of nitrate, nitrite, ammonia and chemical oxygen demand (COD).

RESULTS AND DISCUSSION

1. Characterization of the Open Biocathode MFC in the Presence of Microbes

To manifest the function of microbes presented in the cathode compartment, the electrochemical responses of the open biocathode MFC were recorded under different inoculation conditions, including the blank MFC, the blank MFC fed with influent, the MFC seeded

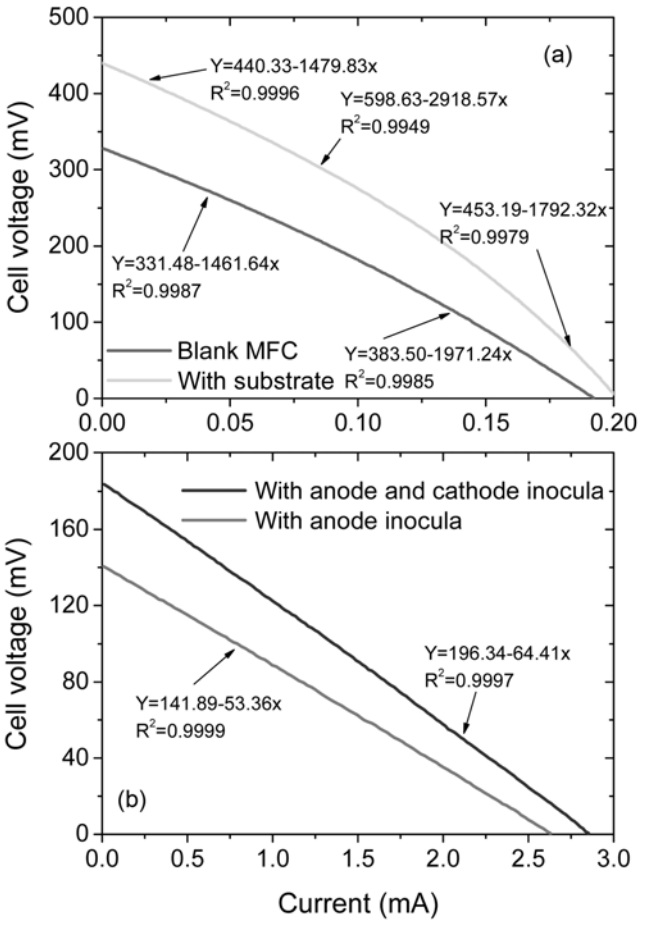

Fig. 2. Polarization curves of blank reactor (a) and inoculated reactor (b) in the open biocathode MFC during startup.

