Korean J. Chem. Eng., **27**(5), 1513-1520 (2010)
DOI: 10.1007/s11814-010-0231-6

Microbial community dynamics and electron transfer of a biocathode in microbial fuel cells

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(*Received 2 September 2009 • accepted 8 December 2009*)

Abstract−Microbial community dynamics and its electron transfer process within a biocathode in a microbial fuel cell (MFC) were investigated in this study. The MFC was operated steadily over 400 days, and the power density reached 1.92 and 10.27 W/m^3 based on the reduction of nitrate and oxygen, respectively. The six major groups of the clones that were categorized among the 26 clone types were *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, *Planctomycetes*, *Firmicutes* and uncultured bacteria. Microbial community dynamics showed that *Betaproteobacteria* was replaced by *Gammaproteobacteria* as the most abundant division among all the clone types with a percentage of 48.86% in the cathode compartment, followed by 20.45% of uncultured bacteria, 17.05% of *Bacteroidetes*, and others. Distinct oxidation and reduction peaks could be observed in the profiles of cathodic effluent during the cyclic and differential pulse voltammetry tests. It confirmed that nitrate and oxygen reduction in the cathode compartment could be significantly enhanced by the presence of microbes, which are able to excrete metabolites to assist the electron transfer process either in the anode or in the cathode compartment.

Key words: Biocathode, Electron Transfer, Microbial Community Dynamics, Microbial Fuel Cell

INTRODUCTION

The microbial fuel cell (MFC) is a promising technology for pollution control and energy recovery [1-7]. There are, however, a few key unresolved problems associated with its practical application, including the high cost of manufacturing, the need for expensive noble metal catalysts for the oxygen-reduced cathode, and the common use of a proton exchange membrane [3,4,8,9].

The application of a biocathode into an MFC is a prospective way to improve the performance of cathode reaction without the need to use any artificial mediators or chemical catalysts, and avoiding the additional investment and potential pollution [10-16]. The seawater biofilm which develops on a stainless steel surface could effectively catalyze the process of oxygen reduction, and the biofilm-covered cathode was able to support a current density up to 1.9 A/m^2 [10]. With the aid of the biocathode enriched on graphite felt or woven carbon fibers, the current densities of up to 2.2 A/m^2 on a cathode projected surface could be obtained when oxygen was used as the ultimate electron acceptor [16]. By applying a biocathode, Clauwaert et al. [12] 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 about 10 W/m^3. Moreover, as one of the key factors that affect the performance of the biocathode, the microbial community of the cathodic microbes was also of significant interest. In another study, by using an electrode as a direct electron donor in the biofilm-electrode reactor, nitrate was reduced in the absence of organic substances by accepting electrons from the electrode, and the major populations of biofilm were identified as *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria* and *Flavobacteria* [17]. Unfortunately, limited information on this area is available, especially the microbial dynamics in the cathode compartment.

However, it is essential to manifest the electron transfer process that occurs on the cathode in the presence of microbes. Electrodes can serve either as electron donors or as electron acceptors for microorganisms, depending on whether the electrode is functioning as a cathode or anode, respectively [18]. It is well known that microbes are able to catalyze the conversion of substrates directly into electricity on the anode of MFCs [1-7,19]. Various examples of extracellular respiration have been illustrated and more particular attention has been paid to what is known about the molecular mechanisms underlying these processes [18,20,21]. It has also been proven that a non-proteinaceous small compound that has characteristics similar to a quinone and can be excreted into the medium is involved in electron transfer to anthraquinone-2,6-disulphonate (AQDS) to humic acid in extracellular electron transfer [20]. Compared to the studies on the anodic microbes, there is only a nominal amount of information available on the electron transfer process in the presence of microbes on the cathode. The microbial dynamics of the biocathode in an MFC was therefore investigated in this study, and more information is provided about the microbial roles and electron transfer process involved in the cathode compartment.

MATERIALS AND METHODS

1. MFC Construction and Operation

A two-chamber MFC was constructed as described in a previous study [11]. An anode and a cathode compartment were joined

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with an Ultrex cation exchange membrane (Membrane International Inc., USA) [22]. The volume of the anode and cathode compartments was 319 ml. Plain granular graphite with a porosity of 0.55 served as the electrode in each compartment. The two compartments were connected to each other by an external resistor of 30 Ω. Sludge from an anaerobic and anoxic tank of a lab-scale aerobic/anoxic/oxic wastewater treatment process (Pusan National University, Busan, South Korea) was seeded into the anode and cathode compartment, respectively [11].

Synthetic wastewater, composed of sodium acetate (1.0 g/*l*) as the sole carbon source, was used for anode cultivation, while nitrate (2.5 g/*l*) was used as the terminal electron acceptor in the cathode compartment, as well as other nutrient solutions (KH_2PO_4 6.2 g/*l*, K_2HPO_4 10.0 g/*l*, $NaHCO_3$ 2.0 g/*l*, NaCl 0.5 g/*l*, Na_2SO_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, $FeCl_3$ 1000, 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. Anode and cathode flowrates of 1.3 ml/min were adopted. After 350-d operation, the biocathode MFC switched the electron acceptor from nitrate to oxygen. It was then operated during the next 40 days using oxygen as terminal electron acceptor in the cathode compartment. The MFC and all the experiments were operated at an ambient temperature of 22 °C using air conditioning.

2. Electrochemical Monitoring 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 cell voltage (mV), Q is the charge (C), and t is the time (s). The power output of the cell (P) (W) was calculated as follows: P=I×V. The power density and current density were normalized by the effective volume of the cathodic compartment, which was the key factor in this biocathode MFC [12].

