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Application of Single-Compartment Bacterial Fuel Cell (SCBFC) Using Modified Electrodes with Metal Ions to Wastewater Treatment Reactor

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Abstract The SCBFC was composed of bilayered cathode, the outside of which was modified with Fe^{3+} (graphite-Fe(III) cathode) and the inside of which was porcelain membrane, and of an anode which was modified with Mn^{4+} (graphite-Mn(IV) anode). The graphite-Fe(II), graphite-Mn(IV), and porcelain membrane were designed to have micropores. The outside of the cathode was exposed to the atmosphere and the inside was contacted with porcelain membrane. In all SCBFCS the graphite-Fe(III) was used as a cathode, and graphite-Mn(IV) and normal graphite were used as anodes, for comparison of the function between normal graphite and graphite-Mn(IV) anode. The potential difference between graphite-Mn(IV) anode and graphite-Fe(III) cathode was about 0.3 volt, which is the source for the electron driving force from anode to cathode. In chemical fuel cells composed of the graphite-Mn(IV) anode and graphite-Fe(III) cathode, a current of maximal 13 mA was produced coupled to oxidation of NADH to NAD^+; the current was not produced in SCBFC with normal graphite anode. When growing and resting cells of *E. coli* were applied to the SCBFC with graphite-Mn(IV) anode, the electricity production and substrate consumption were 6 to 7 times higher than in the SCBFC with normal graphite anode, and when we applied anaerobic sewage sludge to SCBFC with graphite-Mn(IV) anode, the electricity production and substrate consumption were 3 to 5 times higher than in the SCBFC with normal graphite anode. These results suggest that useful electric energy might possibly be produced from SCBFC without electron mediators, electrode-active bacteria, and extra energy consumption for the aeration of catholyte, but with wastewater as a fuel.

Key words: Single-compartment bacterial fuel cell (SCBFC), graphite-Mn(IV) anode, graphite-Fe(III) cathode, cyclic voltammetry, wastewater treatment

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Progress has been made in the development of bacterial fuel cells by changing various bacteria, electron mediators, and substrates and by modifying electrodes [1, 2, 4, 6, 25]. In bacterial fuel cells, a bacterium functions as a catalyst for the production of biochemical reducing power from substrates, an electron mediator functions as a converter of bacterial reducing power to electricity, and a substrate serves as a fuel [22]. An anode functions as an oxidant for the electron mediators (e.g., neutral red, thionin, hydroquinone, and viologen dyes) that are reduced coupled to biochemical oxidation of substrate, and a cathode functions as a reductant for electron acceptors (e.g., O_2, nitrate, sulfate, and carbonate), respectively. Biochemical energy can be converted into electrical energy by coupling biocatalytic oxidation of substrate to the chemical reduction of the oxidant at the interface between anode and cathode [28]. An electron mediator has been an essential requirement for the promotion of electron transfer from bacteria to electrodes because bacterial membrane acts as an electron barrier [18] and direct electron transfer from bacteria to electrodes occurs only at very low efficiency [1]. Park *et al*. [17] reported that viologen dye cross-linked with carbon polymers could be absorbed on the cytoplasmic membrane of *Desulfovibrio desulfuricans* and could mediate electron transfer from bacterial cells to electrodes or from electrodes to bacterial cells. The electron transfer efficiency from bacterial cells to the electrodes in bacterial fuel cells could be improved if more suitable electron mediators were used.

Until now, most researchers have concentrated on finding a better electron mediator for the improvement of electron transfer from bacteria to electrodes, and better bacteria such as *Shewanella putrefacience*, with a special function for electron transfer from bacterial cells to electrodes without using an electron mediator [9, 19, 26, 27]. No one, however, has tried to develop an anode with a higher affinity

to bacteria than soluble electron mediators and a cathode with a higher affinity to oxygen than oxidants such as soluble ferricyanide [2]. For the development of bacterial fuel cells without the assistance of electron mediators, Kim *et al*. [9] applied an electrode-active bacterium *Shewanella putrefaciens* to bacterial fuel cells, and reported that *S. putrefaciens* can produce electricity without an electron mediator, but the electricity produced was lower than that produced in bacterial fuel cells with a soluble electron mediator and a non electrode-active bacterium such as *E. coli*. In order to improve the bacterial fuel cell with a soluble electron mediator, Park *et al*. [20, 21, 22] modified the electrodes with neutral red and reported that the modified electrodes were more efficient for electricity production than unmodified (normal) electrodes were. This might be a useful tool for improving the bacterial fuel cell because some electron mediators can be covalently bound to carbon or graphite electrodes.

Recently, we have developed new electrodes modified with metal ions and a single-compartment bacterial fuel cell (SCBFC). The purpose of this report is to test the ability of these modified electrodes with metal ions to enhance electron transfer from bacterial cells to electrodes, in order to develop a less complex and new fuel cell system and to apply a new bacterial fuel cell to wastewater treatment reactors. In this paper, we compared electricity production and substrate consumption as between SCBFCs with graphite-Mn(IV) anode and those with normal graphite anode and tried to apply the SCBFC to wastewater treatment systems for the improvement of treatment efficiency and electricity production without any extra electric energy consumption for the aeration of catholyte.