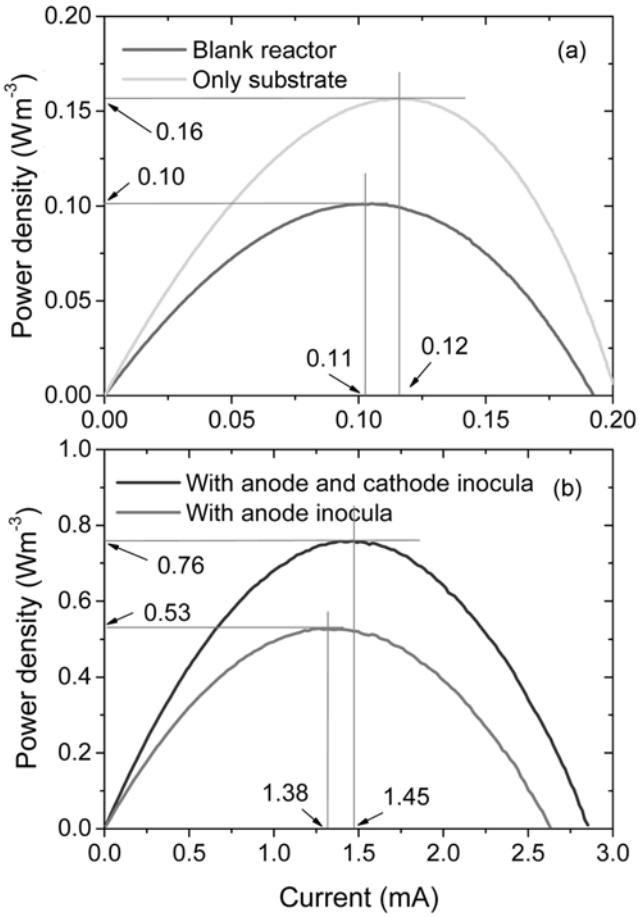

Fig. 3. Power curves of blank reactor (a) and inoculated reactor (b) in the open biocathode MFC during startup.

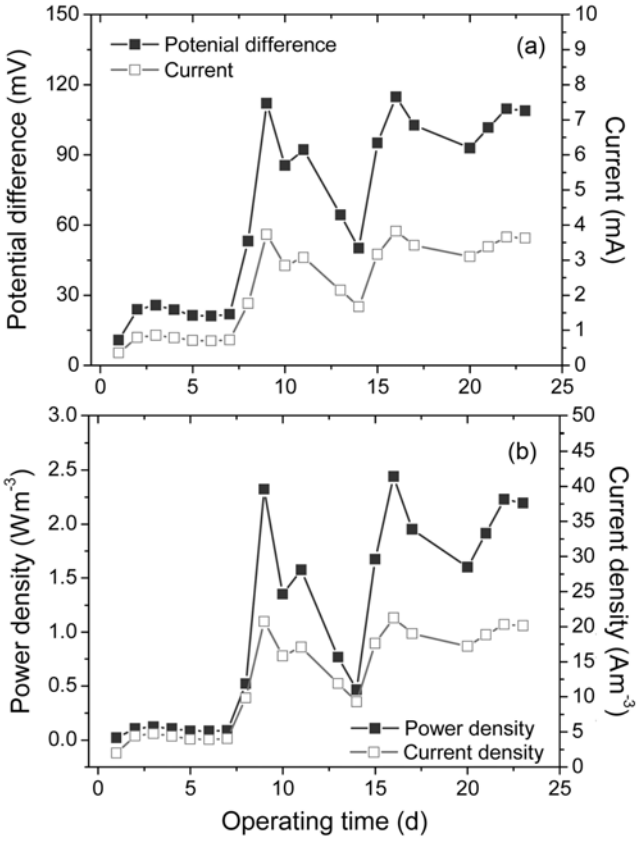

Fig. 4. Performance of the open biocathode MFC during startup: Potential difference and current (a), Power density and current density (b).

with anodic bacteria, and the MFC seeded with anodic and cathodic bacteria. As shown in Figs. 2 and 3, before the MFC was running continuously, the properties of the cell were checked carefully. According to the cell voltage and power density data shown in the polarization curves, it is obvious that the presence of microbes in both compartments was beneficial for the performance of the MFC. Without the presence of microbes in both compartments, the blank MFC exhibited a higher internal resistance ranging from 1,480 to 2,919 Ω, which significantly affects the electricity generation. As substrate was fed into the system, the system internal resistance decreased slightly, within the range of 1,461 to 1,971 Ω. When microbes were introduced into the system, the internal resistance decreased substantially, especially when the biocathode was applied. As shown in Fig. 2(b), when the anode was seeded with an inoculum, the internal resistance of the MFC decreased to 53 Ω. Similarly, when the inoculum was seeded into the cathode compartment, the internal resistance remained at a low level of 64 Ω. The decrease of internal resistance of the MFC systems in the presence of microbes proved that the application of a biocathode is a promising way to enhance the electricity generation in an MFC.