The cyclic and differential pulse voltammograms were carried out by a potentiostat (Model KST-P1, Kosentech, South Korea) [3, 23]. The cyclic and differential pulse voltammograms were adopted to detect the function of the microorganism by the classic three electrodes method in 25-ml samples [24]. Cyclic voltammetry was generally performed in a CV test kit (Cell stand C3, BAS, Warwickshire, United Kingdom) by starting at −800 mV and increasing to 800 mV and back at a scan rate of 50 mV/s. A differential pulse voltammogram was generally performed in a CV test kit within a range of −1,200 mV to 1,200 mV at a scan rate of 5 mV/s. All data of the cyclic and differential pulse voltammograms were logged by a personal computer which 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.

3. DNA Extractions

The sludge samples were collected from the cathode compartment of the microbial fuel cell. After 350-d operation, DNA was extracted from the MFC samples by using the Powersoil™ DNA isolation kit (MoBio, USA). DNA extracts were examined by agarose (1% w/v) gel electrophoresis and were compared with 1 Kb+ DNAladder (SolGent, Korea).

4. PCR Amplification of 16S rDNA Gene Sequences

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 reaction was performed with 2.5 μl of 10× Taq buffer, 0.5 μl of 10 mM dNTP, 1 μl of each primer (10 pmol), 2 μl of DNA template, 0.125 μl Taq DNA polymerase (SolGent, Korea) and deionized water in a 25 μl reaction volume. 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. PCR products were purified using a PCR purification kit (Bioneer, Korea).

5. 16S rDNA Gene Cloning, Sequencing and Phylogenetic 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, Madison, USA) were transformed with the ligated DNA. All procedures were carried out according to the supplier's instruction. White colonies were selected at random and plasmid DNAs were extracted. Verification PCR for DNA inserts was performed using primers M13F (5'-AGTCACGACGTTGTA-3'), and M13R (5'-CAGGAAACAGCTATGAC-3'). PCR products of the correct size were selected for sequencing by SolGent (Korea), and were then also subjected to the National Centre for Biotechnology (NCBI) BLAST (http://www.ncbi.nlm.nih.gov/blast/) search to identify the sequences with the highest similarity.

6. Chemical Analysis

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

RESULTS AND DISCUSSION

1. Performance of the Biocathode MFC

The MFC with a biocathode was operated steadily over 400 days, and its performance was recorded continuously with nitrate or oxygen serving as the terminal electron acceptor, as summarized in Table 1. At the anodic flowrate of 1.3 ml/min, the corresponding volumetric loading rate of the anode compartment was calculated as 7.5 kg-COD/m^3·d, while the hydraulic retention time was set as 2.25 h. For the nitrate-reduced cathode, the anodic and cathodic potentials were maintained at around −531 mV and −435 mV, respectively, resulting in a cell voltage of 101 mV with a 30 Ω external resistor served. The power density reached 1.9 W/m^3 with current density of 18.9 A/m^3. Under these conditions, 54.22% of COD in the anodic compartment was removed, while 35.21% of the nitrate was reduced in the cathodic compartment.

Meanwhile, the biocathode enriched by nitrate reduction was also found to be surprisingly helpful to oxygen reduction. When nitrate was replaced by oxygen as the terminal electron acceptor in the cathodic compartment, the cathodic potential increased rapidly and then

Table 1. Performance of the MFC with a biocathode in the presence of nitrate or oxygen as electron acceptor

Electron acceptor	Electricity generation					Removal efficiency	
	Anodic potential (mV)	Cathodic potential (mV)	Cell potential (mV)	Power density (W/m^3)	Current density (A/m^3)	COD removal in anodic compartment (%)	Nitrate removal in cathodic compartment (%)
Nitrate	−531±6	−435±9	101±9	1.9±0.3	18.8±1.6	54.22±13.45	35.21±7.41
Oxygen	−463±11	−231±11	233±1	10.3±0.1	44.2±0.2	71.24±6.40	N.A.

N.A.: Not available

reached −231 mV, with an anodic potential at −463 mV. The cell voltage was then maintained at 233 mV with a 30 Ω external resistor, and the electricity generation was therefore highly improved. The power density and current density reached 10.3 W/m^3 and 44.2 A/m^3, respectively, which was 5.4 and 2.4 times more, respectively, than that of nitrate reduction. Moreover, the COD removal efficiency in the anodic compartment increased rapidly to 71.24%.

2. Microbial Community Analysis of Biocathode with Nitrate Reduction

After 350-d operation, microbial samples were extracted from the biocathode compartment, and the microbial community was analyzed. The results of a 16S rDNA gene clone library analysis of the bacterial population in the cathode compartment are summarized in Table 2, which shows the compositions of the bacterial community in terms of the major phyla and subdivisions. Twenty-six different clone types were identified from 88 sequenced random clones which were derived from the biocathode in the MFC. Six major groups of the clones were categorized among the 26 clone types. The bacterial community consisted of *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, *Planctomycetes*, *Firmicutes* and uncultured bacteria. Among the above phylotypes, *Bacteroidetes* were the most diverse, consisting of 9 of the 26 identified clone types, while *Gammaproteobacteria* was confirmed as the most abundant division with 48.86% (43 of 88) of the sequenced clones in the cathode compartment, followed by 20.45% (18 of 88) of uncultured bacteria, 17.05% (15 of 88) of *Bacteroidetes*, 3.41% (3 of 88) of *Alphaproteobacteria*, 3.41% (3 of 88) of *Actinobacteria*, 2.27% (2 of 88) of *Betaproteobacteria*, 2.27% (2 of 88) of *Planctomycetes*,