MATERIALS AND METHODS

Chemicals and Reproducibility of Results

All the chemicals used in the experiments were of reagent grade, and all individual experiments were repeated three to five times with identical results.

Growth of Organisms

Escherichia coli k12 was grown in LB medium (Peptone 10 g/l, Yeast Extract 5 g/l, NaCl 10 g/l) and the culture was used as a biocatalyst. Resting cells of *E. coli* were prepared by harvesting the early stationary-phase cultures at 4°C by centrifugation at 5,000 ×*g* for 30 min. The cells were resuspended in cultivation medium (LB medium with 10 g/l glucose and 100 mM phosphate buffer, pH 7.0) [11].

Anaerobic Sewage Sludge

Anaerobic sewage sludge was obtained from Jungrang wastewater treatment plant (Jangan-dong Dongdaemun-gu, Seoul Korea) and used while still fresh, 24 h after being transported from the plant. The fresh anaerobic sludge was allowed to settle at room temperature under N_2 atmosphere for 12 h to precipitate solid particles. The supernatant was an anaerobic bacterial consortium that was used as a biocatalyst. For the preparation of resting cells of the anaerobic bacterial consortium, 20 g/l glucose and 100 mM potassium phosphate buffer (pH 7.0) were added to settled supernatant of sewage sludge. The settled supernatant with glucose and phosphate buffer was used as an anolyte for the bacterial fuel cell and an anaerobic bacterial consortium autogenously grown in sewage sludge was used as a biocatalyst. The bacterial density of the anaerobic bacterial consortium in the settled supernatant as measured by spectrophotometery was 4.6 OD at 660 nm.

Electrode Composition

A graphite-Fe(III) cathode was made from a mixture of 60% (w/w) fine graphite powder (mean particle size 1–2 μm, Sigma-Aldrich, St. Louis, MO), 37% (w/w) inorganic binder (white clay mainly composed of Kaolin of which the mean particle size is 1–2 μm), and 3.0% (w/w) ferric ion. One side of the cathode was coated with a 2-mm thick porcelain septum made from 100% white clay. A graphite-Mn(IV) anode was made from a mixture of 60% (w/w) fine graphite powder, 37% (w/w) inorganic binder, and 3.0% (w/w) manganese ion. A normal graphite-cathode was made from a mixture of 60% (w/w) fine graphite powder and 40% (w/w) inorganic binder. The gaps among particles can make micropores with diameters below 1 μm. A proper amount of distilled water was added to the mixture in order to make the graphite mixture paste, and the paste was configured to a square-shaped plate (20 cm× 20 cm×1 cm thickness) by pressing at 44 kg/cm$_2$, drying on air for two weeks at room temperature, and solidified by baking at 1,200°C for 12 h under anaerobic condition using an electric Kiln (Red Corona Model 50L, U.S.A.). After baking, the anode and cathode were confirmed to have absorbed water but not to have leaked water through the micropores.

Electrical Measurement

Potential was measured with a voltameter that was connected to the recorder under a closed circuit configuration, and the current was measured with an ammeter in a closed circuit configuration every 2 h to obtain the maximal current value. The current can be controlled with external variable resistance. The data reported are means based on values that were obtained in triplicate experiments and were within 1 standard deviation of each other. The current and potential were nearly identical in the replicated experiments.

Cyclic Voltammetry

The cyclic voltammograms were obtained using the graphite-Mn(IV) electrode or graphite-Fe(III) electrode transformed into rod type (diameter 5 mm, length 4 cm) as a working electrode, platinum wire as a counter electrode, and Ag/

AgCl as a reference electrode in 50 mM phosphate buffer (pH 7.0). Cyclic voltammetry was performed using a cyclic voltammetric potentiostat (model CV50W, BAS, U.S.A.) linked to an IBM personal computer data acquisition system. Prior to use, the electrodes were cleaned using an ultrasonic cleaner. The scanning rate used was 10 mVs^{-1} over the range of +2.0 volt to –2.0 volt.

Chemical Fuel Cell

A chemical fuel cell was designed to confirm the electrochemical function of graphite-Mn(IV) anode and graphite-Fe(III) cathode. A 100 ml volume of SCBFC system (Fig. 1B) was used to produce electricity from NADH, which was used as a fuel. Fifty mM of Tris-HCl buffer (pH 7.2) was used as anolyte and the reaction was started by the addition of 10 times concentrated NADH solution to the anolyte. The final concentration of NADH was adjusted to 0.25 mM and 2 ml of NADH solution was taken out for measurement of the spectrum every 20 min. The surface area of both electrodes was 48 cm^2. The current was measured with an ammeter in closed circuit configuration every 20 min to obtain the maximal current value. In this system, NADH was used as a fuel of bacterial fuel cell instead of bacterial cell for confirmation of the catalytic oxidation of NADH on graphite-Mn(IV) linked to electricity production.