The power curves of the MFC under different configurations are shown in Fig. 3. To eliminate the effect of plain graphite, the blank MFC or the MFC feed only with a substrate is given in Fig. 3(a). Without the presence of the microbes, the MFC produced very low electricity of 0.10 and 0.16 W/m^3 for the blank MFC and the MFC fed only with substrate, respectively. As soon as the microbe was seeded into the anode chamber, the power density increased to 0.53 W/m^3. When the biocathode was applied by seeding microbes into the cathode chamber, the power density slightly increased to 0.76 W/m^3. This demonstrated that the presence of microbes apparently enhanced the electrochemical response of the cell, especially under the conditions of a biocathode MFC.

2. Startup and Optimization of the Open Biocathode MFC

To start the open biocathode MFC with a biocathode, a low flowrate of 0.1 mL/min was used, which had an organic loading rate of 0.29 kg-COD/m^3 d. As shown in Fig. 4, the startup of the passive aerated MFC with a biocathode was quickly accomplished, and after 10 days, the output of electricity reached around 100 mV and 3.3 mA when a 30 Ω external resistor was used. The corresponding current and power density also increased to 20 A/m^3 and 2.0 W/m^3, respectively. After 25 days, the outputs of the open biocathode MFC reached a stable state at a flowrate of 0.10 mL/min. Fig. 5 shows the electricity generation during this period in detail. With an external resistor of 30 Ω, the cell potential voltage was around 90 mV with a current of 3.5 mA. The current and power densities at this steady state were maintained at 20 A/m^3 and 2.0 W/m^3, respectively.

To obtain the maximum output of the cell, it was necessary to verify the effect of the loading rate of the cell in this type of MFC. After 75-d operation, the MFC was fed with different flowrates and the results are listed in Table 1. Five different flowrates were tested

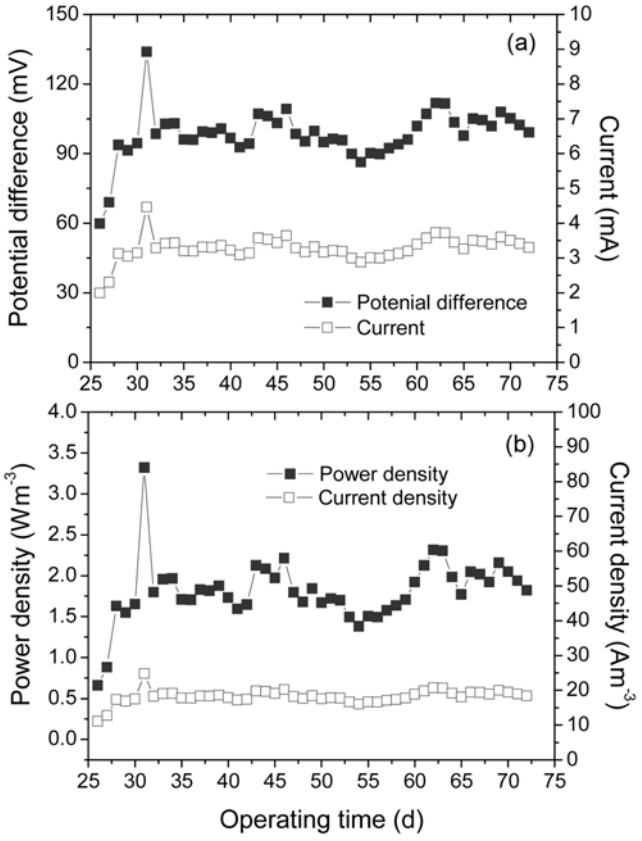

Fig. 5. Performance of the open biocathode MFC: Potential difference and current (a), Power density and current density (b).

in this study, from 0.1 to 0.3 mL/min, where the corresponding organic loading rates were in the range of 0.29 to 0.88 kg-COD/m^3 d. When the flowrate increased from 0.1 to 0.3 mL/min, the anodic potential decreased from −327 to −437 mV, and the cathodic potential decreased from −241 to −343 mV. However, the magnitudes of the decreasing values were not the same; thus a maximum cell potential was achieved at 116 mV with a current of 3.86 mA at the flowrate of 0.20 mL/min. Similarly, the power density reached its maximum value of 2.55 W/m^3, which was slightly higher than the performance previously reported by Rabaey et al. [20] of a biocathode developed on a graphite felt (2.1 W/m^3) or woven carbon fiber (1.3 W/m^3).