Table 2. Phylogentic affiliations, closest matches and percentage of clones from cathode compartment for 350-d operation

Phylum (class)	Percentage (%)	Clones	Genbank closest match (acession number)	Similarity (%)
Actinobacteria	3.41%	NC42	*Leifsonia* sp. MSL 02 (EF527429)	98
		NC06	Uncultured *Actinobacterium* (DQ828052)	99
Bacteroidetes	17.05%	NC18	Uncultured *Bacteroidetes* bacterium (EF665122)	93
		NC26	Uncultured *Bacteroidetes* bacterium (AJ318191)	98
		NC54	Uncultured *Bacteroidetes* bacterium (EF111203)	97
		NC23	Uncultured *Bacteroidetes/Chlorobi* group bacterium (EU266899)	98
		NC59	Uncultured *Bacteroidetes* bacterium (EU426949)	100
		NC22	Uncultured *Bacteroidetes* bacterium (AJ318191)	94
		NC80	Uncultured *Bacteroidetes* bacterium (DQ640712)	96
		NC08	Uncultured *Flexibacteraceae* bacterium (DQ507161)	94
		NC65	Uncultured *Bacteroidetes* bacterium (EF220122)	97
Firmicutes	1.14%	NC50	*Clostridium* sp. D3RC-2 (DQ852338)	100
Planctomycetes	2.27%	NC81	Uncultured *Planctomycete* (AM902598)	92
		NC12	Uncultured *Planctomycetales* bacterium (AY293515)	90
Alphaproteobacteria	3.41%	NC49	*Alpha proteobacterium* CRIB-02 (DQ123619)	97
Betaproteobacteria	2.27%	NC40	Uncultured *beta proteobacterium* (EF074259)	98
Deltaproteobacteria	1.14%	NC91	Uncultured *Myxococcales* bacterium (DQ646301)	94
Gammaproteobacteria	48.86%	NC84	Uncultured *gamma proteobacterium* (AY875911)	96
		NC44	*Fulvimonas soli* (AJ311653)	95
		NC63	*Xanthomonas* sp. ML-122 (AF139997)	98
Uncultured bacteria	20.45%	NC36	Uncultured bacterium (EU704650)	99
		NC53	Uncultured bacterium (FJ230924)	100
		NC72	Uncultured bacterium (FJ230910)	99
		NC73	Uncultured bacterium (DQ661739)	100
		NC74	Uncultured bacterium (DQ988286)	98
		NC78	Uncultured bacterium (EU083501)	98

1.14% (1 of 88) of *Deltaproteobacteria* and 1.14% (2 of 88) of *Firmicutes*.

3. Electrochemical Characterization of Electron Transfer Process in Anode Compartment

Cyclic voltammetry is a type of potentiodynamic electrochemical measurement that is generally used to study the electrochemical properties of an analyte in solution. Meanwhile, compared to other 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. To characterize the electron transfer process involved in the anode and cathode compartments, cyclic voltammetry and differential pulse voltammetry were adopted in this study.

Fig. 1 shows the cyclic voltammograms of anodic influent and effluent from the biocathode MFC. As a control test, no distinct peaks (Fig. 1(a)) could be detected from the anodic influent, indicating that there was no electrochemical active substance present. However, the cyclic voltammogram from the anodic effluent (Fig, 1(b)) exhibited a different pattern, regardless of the magnitude of current or the peaks. Two weak symmetrical peaks could be observed around 0 mV on both scan directions, which was attributed to an electrochemically active substance responsible for the reversible reactions in the tested solution. The difference between the anodic influent and effluent in the cyclic voltammogram was due to the function

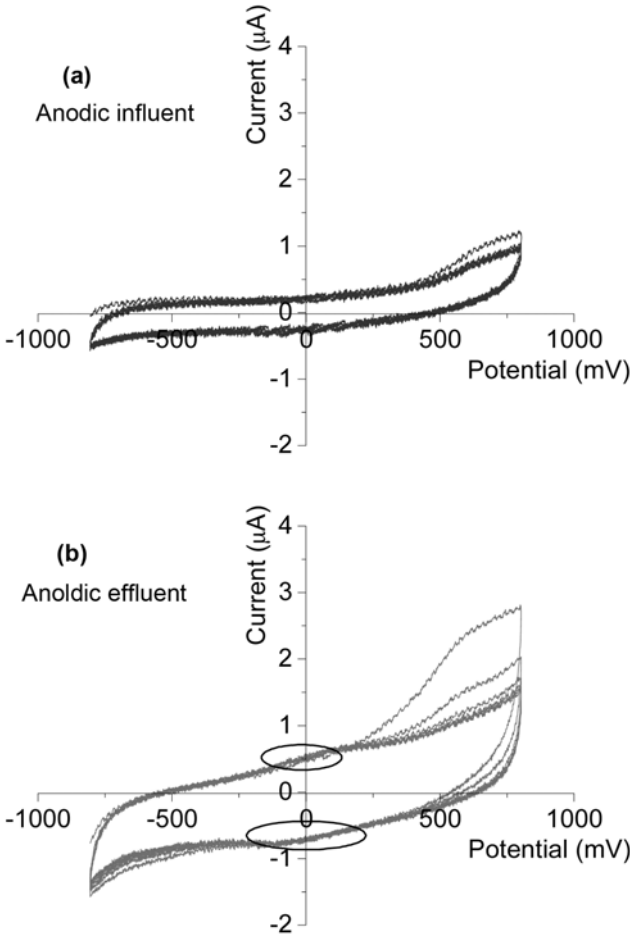

Fig. 1. Successive cyclic voltammograms of the influent and effluent from anodic compartment in the biocathode MFC.