Biofuel Cell

A 800 ml volume of SCBFC (Fig. 1) was used to compare bacterial growth, electricity (potential and current) production, and substrate consumption using different biocatalysts, electrodes, and substrates. Graphite-Fe(III) cathode was used as a cathode and graphite-Mn(IV) anode and the normal graphite electrode were used as an anode. The surface area of both the graphite-Mn(IV) and the normal

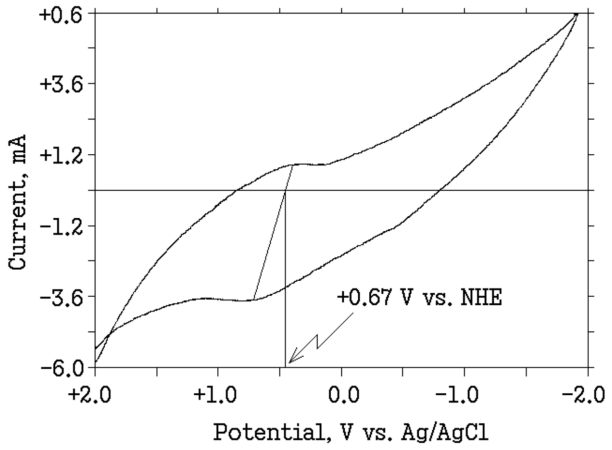

Fig. 1. Cyclic voltammogram of graphite-Fe(III) cathode during 2 successive cycles following the introduction of the electrode into a phosphate buffer (50 mM, pH 7.0)

The scan rate was 10 mV/s, the working electrode was the graphite-Fe(III) cathode, the reference electrode was Ag/AgCl, and the counter electrode was platinum wire. The oxidation potential is +0.58 volt vs. Ag/AgCl (+0.78 volt vs. NHE), reduction potential is +0.47 volt vs. Ag/AgCl (+0.67 volt vs. NHE), and the half redox potential (*Eh*) is +0.493 volt vs. Ag/AgCl (0.69 volt vs. NHE).

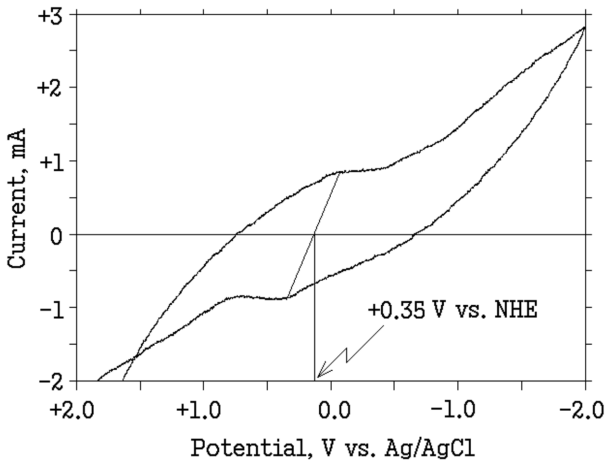

Fig. 2. Cyclic voltammogram of graphite-Mn(IV) anode during 2 successive cycles following the introduction of the electrode into a phosphate buffer (50 mM, pH 7.0).

The scan rate was 10mV/s, the working electrode was the graphite-Mn(IV) anode, the reference electrode was Ag/AgCl, and the counter electrode was platinum wire. The oxidation potential is +0.35 volt vs. Ag/AgCl (0.55 volt vs. NHE), reduction potential is -0.03 volt vs. Ag/AgCl (+0.17 volt vs. NHE), and the half redox potential (*Eh*) is +0.15 volt vs. Ag/AgCl (0.35 volt vs. NHE).

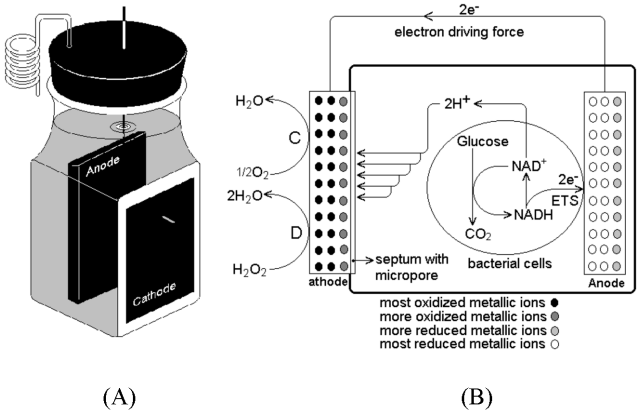

Fig. 3. Real shape [A] and schematic structure [B] of the single-compartment bacterial fuel cell system.