The substrate removal performance was another key factor of this MFC system. At different influent flowrates, the system exhibited similar removal efficiency. The quality of the effluent from the MFC system was excellent at all tested flowrates, within a COD range of 15 to 48 mg/L. However, it should be pointed out that a slight difference was found in the anodic effluent that was sampled before it flowed into the cathode compartment. At the influent flowrate of 0.20 mL/min, the anode compartment performed a relatively good function, and the anodic effluent COD was kept at a low level. Since the COD concentration in the cathode compartment significantly affected the cathodic potential, it was beneficial to maintain a low COD of anodic effluent to achieve a higher electricity output for the system. Consequently, at the influent flowrate of 0.20 mL/min, the system reached its maximum electricity generation.

3. Microbial Divisions of the Open Biocathode in MFC

As previously mentioned, the presence of the microbe on the biocathode in the cathode compartment could significantly enhance the electrochemical responses of the system. Hence, it is very important to determine which bacteria performed as a biocatalyst in this MFC system. After 180-d operation, the microbial analysis was carried out of a clone library based on a PCR and 16s rDNA, and the results of the phylogentic affiliations, closest matches, and relative percentage of clone numbers from the cathode compartment are summarized in Table 2. Twenty-eight different clone types were identified from 88 sequenced random clones, revealing the compositions of the bacterial community in terms of the major phyla and subdivisions. The bacterial community consisted of *Proteobacteria*, *Bacteroidetes*, *Firmicutes* and unclassified bacteria. Among these phylatypes, *Deltaproteobacteria* was the most abundant division with 25.0% of total clones (22 of 88), followed by 22.7% of unclassified bacteria (20 of 88), 17.0 % of *Firmicutes* (15 of 88), 11.4% of *Bacteroidetes* (10 of 88), 10.2% of *Alphaproteobacteria* (9 of 88), 8.0% of *Gammaproteobacteria* (7 of 88), and 5.7% of *Betaproteobacteria* (5 of 88).

This result showed the different microbial composition obtained from the graphite felt and woven carbon fiber, which were dominated by *Sphingobacterium*, *Acinetobacter* and *Acidovorax* sp. [20]. It was assumed that the difference was due to the different inoculum used in the reported experiment. In the study, rather than using a single composition of anaerobic sludge, the cathode compartment was inoculated with a mixture of environmental samples obtained from rusted metal poles in the Brisbane River at a freshwater section, sediment from a pond and mixed liquor from a domestic activated sludge plant.

According to the electricity generation, it was obvious that the presence of these bacteria was beneficial to the oxygen reduction in the cathode. An explanation of electron transfer by anodic bacte-

Table 1. Optimization of cell performance under various influent flowrates

Flowrate (mL/min)	Anodic potential (mV)	Cathodic potential (mV)	Cell potential (mV)	Current (mA)	Power density (W/m^3)	Organic loading rate (kg-COD/m^3 d)	Influent COD (mg/L)	Anodic effluent COD (mg/L)	Effluent COD (mg/L)
0.10	−326.95±6.31	−240.90±6.31	86.05±3.22	2.87±0.11	1.41±0.34	0.29	349.03	222.68	41.20
0.15	−324.41±58.04	−215.15±57.70	109.26±1.36	3.64±0.05	2.27±0.07	0.46	372.49	236.60	18.49
0.20	−397.06±5.93	−281.28±6.14	115.78±2.16	3.86±0.07	2.55±0.02	0.61	371.64	179.31	22.28
0.25	−416.87±7.62	−310.27±11.85	106.60±4.66	3.55±0.16	2.17±0.08	0.74	358.91	201.90	15.00
0.30	−437.73±13.95	−343.15±14.43	94.58±1.99	3.15±0.07	1.70±0.14	0.88	358.91	229.53	47.58