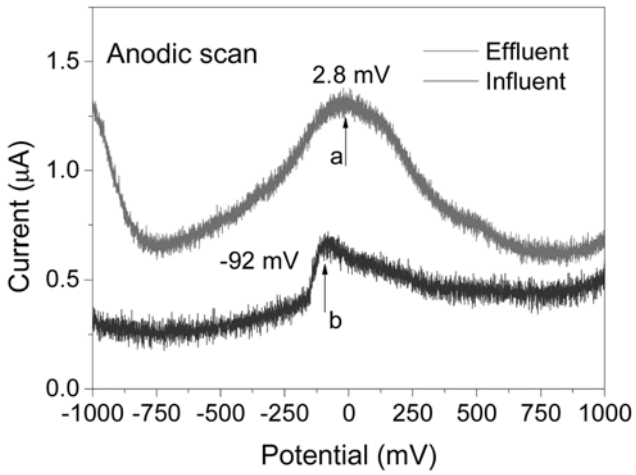

Fig. 2. Anodic scan of differential pulse voltammograms (5 mV/s) of the influent and effluent from the anode compartment in the biocathode MFC.

of microbes, which were assumed to produce the electrochemically active substance. Similar results have been reported in a previous study for *Pseudomonas aeruginosa*, which was able to excrete redox mediators, such as pyocyanin, assisting electron transfer process in the anode compartment [22]. The peaks occurring at high potentials were produced due to the small amount of remnant oxygen in the solution, and disappeared after 2-3 cyclic scans.

Two peaks were observed in the differential pulse voltammogram profiles of anodic influent and effluent, as shown in Fig. 2. A small asymmetrical peak (b) in the differential pulse voltammogram of anodic influent indicated that an irreversible oxidation reaction occurred at the potential of –92 mV. Meanwhile, a symmetrical peak (a) was observed in the profiles of anodic effluent at a potential of 2.7 mV. This demonstrated that an electrochemical active species, which was able to carry out reversible redox reactions, was found in the anodic effluent. Compared with the influent profile, the electrochemically active species found in the effluent was assumed to be excreted by the microbes present in the anode compartment. The results obtained from the differential pulse voltammograms were consistent with those obtained from the cyclic voltammograms.

4. Electrochemical Characterization of Electron Transfer Process in Cathode Compartment

Since the biocathode was applied into the cathode compartment to assist the electron transfer, it was naturally assumed that cathodic microbes could perform similar functions in the electron transfer process as the anodic microbes. Accordingly, the electrochemical properties of cathodic influent and effluent were examined. Similarly, to eliminate the effect of influent composition on the electrochemical tests, the cathodic influent was checked as a background value. The cyclic voltammograms of cathodic influent and effluent are recorded in Fig. 3. An oxidation peak could be observed around the scan potential of 510 mV in the profiles of cathodic effluent. For the nitrate reduction biocathode, the peak was responsible for the nitrate/nitrite redox couple, which had a redox potential of 420 mV (vs. standard hydrogen electrode) under standard conditions [3]. This indicated that the presence of microbes in the cathode compartment significantly improved the electrochemical response of ni-

trate reduction.

The differential pulse voltammogram profiles are shown in Fig. 4. Four peaks were identified from the cathodic influent and effluent. It is obvious that peak (a) and peak (b) (both at a potential of 510 mV) were responsible for nitrate reduction. As the corresponding current of peak was increased, the nitrate reduction became easier. Therefore, the small scale of peak (a) proved the limited ability of the abio-cathode in nitrate reduction, while the large peak (b) proved that the cathodic effluent was electrochemically active for the nitrate reduction rather than the influent, which could be attributed to the redox mediator excreted by the cathodic microbes. It was demonstrated that nitrate reduction and electricity production in the cathode compartment were significantly enhanced by the presence of microbes. The peak c (at potential of 104 mV) and peak d (at potential of 123 mV) in the profiles are assumed to be caused by other ingredients of the influent, such as Fe^{3+}/Fe^{2+} (pH=7.0).

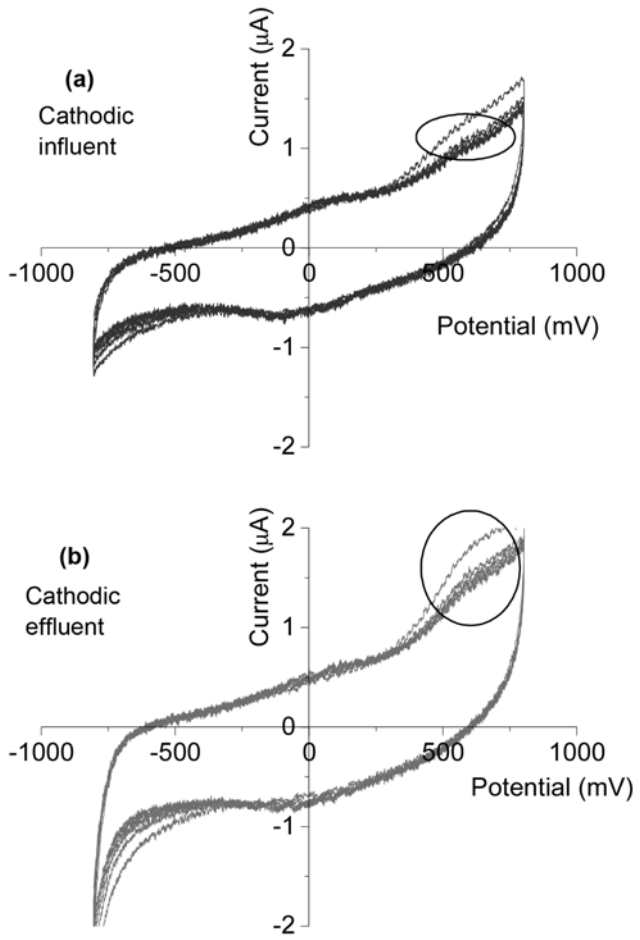

Fig. 3. Successive cyclic voltammograms of the influent and effluent from cathodic compartment in the biocathode MFC.