The cathode was made from a mixture of fine graphite power, clay powder, and Fe^{3+}, and the anode was made from a mixture of fine graphite power, clay powder, and Mn^{4+}. A septum located between cathode and anolyte was made from porcelain membrane to which cathode was completely attached. Both cathode and septum have micropores. Proton can move through micropores of both septum and cathode to reach outside of the cathode at which protons and electrons react with oxygen to be oxidized to H_2O. The cathode can be spontaneously oxidized by oxygen in atmosphere (reaction C) and oxdiation of the cathode can be activated by hydrogen peroxide (reaction D).

graphite anode was 120 cm^2 and the surface area of the graphite-Fe(III) cathode was 150 cm^2. As shown in Fig. 3, one side of the graphite-Fe(III) cathode was exposed to air by means of which the ferrous ion contained in cathode is reduced coupled to oxidation of the Mn(II) contained in the anode, which can be re-oxidized into ferric ion to act as an oxidant, and another side coated with porcelain membrane (2 mm thickness) made contact with the anolyte (bacterial culture). The porcelain membrane prevents the bacterial cell against contact with the graphite-Fe(III) cathode. The proton can move through the micropores of the porcelain membrane from the anolyte to the surface of the cathode to where the proton was oxidized in water following reaction with electrons released from the anode and oxygen molecules released from the atmosphere. The growing cells and resting cells of *E. coli* and anaerobic sewage sludge were used as a biocatalyst for composing a bacterial fuel cell. Five % (v/v) of pre-cultivated *E. coli* was inoculated into bacterial fuel cell with fresh medium, an anolyte, in which the initial cell density was adjusted to 0.3 OD at 660 nm. The harvested *E. coli* suspended in LB medium with 10 g/l glucose and 100 mM phosphate buffer (pH 7.0) and the settled supernatant of sewage sludge with 20 g/l glucose and 100 mM potassium phosphate buffer (pH 7.0) were used as a reactant for the bacterial fuel cell with resting cells, in which the initial bacterial density of *E. coli* and sewage sludge was adjusted to 4.3 OD and 4.6 OD at 660 nm.

Influence of Oxidant on the Function of the Cathode

The hydrogen peroxide was used as an oxidant to test the cathode function in single-compartment bacterial fuel cells. Theoretically, in a single-compartment bacterial fuel cell system, the proton moves from the anolyte to the graphite-Fe(III) cathode surface through the porcelain membrane and graphite-Fe(III) cathode, and the electron moves from the anode to the cathode surface on which the electrons and protons are oxidized to H_2O by reaction with molecular oxygen or some other oxidant. The oxidant can activate the oxidation of protons and electrons and electricity production may be increased in this way. One % of hydrogen peroxide solution was continuously spread on the surface of the graphite-Fe(III) cathode at intervals of 20 min while the bacterial fuel cell was working, as shown in reaction D of Fig. 3B.

RESULTS AND DISCUSSION

The two-compartment fuel cell system with normal graphite electrodes has been typically used for testing biofuel cells but problems have arisen with respect to its application to real bioreactors, namely, with respect to the continuous aeration of catholyte, the continuous addition of electron mediators to anolyte, and the maintenance of catholyte volume and structural complexity [18, 19, 21, 22]. The electron mediator has been used to activate electron transfer from bacterial cells to electrodes and has to be continuously added to bacterial cultures in bacterial fuel cell because only some of them can be contacted by to both bacterial cells and the electrode surface [24]. In bacterial fuel cells with a soluble electron mediator, the current production was reported to be insufficient for useful energy production because current production is proportional to the number of reduced electron mediators coupled to the oxidation of bacterial reducing power and the penetrating efficiency of electron mediator through the bacterial membrane [1, 17, 18, 25]. In fact, since it is difficult for any electron mediator to transfer freely across the bacterial membrane, it is difficult for current productivity to exceed 3–5 mA per fuel cell system (below 100 mA per 100 cm^2 anode surface area) in a bacterial fuel cell with a soluble electron mediator [26]. For the production of useful electric energy from biofuel cell, the electron density has to exceed 5 mA per 100 cm^2 anode surface area and the potential difference between anodes and cathodes has to exceed 0.5 volt. The only way to solve the problems of two-compartment bacterial fuel cell systems is to modify the anodes and cathodes with a material with an affinity to bacterial cells (e.g., neutral red or Mn^{4+}) and O_2 (e.g., ferricyanide or Fe(III)-OOH), [22], and to eliminate the cathode compartment. Various aerobic bacterial strains such as *E. coli*, *Bacillus* sp., and *Pseudomonas* sp. and most of the anaerobic bacterial consortium such as *Clostridium* sp., *Shewanella* sp., sulfate-reducing bacteria, and the denitrifying bacterial consortium have been reported to reduce various metal ions [5, 12, 13]. The metal-reducing bacteria have been reported to expend their reducing power coupled to the reduction of water-insoluble metal ions such as Mn^{4+} and ferric ion Fe^{3+} located outside the bacterial cells [14, 15, 16]. It seems possible that the Mn^{4+} immobilized into anodes may be reduced to Mn^{2+} coupled to oxidation of bacterial reducing power and that in this way electrons may be transferred from bacterial cells to anodes. On the other hand, the Fe^{3+} immobilized into cathodes may be reduced to Fe^{2+} coupled to oxidation of Mn^{2+} into Mn^{4+} immobilized into anode, thus enabling electrons to transfer from anodes to cathodes. The Fe^{2+} immobilized into cathode has to be spontaneously reoxidized into Fe^{3+} by reaction with O_2, which acts as a final electron acceptor. The potential difference between anodes and cathodes is the electron-driving force that was increased by the modification of the anodes and cathodes with Mn^{4+} and Fe^{3+}, respectively. As shown in the cyclic voltammograms of Figs. 1 and 2, the *Eh* of graphite-Fe(III) electrode is 0.693 volt vs. NHE and the *Eh* of graphite-Mn(IV) electrode was about 0.34 volt vs. NHE. In bacterial fuel cells the voltage is proportional to the *Eh* difference between the anodes and cathodes, and current production is proportional to the electron density on the anode surface which may be