Table 2. Phylogenetic affiliations, closest matches and percentage of clones from the cathode compartment of the open biocathode MFC

Phylum (class)	Percentage (%)	Clones	Numbers	Genbank closest match (accession number)	Similarity (%)
Bacteroidetes	11.4%	G01	5	*Bacteroides* sp. 22C (AY554420)	98
		G02	3	*Flavobacterium* sp. 3A5 (AF368756)	99
		G04	2	*Pedobacter* sp. B9 (EU109722)	86
Firmicutes	17.0%	G08	3	*Clostridium* sp. D3RC-2 (DQ852338)	99
		G09	2	*Clostridium* sp. FF08 (AB276319)	99
		G10	6	*Soehngenia* sp. enrichment culture clone (EU498372)	100
		G13	4	*Fusibacter* sp. enrichment culture clone 22-7A (EU517558)	97
Alpha-proteobacteria	10.2%	G19	4	*Alpha proteobacterium* BAL284 (AY972871)	99
		G26	1	uncultured *Hyphomonas* sp. (EU305586)	76
		G07	4	*Hyphomonas polymorpha* (AJ227813)	99
Beta-proteobacteria	5.7%	G18	3	uncultured *beta proteobacterium* (AB076869)	97
		G25	1	*Thiobacillus* sp. K6.2 (EF079668)	95
		G29	1	*Dechloromonas* sp. R-28400 (AM084133)	96
Delta-proteobacteria	25.0%	G27	1	*Desulfobulbus elongatus* (X95180)	96
		G20	1	uncultured *Geobacter* sp. clone KB-1 (AY780563)	95
		G05	5	*Pelobacter propionicus* (X70954)	97
		G03	15	uncultured *delta proteobacterium* (EU104835)	99
Gamma-proteobacteria	8.0%	G30	1	uncultured *Chromatiales bacterium* (EU375037)	86
		G06	2	*Halomonas variabilis* (AY204638)	99
		G16	2	*Pseudomonas* sp. G3DM-15 (EU037276)	98
		G17	2	*Stenotrophomonas* sp. YC-1 (DQ537219)	99
Unclassified bacteria	22.7%	G21	1	uncultured bacterium (AB374123)	100
		G11	15	uncultured bacterium (DQ266900)	99
		G22	1	uncultured bacterium (AJ853567)	97
		G23	1	uncultured bacterium (AF338764)	99
		G24	1	uncultured bacterium (EU509144)	93
		G28	1	uncultured bacterium (DQ088761)	88

ria has been reported by various researchers in recent studies as follows. Without the presence of artificial electron mediators, the bacteria may produce an endogenous mediator to assist the electron transfer outside of the cell, for example, in primary or secondary metabolic products [25,29,30]. However, the biocatalysis function of the cathodic bacteria is not yet very clear.

4. Characterization of Electron Transfer Process by the Open Biocathode in MFC

According to explanations of anodic electron transfer by anodic bacteria, it was assumed that similar metabolic products were excreted by the cathodic bacteria to assist the electron transfer from bacteria to the cathodic electrode. Cyclic voltammetry is generally