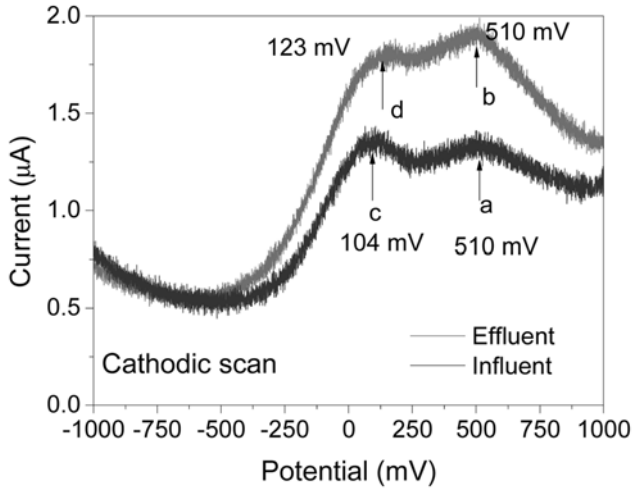

Fig. 4. Cathodic scan of differential pulse voltammograms (5 mV/s) of the influent and effluent from cathode compartment in the biocathode MFC.

5. Electrochemical Characterization of Electron Transfer Process with the Presence of Oxygen

Surprisingly, the biocathode electrode enriched by nitrate as the terminal electron acceptor could also achieve excellent oxygen reduction and electricity production performance, as explained in Table 1. Therefore, to manifest this phenomenon, it was essential to characterize the electrochemical properties of catholyte in the presence of oxygen. The successive cyclic voltammograms of the cathodic effluent in the presence of oxygen, as well as the influent profile, are illustrated in Fig. 5.

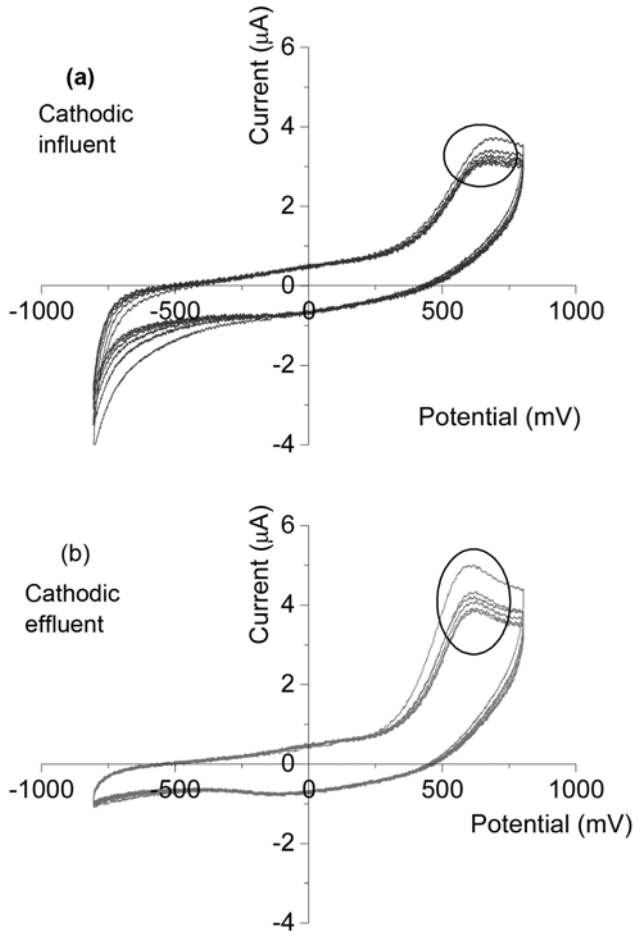

Fig. 5. Successive cyclic voltammograms of the influent and effluent from cathodic compartment in the biocathode MFC in the presence of oxygen.

First, the abio-cathode was tested by examining the electrochemical responses of the cathodic influent. Without the presence of cathodic microbes, the peaks could also be found in its profile (Fig. 5(a)). These phenomena proved the electrochemical activity of plain carbon electrode, which were able to carry out oxygen reduction without any catalyst. Freguia et al. [14] also reported that significant stable currents can be delivered by using a non-catalyzed cathode made of granular graphite in a continuous MFC fed with acetate, and a power production of 21 W/m^3 was attained. On the other hand, compared to Fig. 3(a), the corresponding scale of the peaks obtained from the cathodic influent was significantly higher, demonstrating that it was more difficult for nitrate to be reduced on the plain carbon cathode without a catalyst than it was for oxygen.