determined by the size of the bacterial population in contact with the surface of the anode and the efficiency of electron transfer from bacterial cells to anodes [19, 21].

Generally, the two-compartment bacterial fuel cell has been developed and improved by alteration of the electrodes, cation-selective membranes, bacterial strains, substrates, and electron mediators, respectively. This fuel cell has two problems; one is that it expends much more electrical energy on the aeration of the cathode compartment than is produced by the fuel cell system, and another is that it contains two electron barriers, namely the cytoplasmic membrane of biocatalysts and the cation-selective membrane of bacterial fuel cells. Therefore, only SCBFC can be a practical bacterial fuel cell capable of application to a real system, for example, a wastewater treatment system, a space station, submarine, and isolated locations such as islands with severely restricted access to electricity. By using the graphite-Mn(IV) anode with lower redox potential than the graphite-Fe(III) electrode and the graphite-Fe(III) cathode with a higher affinity with oxygen, we composed a SCBFC that has not been obtained until now. The SCBFC system (Fig. 3) with the graphite-Fe(III) cathode and the graphite-Mn(IV) anode is simpler and more practical than the two-compartment fuel cell systems because the electrode-active bacterium, the electron mediators, the aeration of catholyte, and the maintenance of catholyte volume are not essential requirements. Figure 3B is the schematic structure for the explanation of the mechanism of SCBFC. The septum contacted to the inside of the cathode was made from porcelain membrane with micropores. The test of permeation of Na^+ and H^+ through the micropores confirmed that Na^+ was not transmitted through the micropores but that H^+ was (result not shown). As shown in Fig. 3B, the graphite-Mn(IV) anode is thought to function as a catalyst for the oxidation of bacterial reducing power such as NADH, and the graphite-Fe(III) is thought to function as a catalyst for the reduction of O_2 to H_2O. The outside surface of the cathode is thought to be a reaction center for the oxidation of protons and electrons, in which O_2 (reaction C in Fig. 3B) and H_2O_2 (reaction B in Fig. 3D) may be reduced to H_2O. Under atmosphere, O_2 pressure is theoretically 0.2 atm, which may be enough for oxidation of graphite-Fe(III) cathode, but under conditions with higher O_2 pressure or oxidant the electricity production may be increased more sharply than under atmospheric conditions. If the graphite-Fe(III) cathode could function as an oxidant for the oxidation of electrons and protons to H_2O, when H_2O_2 is spread on the cathode surface, it is possible for it to be an activator for oxidation of electrons and protons into H_2O, which would result in an increase in electricity production. As shown in Fig. 4, electricity production was increased approximately two-fold by spraying oxidant (H_2O_2) on the cathode surface. This is definite evidence that the graphite-Fe(III) cathode can function as a catalyst for the oxidation of protons and electrons into H_2O and that protons can reach the surface of the cathode from the anolyte through the micropores inside both the cathode and the porcelain membrane. The NADH acts as a primary electron donor in the electron transport system whose redox potential is –0.32 volt. NADH cannot be spontaneously and electrochemically oxidized without a catalytic enzyme or an appropriate electron mediator and it has been reported that NAD^+ is not electrochemically reduced and acts as a dimer in reaction with an anode. The NAD^+ dimer may lose its function as a redox carrier for biochemical reaction. It was confirmed, however, that graphite-Mn(IV) catalyzes oxidation-reduction of NADH-NAD^+ without an electron mediator or catalytic enzyme. As shown in Fig. 5, when NADH is used as a fuel in SCBFC, electricity is produced coupled to oxidation of NADH (Fig. 5A), and the amount of electricity produced is proportional to the concentration of the NADH (Fig. 5B). Hoogstraten *et al.* [7] have reported that the Mn(II) ion is bound to a specific site within the framework of nucleotides (e.g., NAD^+ and ribonucleic acid) and catalyzes reactions such as RNA splicing and aminoacyl-tRNA synthesizing. This suggests that electrochemically reduced Mn(II) may be bound to NAD^+ and may catalyze the reduction of NAD^+ to NADH (result not shown), or the Mn(IV) may be bound to the NADH and reduced coupled to oxidation of the NADH. This serves as strong evidence that the graphite-Mn(IV) anode can function as a catalyst for oxidation-reduction of NADH-NAD^+ without an enzyme or electron mediator. Figure 6 is a result obtained from a test with SCBFC for comparison of two different anodes, graphite-Mn(IV) and normal graphite. As shown in Fig. 6, in the