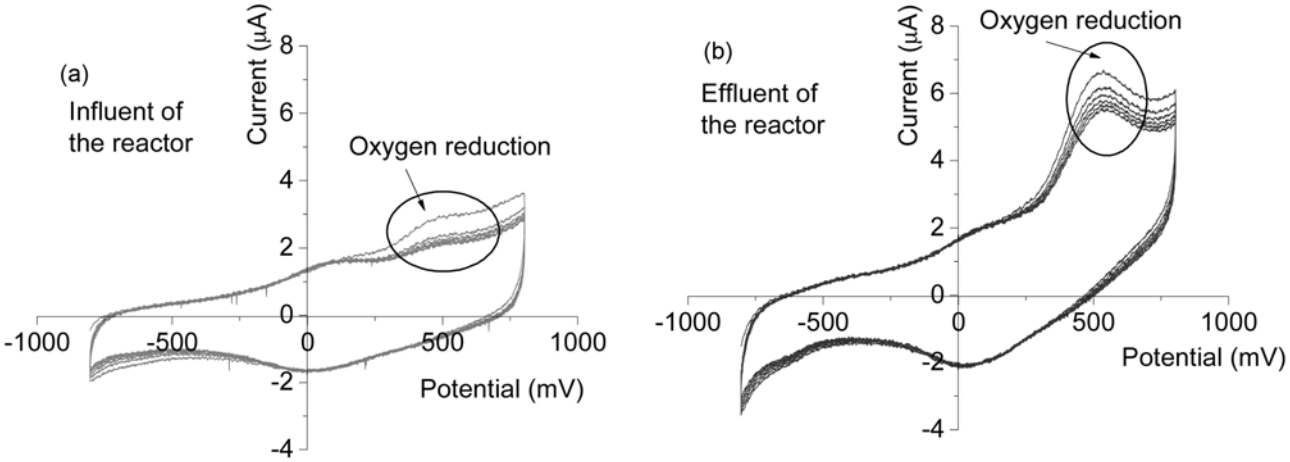

Fig. 6. The successive cyclic voltammogram of influent (a) and effluent (b) in the open biocathode MFC.

used to study the electrochemical properties of an analyte in solution. Meanwhile, compared to cyclic voltammetry methods, differential pulse voltammetry has an excellent detection limitation of 10^{-8} M, which can be used to study the redox properties of extremely small amounts of chemicals [26]. To characterize the electron transfer process involved in the cathode compartment, the cyclic and differential pulse voltammetry were adopted in this study.

The cyclic voltammogram was carried out and the results are shown in Fig. 6. To identify the function of the cathodic bacteria, the influent of the MFC was tested as a background value, as shown in Fig. 6(a). In agreement with the results shown in Figs. 2 and 3, the cyclic voltammogram of the influent showed a small peak of oxygen reduction, which proved that even without the presence of bacteria in the cathode compartment, significantly lower electricity can be produced by the plain graphite electrode. It was also consistent with the study carried out by Freguia et al. [31] of the MFC using a non-catalyzed cathode made of granular graphite, which also achieved significant stable currents. With the presence of cathodic bacteria, the peak of oxygen reduction was much higher than the background value, implying that the cathodic bacteria performed as a biocatalyst to enhance electricity generation in the MFC system.

Furthermore, the pulse differential voltammogram was used to verify in detail the solution responses for the electrochemical changes.