When the microbe was used as a catalyst in the cathode compartment, the functions of microbes were checked based on the electrochemical responses of the cathodic effluent. As illustrated in Fig. 5(b), distinct peaks could be detected from the cyclic voltammogram of the cathodic effluent, which were considerably higher than those of the cathodic influent. This indicated that catholyte was highly electrochemically active for the oxygen reduction after it flowed through the microbes on the cathode. In other words, the application of the biocathode was beneficial to oxygen reduction. The presence of the seawater biofilm on the stainless steel surface was reported to result in an efficient catalysis of oxygen reduction, and the biofilm-covered cathode was able to support a current density up to 1.89 A/m^2 [10].

Furthermore, the differential pulse voltammogram profiles of the cathodic influent and effluent in the presence of oxygen are shown in Fig. 6. Four peaks were identified from the cathodic influent and effluent. The peak (a) (at potential of 550 mV) and peak (b) (at potential of 530 mV) were responsible for the oxygen reduction. The difference in the corresponding current between peak (a) and peak (b) proved the function of cathodic microbes, and the capacity of oxygen reduction was obviously enhanced by the presence of microbes on the biocathode in the MFC. The results of the differential pulse voltammogram agreed well with those of the cyclic voltammogram.

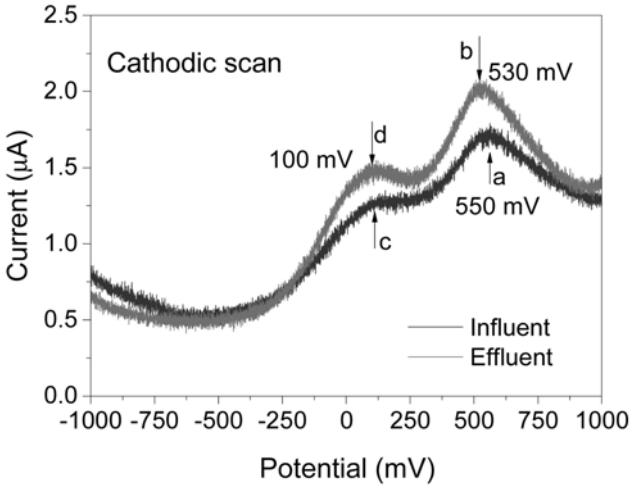

Fig. 6. Cathodic scan of differential pulse voltammograms (5 mV/s) of the influent and effluent from cathode compartment in the biocathode MFC in the presence of oxygen.

6. Electricity Production and Microbial Activity

Quantitative population dynamics of microbial communities from the anodes of plankton-fed microbial fuel cells show that differences in growth, succession and population dynamics of key phylotypes were due to anode potential, which may relate to their ability to exploit the anode as an electron acceptor [25]. In this study, the biocathode MFC operated for over 400 days from the startup, and the population dynamics of microbial communities from the cathode were investigated, too. Based on the microbial community analysis results of 80-d [11] and 350-d, the changes within the microbial communities were compared. As shown in Fig. 7(a), at 80-d operation, four major phyla of bacteria were found in the cathode chamber, which consisted of *Proteobacteria*, *Bacteroidetes*, *Actinobacteria* and *Chlorobi*. Among the above phylotypes, *Betaproteobacteria* was the most abundant division with 50.0% of the sequenced clones in the cathode compartment, followed by 21.6% *Bacteroidetes*, 9.5% *Alphaproteobacteria*, 8.1% *Chlorobi*, 4.1% *Deltaproteobacteria*, 4.1% *Actinobacteria*, and 2.7% *Gammaproteobacteria*. However, after 350-d operation, the microbial community and its distribution

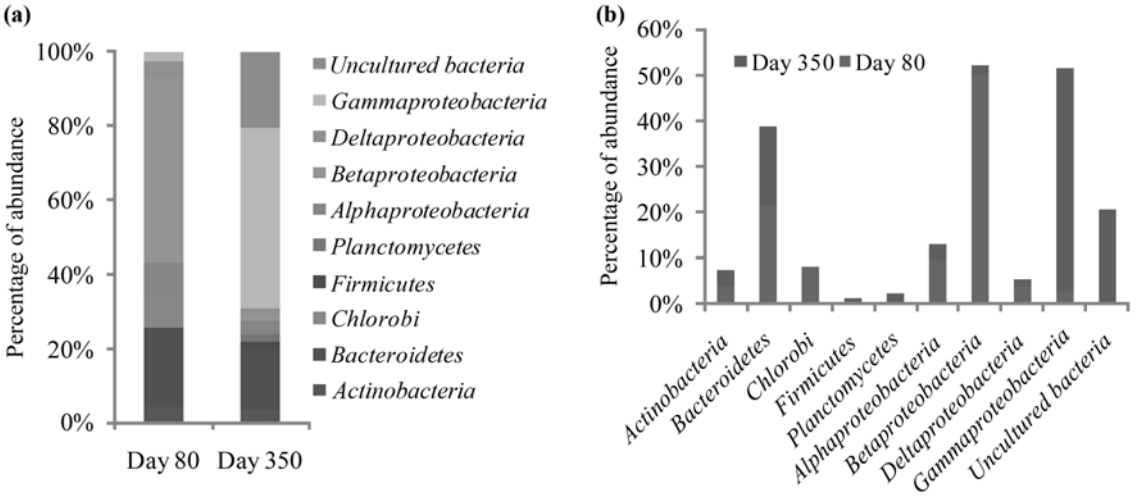

Fig. 7. Frequency distribution of cathodic microbial phylotypes from analysis of cloned rDNA fragments after 80 and 350 days of enrichment.