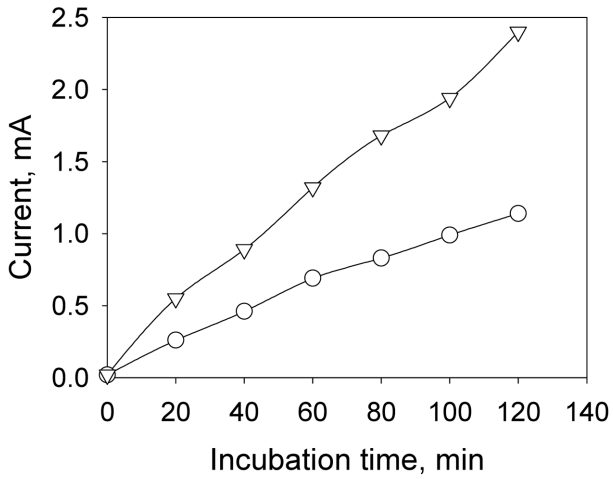

Fig. 4. Effect of hydrogen peroxide on electricity production in a single-compartment bacterial fuel cell system in which *E. coli* was used as a biocatalyst.
Under condition with hydrogen peroxide (△), the electricity productivity was two times higher than that without hydrogen peroxide (○). The 1% hydrogen peroxide was spread on the cathode surface during the reaction at intervals of 20 min under aerobic atmosphere.

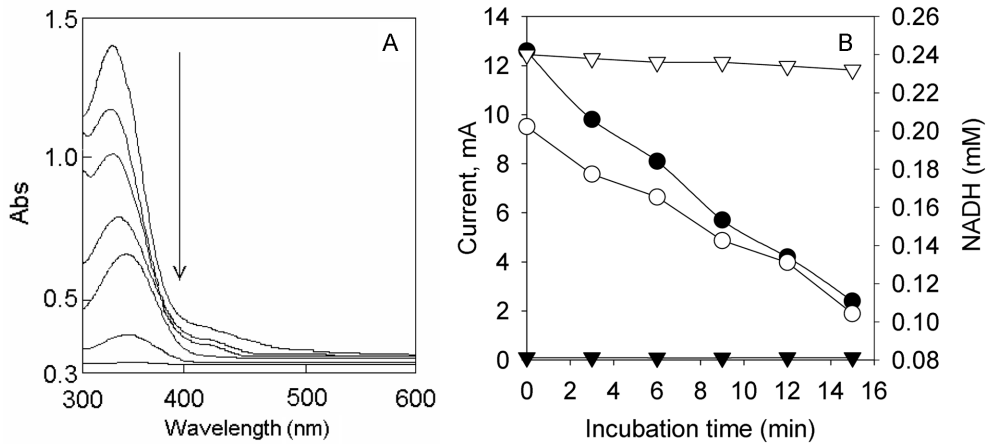

Fig. 5. The spectrum of of NADH (A) decreased in coupling with electricity production (B) in the course of incubation time.
The NADH concentration (○) was decreased in the coupling with electricity production (●) in SCBFC with graphite-Mn(IV) anode and graphite-Fe(III) cathode, however, NADH (▽) was not oxidized and electricity (▼) was not produced in SCBFC with normal graphite anode and graphite-Fe(III) cathode. The initial concentration of NADH used was 0.25 mM.

test with a growing *E. coli* cell, the bacterial growth, electricity (potential and current) production, and substrate consumption were more active in SCBFC with the graphite-Mn(IV) anode than with the unmodified anode. This is a clue that *E. coli* dependent on the respiratory metabolism may be actively grown using Mn(IV)-ion immobilized to an anode as a substitutive electron acceptor instead of oxygen under anaerobic conditions. The Mn(IV)-ion of an anode may be reduced to Mn(II)-ion coupling with bacterial metabolism and may be re-oxidized into Mn(IV)-ion coupled with the reduction of Fe(III)-ion into the Fe(II)-ion of a cathode, which is spontaneously re-oxidized into Fe(III)-ion by contact with oxygen in the atmosphere. The electrons from *E. coli* in the graphite-Mn(IV) anode can be spontaneously transferred by potential gradient from bacterial electron carriers such as NADH (*Eh*=– 0.32 volt vs. NHE) to graphite-Mn(IV) anodes (*Eh*=0.35 volt vs. NHE), and the electrons from the graphite-Mn(IV) anode in the graphite-Fe(III) cathode may be spontaneously transferred by potential gradient from graphite-Mn(IV) anode (*Eh*=0.35 volt vs. NHE) to the graphite-Fe(III) cathode (*Eh*=0.693 volt vs. NHE), which is an electron-driving force for the production of electricity in SCBFC. When the resting cells of *E. coli* were used as a biocatalyst, substrate consumption and electricity production were increased 1.5– 2.0 times and 4– 5 times, respectively, as compared to the rates obtained when the growing cells of *E. coli* were used. Because the bacterial cell functions as a biocatalyst for the production of reducing power, the amount of biomass is one of the key factors in electricity productivity and substrate consumption in SCBFC. Anaerobic bacterial consortia were more active in the reduction of metal ion under anaerobic conditions because they are a mixed culture of anaerobic respiratory and anaerobic fermentative bacteria. Under anaerobic conditions without an electron acceptor, the energy production for bacterial growth is dependent on fermentative metabolism but energy metabolism can be changed in respiratory metabolism by the addition of an electron acceptor such as Mn(IV), Fe(III), NO_3^-, or SO_4^{2-}. This shows that the graphite-Mn(IV) anode acts as an electron acceptor and the graphite-Fe(III) cathode acts as an oxidizer for the re-oxidation of reduced Mn(II) coupled to bacterial metabolism, thereby increasing substrate consumption. In wastewater treatment plants, anaerobic digestive reactors have to be operated for a long period because bacterial metabolism is