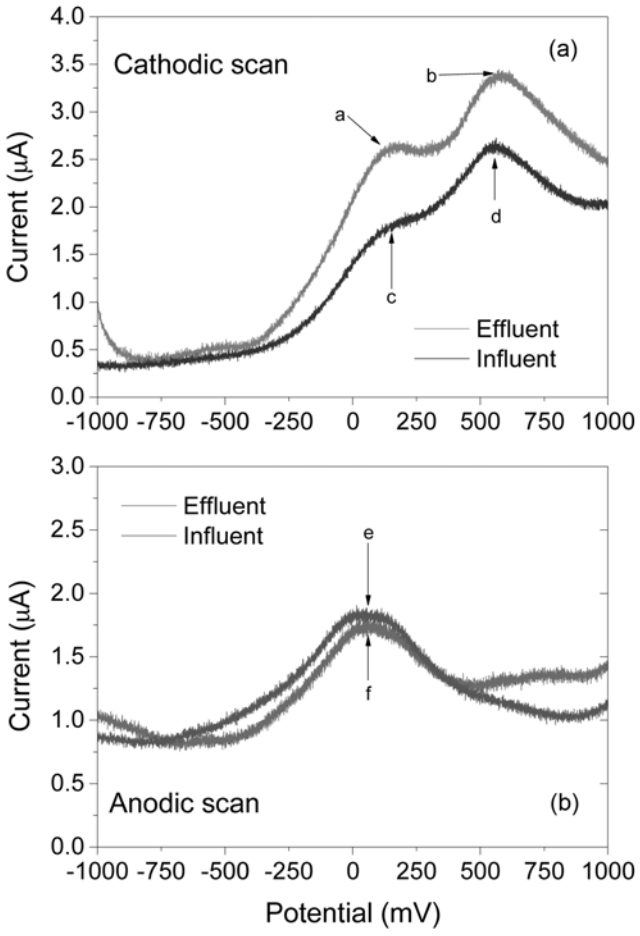

Fig. 7. The cathodic (a) and anodic (b) scan (5 mV/sec) of pulse differential voltammograms of the influent and effluent in the open biocathode MFC.

Fig. 7 shows the cathodic and anodic scans of pulse differential voltammograms of the influent and effluent in the open biocathode MFC. When the cathodic scan was carried out, distinct peaks were found in both solutions. The peaks (a, b) of the effluent were much higher than those of the influent (c, d), which indicates that the effluent solutions maybe contained electron mediators enhancing the electrochemical responses. For comparison, while the anodic scan was applied, two peaks (e, f) of the same scale were found at different potential values from that of the cathodic scan. Since the anodic effluent was used as catholyte in this study, it shows the same oxidation peaks of organic matters. According to the results of the above electrochemical tests, the function of bacteria present in the cathode compartment was confirmed.

CONCLUSION

The need for a noble metal or other chemical catalyst increases the manufacturing cost of MFC technology and limits its practical application. The use of a biocathode in an MFC is a promising method for the practical wastewater treatment process. Compared to the plain graphite granules, the presence of cathodic bacteria significantly enhances the electricity generation. Microbial community analysis demonstrated that four major groups of the clones were categorized among these 28 clone types derived from the cathode microorganisms. *Deltaproteobacteria* was the most abundant division of the sequenced clones in the cathode compartment. Further electrochemical tests confirmed the function of cathodic bacteria. This study provides direct evidence of the possibility of biocathode application in MFC technology.

ACKNOWLEDGMENTS

This work was financially supported by the Ministry of Environment as "Eso-Technopia 21 project" and "Human resource development Project for Energy from Waste & Recycling". It also was supported by the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea government (MEST) (No. 2009-0060376). The authors would also like to thank the support provided by the Second stage of Brain Korea 21 Project in 2008.

REFERENCES

1. L. T. Angenent, K. Karim, M. H. Al-Dahhan, B. A. Wrenn and R. Domiguez-Espinosa, *Trends Biotechnol*., **22**, 477 (2004).
2. B. H. Kim, I. S. Chang and G. M. Gadd, *Appl. Microbiol. Biot.*, **76**, 485 (2007).
3. B. Logan, B. Hamelers, R. Rozendal, U. Schroder, J. Keller, S. Freguia, P. Aelterman, W. Verstraete and K. Rabaey, *Eviron. Sci. Technol.*, **40**, 5181 (2006).
4. K. Rabaey, W. Ossieur, M. Verhaege and W. Verstraete, *Water Sci. Technol.*, **52**, 515 (2005).
5. L. M. Tender, C. E. Reimers, H. A. Stecher, D. E. Holmes, D. R. Bond, D. A. Lowy, K. Pilobello, S. J. Fertig and D. R. Lovely, *Nat. Biotechnol.*, **20**, 821 (2002).
6. S. Freguia, K. Rabaey, Z. Yuan and J. Keller, *Water Res.*, **42**, 1387 (2008).
7. F. Zhao, F. Harnisch, U. Schrorder, F. Scholz, P. Bogdanoff and I.