changed significantly. The relative ratio of each phylotype presented in different operation period is illustrated in Fig. 7(b). *Gammaproteobacteria* became the most abundant division among all clone types with a percentage of 48.86%, while *Betaproteobacteria* was now found to be scarcely presented. *Chlorobi* disappeared over the long-term operation, while *Planctomycetes* and *Firmicutes* became present as minor divisions with quite low percentages, as well as uncultured bacteria. The *Actinobacteria*, *Bacteroidetes* and the total *Proteobacteria* were kept at a relatively stable percentage at both periods. Among *Proteobacteria*, *Alphaproteobacteria*, *Betaproteobacteria* and *Deltaproteobacteria* decreased their representation in microbial distribution, with the exception of *Gammaproteobacteria.* Similarly, Phylotypes recovered from cathod library of two benthic microbial fuel cells deployed at a deep-ocean cold seep were dominated by genes from *Pseudomonas flourescens*-like phylotypes of 49% of 160 clones, and genes from phylotypes related to *Janthinobacterium lividum* and *Aeromonas encheleia* constituted 27% and 7% of the cathode library, respectively [26].

Various electron acceptors could be utilized on the cathode in the MFC, including oxygen, nitrate, nitrite, tetrathionate, fumarate etc. [3,5,20]. The transcription activator Fnr (fumarate and nitrate reductase) is responsible for oxygen reduction, while reduction of nitrate and nitrite is controlled by the nitrate reductase (Nar) two-component regulatory systems. These respond to nitrate and/or nitrite by autophosphorylating the conserved histidyl residue in the transmitter module [27]. The regulation of respiratory enzyme synthesis is controlled by the electron acceptors whose couples are the most electropositive. Nitrate and nitrite are the preferred respiration oxidants during anaerobic conditions.

When nitrate served as the terminal electron acceptor on the cathode, the microbial community dynamics of the biocathode were recorded, as described in the above section. In the earlier enrichment period, the most abundant phylotype was *Betaproteobacteria* in the cathodic compartment. After long-term operation *Betaproteobacteria* was replaced by *Gammaproteobacteria* as the most abundant division. Similarly, in a biofilm-electrode reactor, where nitrate was reduced in the absence of organic substances by accepting electrons from the electrode, the major populations of microbes were *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria* and *flavobacteria* [17]. *Betaproteobacteria*, which was reported as the most abundant potential denitrifiers, made up 20-49% of all bacteria in most of the 17 nitrogen removal wastewater treatment plants and were barely present in four plants without denitrification [28]. Either *Beta* or *Gamma proteobacteria* proved to be able to carry out denitrification [29-31]. Anaerobic enrichment culture with thiocyanate as an electron donor and nitrate as an electron acceptor resulted in a selection of sulfur-oxidizing bacteria capable of the complete denitrification of nitrate, which was identified as new lineages within the *Gammaproteobacteria* [32]. A heterotrophic, non-fermentative, denitrifying isolate strain of *Gammaproteobacteria*, was obtained from a seawater sample, which grew aerobically and could achieve anaerobic growth by adopting a denitrifying metabolism with nitrate or nitrous oxide as the terminal electron acceptor [29]. Chemolithoautotrophic nitrate reduction was also demonstrated to be an important process for anaerobic or microaerophilic chemolithoautotrophic bacteria, which were identified as *Gammaproteobacteria* [33]. The presence of a much more diverse denitrifying community was demonstrated by isolates from a cultivation-dependent research on activated sludge, which included *Betaproteobacteria*, *Alphaproteobacteria*, *Gammaproteobacteria*, *Epsilonproteobacteria*, *Firmicutes* and *Bacteroidetes* [34]. To date, NarX and NarQ genes are confined to species classified in the *Gamma* and *Beta* subdivisions of the *proteobacteria* [27]. Comparative genomics analysis showed that only NarP is responsible for regulation in most *Gammaproteobacteria*. It was assumed on the basis of this evidence that the role of NarP in regulating respiration changed during evolution [35].

The mechanism of electron transfer to or from extracellular substrates has been manifested to some extent [18,21,22]. Direct or indirect electron transfer was responsible for anodic electron transfer in the microbial fuel cells, including the physical contact of the bacterial cell membrane or a membrane organelle with the fuel cell anode, electronically conductive molecular pili (nanowires), as well as the assistance of primary or secondary metabolites [21,22,35,36]. For the practical application of MFC, the presence of secondary metabolites excreted by bacteria (bacterial phenazines like pyocyanine and 2-amino-3-carboxy-1,4-naphthoquinone), is of great interest [20-22]. The cyclic and pulse differential voltammograms of the anode effluent in our study also proved that certain metabolites could be detected in anolyte, which played a key role in the electron transfer involved in the anode compartment. A similar extracellular electron transfer mechanism of microbially produced shuttle could be employed by the cathode. When the microbes were adopted onto the cathode, distinct electrochemical responses could be detected regardless of whether nitrate or oxygen was used as the electron acceptor. This proved that some microbial excreted metabolites were produced, and that the electron transfer was assisted by the excreted mediators or shuttles in the cathode compartment. Further research is required to identify these microbial excreted metabolites.

CONCLUSIONS

An MFC with a biocathode was operated steadily over 400 days in this study. Meanwhile, the microbial community dynamics of the cathode and its electron transfer process were examined. The power density reached 1.92 and 10.27 W/m^3 based on the reduction of nitrate and oxygen, respectively. The six major groups of the clones that were categorized among the 26 clone types were *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, *Planctomycetes*, *Firmicutes* and uncultured bacteria. Microbial community dynamics showed that *Betaproteobacteria* was replaced by *Gammaproteobacteria* as the most abundant division among all the clone types, which responded to the electricity production. Cyclic and differential pulse voltammetry confirmed that microbes are able to excrete metabolites to assist the electron transfer process both in the anode and in the cathode compartments.