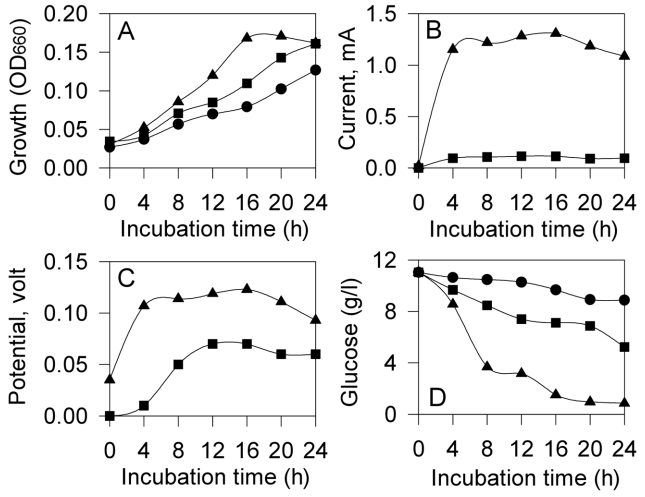

Fig. 6. Comparison of bacterial growth (A), current production (B), potential difference (C), and glucose consumption (D) in bacterial fuel cell with the normal graphite electrode (■) and the modified graphite electrode (▲) in SCBFC.
Growing cells of *E. coli* were used as a biocatalyst and cultivated in the bacterial fuel cell without electrode (●), normal graphite anode (■), and Mn(IV)-graphite anode (▲). The Fe(III)-graphite cathode was used for all bacterial fuel cells used for test. Five % (v/v) of a 12-h-old culture of *E. coli* was used as an inoculum.

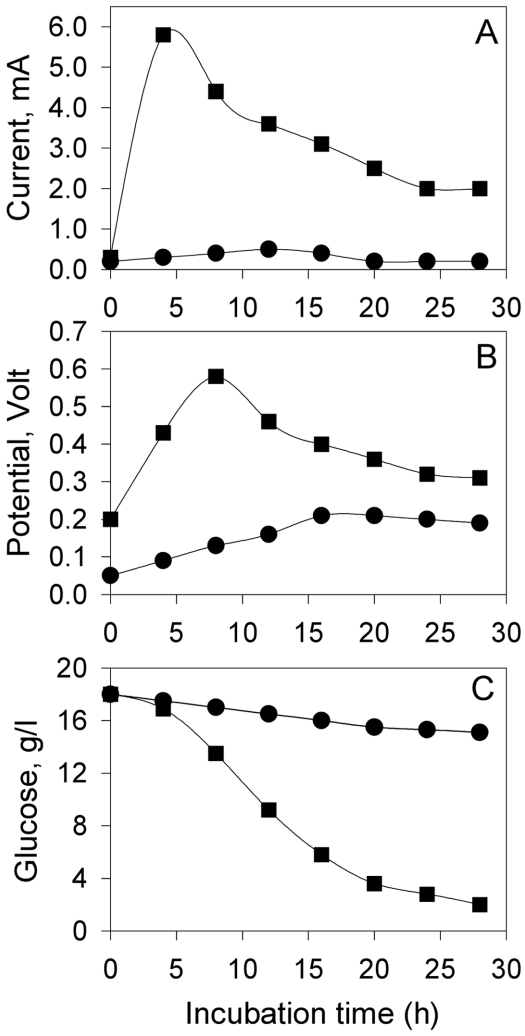

Fig. 7. Comparison of current production (A), potential difference (B), and glucose consumption (C) in bacterial fuel cell with the normal graphite electrode (●) and the modified graphite electrode (■) in SCBFC. Resting cells (OD_{660} 4.3) of *E. coli* were used as a biocatalyst.
The Fe(III)-graphite cathode was used for all bacterial fuel cells used for the test.