Herrmann, *Eviron. Sci. Technol.*, **40**, 5193 (2006).
8. R. A. Bullen, T. C. Arnot, J. B. Lakeman and F. C. Walsh, *Biosens. Bioelectron.*, **21**, 2015 (2006).
9. F. Kargi and S. Eker, *J. Chem. Technol. Biot.*, **82**, 658 (2007).
10. B. Logan, S. Cheng, V. Watson and G. Estadt, *Eviron. Sci. Technol.*, **41**, 3341 (2007).
11. H. I. Park, U. Mushtaq, D. Perello, I. Lee, S. K. Cho, A. Star and M. Yun, *Energy Fuel*, **2**, 2984 (2007).
12. M. Rosenbaum, F. Zhao, U. Schroder and F. Scholz. *Angew. Chem. Int. Edit.*, **45**, 6658 (2006).
13. S. Cheng, H. Liu and B. E. Logan, *Eviron. Sci. Technol.*, **40**, 364 (2006).
14. F. Zhao, F. Harnisch, U. Schrorder, F. Scholz, P. Bogdanoff and I. Herrmann, *Electrochem. Commun.*, **7**, 1405 (2005).
15. G. W. Chen, S. J. Choi, T. H. Lee, G. Y. Lee, J. W. Cha and C. W. Kim, *Appl. Microbiol. Biot.*, **79**, 379 (2008).
16. P. Clauwaert, D. Van der Ha, N. Boon, K. Verbeken, M. Verhaege, K. Rabaey and W. Verstraete, *Eviron. Sci. Technol.*, **41**, 7564 (2007).
17. J. A. Gralnick and D. K. Newman, *Mol. Microbiol.*, **65**, 1 (2007).
18. Z. He and L. T. Angenent, *Electronal.*, **18**, 2009 (2006).
19. A. Bergel, D. Feron and A. Mollica, *Electrochem. Commun.*, **7**, 900 (2005).
20. K. Rabaey, S. T. Read, P. Clauwaert, S. Freguia, P. L. Bond, L. L. Blackall and J. Keller, *ISME J.*, **2**, 519 (2008).
21. P. Clauwaert, K. Rabaey, P. Aelterman, L. De Schamphelaire, T. H. Pham, P. Boeckx, N. Boon and W. Verstraete, *Eviron. Sci. Technol.*, **41**, 3354 (2007).
22. O. Lefebvre, A. Al-Mamun and H. Y. Ng, *Water Sci. Technol.*, **58**, 881 (2008).
23. Y. H. Jia, H. T. Tran, D. H. Kim, S. J. Oh, D. H. Park, R. H. Zhang and D. H. Ahn, *Bioproc. Biosyst. Eng.*, **31**, 315 (2008).
24. P. Aelterman, K. Rabaey, P. Clauwaert and W. Verstraete, *Water Sci. Technol.*, **54**, 9 (2006).
25. K. Rabaey, N. Boon, S. D. Siciliano, M. Verhaege and W. Verstraete, *Appl. Environ. Microbiol.*, **70**, 5373 (2004).
26. A. J. Bard and L. R. *Faulkner Electrochemical method: Fundamentals and applications*, 2nd Ed., John Wiley & Sons Ltd., New York (2001).
27. F. Silveira, D. S. de Sa, Z. N. da Rocha and J. H. Z. dos Santos, *Macromol. React. Eng.*, **2**, 253 (2008).
28. D. E. Holmes, D. R. Bond, R. A. O'Neil, C. E. Reimers, L. R. Tender and D. R. Lovley, *Microbial. Ecol.*, **48**, 178 (2004).
29. D. K. Newman and R. Kolter, *Nature*, **405**, 94 (2000).
30. U. Schroder, *Phys. Chem. Chem. Phys.*, **9**, 2619 (2007).
31. S. Freguia, K. Rabaey, Z. Yuan and J. Keller, *Electrochim. Acta*, **53**, 598 (2007).