ACKNOWLEDGEMENTS

This work was financially supported by the Ministry of Environment as "Eco-Technopia 21 project" and "Human resource development Project for Energy from Waste & Recycling". It also was supported by Mid-career Researcher Program through NRF grant funded by the MEST (No. R01-2008-222-11853-0). 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. E. 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. B. E. Rittmann, *Trends Biotechnol.*, **24**, 261 (2006).
6. 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).
7. N. T. Trinh, J. H. Park and B. W. Kim, *Korean J. Chem. Eng.*, **26**, 748 (2009).
8. S. Freguia, K. Rabaey, Z. Yuan and J. Keller, *Water Res.*, **42**, 1387 (2008).
9. F. Zhao, F. Harnisch, U. Schrorder, F. Scholz, P. Bogdanoff and I. Herrmann, *Eviron. Sci. Technol.*, **40**, 5193 (2006).
10. A. Bergel, D. Feron and A. Mollica, *Electrochem. Commun.*, **7**, 900 (2005).
11. 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).
12. 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).
13. P. Clauwaert, D. Van der Ha, N. Boon, K. Verbeken, M. Verhaege, K. Rabaey and W. Verstraete, *Eviron. Sci. Technol.*, **41**, 7564 (2007).
14. S. Freguia, K. Rabaey, Z. Yuan and J. Keller, *Electrochim. Acta*, **53**, 598 (2007).
15. Z. He and L. T. Angenent, *Electronal.*, **18**, 2009 (2006).
16. K. Rabaey, S. T. Read, P. Clauwaert, S. Freguia, P. L. Bond, L. L. Blackall and J. Keller *ISME J.*, **2**, 519 (2008).
17. H. I. Park, J. S. Kim, D. K. Kim, Y. J. Choi and D. Pak, *Enzyme Microb. Tech.*, **39**, 453 (2006).
18. J. A. Gralnick and D. K. Newman, *Mol. Microbiol.*, **65**, 1 (2007).
19. R. A. Bullen, T. C. Arnot, J. B. Lakeman and F. C. Walsh, *Biosens. Bioelectron*., **21**, 2015 (2006).
20. M. E. Hernandez and D. K. Newman, *Cell Mol. Life Sci.*, **58**, 1562 (2001).
21. U. Schroder, *Phys. Chem. Chem. Phys.*, **9**, 2619 (2007).
22. K. Rabaey, N. Boon, S. D. Siciliano, M. Verhaege and W. Verstraete, *Appl. Environ. Microbiol.*, **70**, 5373 (2004).
23. A. J. Bard and L. R. Faulkner, *Electrochemical method: Fundamentals and applications*, 2nd Ed., Wiley, New York (2001).
24. V. Stewart, *Biochem. Soc. T.*, **31**, 1 (2003).
25. H. K. White, C. E. Reimers, E. E. Cordes, G. F. Dilly and P. R. Girguis, *ISME J.*, **3**, 635 (2009).
26. C. E. Reimers, P. Girguis, H. A. Stecher III, L. M. Tender, N. Ryckerlynck and P. Whaling, *Geobiology*, **4**, 123 (2006).
27. T. R. Thomsen, Y. Kong and P. H. Nielsen, *FEMS Microbiol. Ecol.*, **60**, 370 (2007).
28. S. E. Hoeft, J. S. Blum, J. F. Stolz, F. R. Tabita, B. Witte, G. M. King, J. M. Santini and R. S. Oremland, *Int. J. Syst. Evol. Micr.*, **57**, 504 (2007).
29. N. Fernandez, R. Sierra-Alvarez, J. A. Field, A. Ricardo and J. L. Sanz, *Chemoshpere*, **70**, 462 (2008).
30. T. Osaka, Y. Ebie, S. Tsuneda, A. Hirata1, N. Iwami and Y. Inamori, *FEMS Microbiol. Ecol.*, **64**, 494 (2008).
31. D. Y. Sorokin, T. P. Tourova, E. Y. Bezsoudnova, A. Pol and G. Muyzer, *Archives Microbiol.*, **187**, 441 (2007).
32. M. Labrenz, G. Jost, C. Pohl, S. Beckmann, W. Martens-Habbena and K. Jurgens, *Appl. Environ. Microbiol.*, **71**, 6664 (2005).
33. K. Heylen, B. Vanparys, L. Wittebolle, W. Verstraete, N. Boon and P. De Vos, *Appl. Environ. Microbiol.*, **72**, 2637 (2006).
34. D. A. Ravcheev, A. B. Rakhmaninova, A. A. Mironov and M. S. Gelfand, *Mol. Biol.*, **39**, 832 (2005).
35. Y. A. Gorby, S. Yanina, J. S. McLean, K. M. Rosso, D. Moyles, A. Dohnalkova, T. J. Beveridge, I. S. Chang, B. H. Kim, K. S. Kim, D. E. Culley, S. B. Reed, M. F .Romine, D. A. Saffarini, E. A. Hill, L. Shi, D. A. Elias, D. W. Kennedy, G. Pinchuk, K. Watanabe, S. Ishii, B. Logan, K. H. Nealson and J. K. Fredrickson, *Proc. Nat. Acad. Sci.*, **103**, 11358 (2006).
36. G. Reguera, K. D. McCarthy, T. Mehta, J. S. Nicoll, M. T. Tuominen and D. R. Lovley, *Nature*, **435**, 1098 (2005).