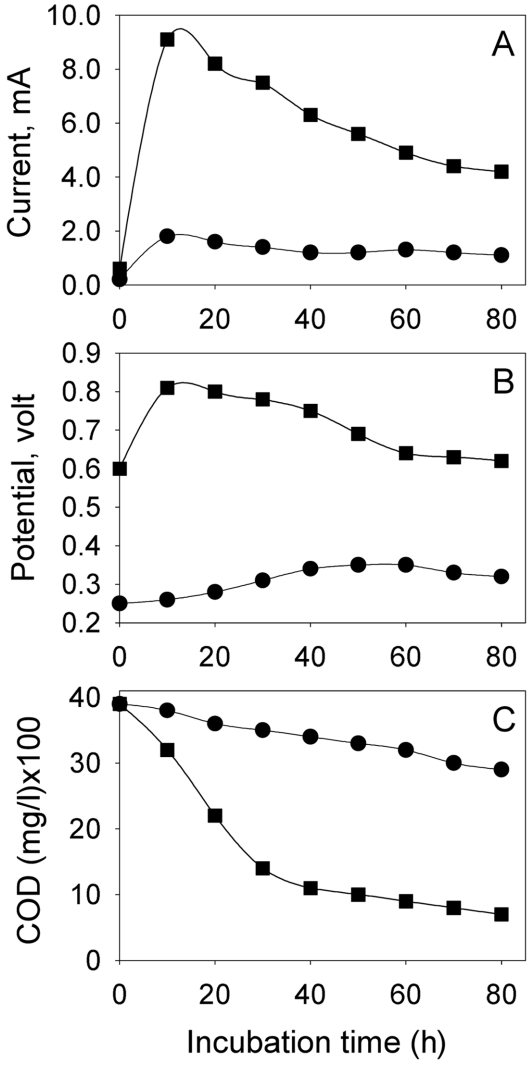

Fig. 8. Comparison of current production (A), potential difference (B), and substrate consumption (C) in bacterial fuel cell with the normal graphite electrode (●) and the modified graphite electrode (■) in SCBFC.
Anaerobic bacterial consortium (OD_{660} 4.6) autogenously grown in sewage sludge was used as a biocatalyst. The Fe(III)-graphite cathode was used for all bacterial fuel cells used for tests.

dependant only on fermentation and methanogenic respiration. The sludge digestive system is an essential requirement for decreasing the quantity of sludge produced from aerobic systems but the reaction is too slow to be effective for the treatment of sludge in a short period [8, 23]. When anaerobic sewage sludge itself with 20 g/l glucose and 100 mM phophate buffer was applied to a bacterial fuel cell system, substrate consumption was increased by about 3 times at 80 h as shown in Fig. 8. In this system, external resistance was not used to test how much maximal electricity can be produced from bacterial fuel cells using the anaerobic sewage sludge itself. For 80 h cultivation, the maximal current and potential was increased to about 3.5 mA and 0.58 volt in the bacterial fuel cells with a graphite-Mn(IV) anode as against 0.4 mA and 0.42 volt in bacterial fuel cells with an unmodified graphite anode.

The growing bacterial cell produces free energy (ATP) and reducing power as does NADH. However, some free energy or reducing power has to be consumed to remove toxicity or inhibitory factors from the environment, as a result of which bacterial growth can be repressed [3], but the consumption of reducing power for the generation of electricity is not an inhibitory factor in bacterial growth because reducing power may be wasted but free energy cannot be wasted as a result of the reduction of electron acceptors such as metal ions. The bacteria growing in

SCBFC with a graphite-Mn(IV) anode may produce more free energy and consume more substrate than in SCBFC with a normal graphite anode, because the bacterial cell has to compensate for the reduction in its power coupled with the reduction of Mn(IV) to Mn(II) in its anode. This is the reason why *E. coli* and the anaerobic bacterial consortium in SCBFC with the graphite-Mn(IV) anode consumed more substrate than in SCBFC with the normal graphite anode during the same incubation time, as is shown in Figs. 6, 7, and 8. The mechanism explaining how bacterial reducing power such as NADH located in bacterial membrane can be oxidized coupled with a reduction of the graphite-Mn(IV) anode is as follows: (1) the potential difference between graphite-Mn(IV) anode and graphite-Fe(III) cathode is enough to activate the electron drive from anode to cathode, (2) Fe(II) in a reduced cathode coupled to oxidation of Mn(II) in the anode can be spontaneously oxidized into Fe(IV) by O_2, and (3) Mn(IV) has been reported to be an electron acceptor for bacterial respiration. Consequently, the Mn(IV) is thought to be a suitable material for making electron channel and maintenance of lower potential of anode, and the Fe(III) is considered to be useful as an oxidant for re-oxidation of Mn(II) to Mn(IV) and maintenance of a higher potential of cathode. The SCBFC serves a possibility that electricity production may be substituted for methane production from anaerobic digestive reactors in sewage sludge treatment plants, which has been impossible when using an electron mediator and two-compartment bacterial fuel cell system.

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