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Development of Single-Compartment Bacterial Fuel Cell Using Modified Graphite Electrode with Metal Ion

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Introduction

The bacterial fuel cell has been progressed by change of bacterial species, electron mediators and substrates [1, 2]. In the bacterial fuel cell, a bacterium functions as a catalyst for production of biochemical reducing power from substrate, an electron mediator functions as a converter of bacterial reducing power to electricity and a substrate serves as a fuel [7]. And anode functions as an oxidant for electron mediators that are reduced coupled to biochemical oxidation of substrate and cathode functions as a reductant for electron acceptors, respectively. The biochemical energy can be converted into electric energy by coupling biocatalytic oxidation of substrate to chemical reduction of the oxidant at interface between anode and cathode [8]. An electron mediator has been absolutely required to promote electron transfer from bacteria to electrode because bacterial membrane acts as an electron barrier [4] and direct electron transfer from bacteria to electrode occurs only at very low efficiency [1]. Park3 *t al.* [3] reported that viologen dye cross-linked with carbon polymers could be absorbed on cytoplasmic membrane of *Desulfrovibrio desulfuricans* and could mediate electron transfer from bacterial cells to electrode or from electrode to bacterial cells. The electron transfer efficiency from bacterial cell to electrode in bacterial fuel cells be improved if more suitable electron mediators were used. For improving the bacterial fuel cell with soluble electron mediator, Park *et al.* [6] tried to modify electrode with neutral red and reported that the modified electrode was confirmed to be more active for electricity production than unmodified (normal) electrode. Recently, we developed the modified electrode with metal ions and a single-compartment bacterial fuel cell capable of electricity production without electron mediators. The purpose of this research is to test ability of modified electrodes with metal ion, to develop less complex bacterial fuel cell and to apply new bacterial fuel cell to wastewater treatment reactor. In this research, we compared substrate consumption and electricity production in single-compartment bacterial fuel cell with normal electrode and the modified electrode, and tried to apply the single compartment bacterial fuel cell to wastewater treatment system for improvement of treatment efficiency.

Materials and Methods

Electrode

Graphite felt modified with neutral red and graphite modified with Mn(IV) and Fe(III) ion.

Cyclic voltammetry

The cyclic voltammograms was obtained using graphite working electrode modified with Mn^{4+} and Fe^{3+} which was transformed into rod type (diameter 5mm, length 4cm), platinum wire counter electrode and an Ag/AgCl reference electrode in 50 mM phosphate buffer (pH 7.0). Cyclic voltammetry was performed using a cyclic voltammetric potentiotat (model CV50W, BAS, USA) linked to an IBM personal computer data acquisition system. Prior to use, the electrodes were cleaned using ultrasonic cleaner. The scanning rate used was $25mVs^{-1}$ over the range of +2.0 volt to –2.0 volt.

Biofuel cell

A 800 ml volume of single-compartment bacterial fuel cell (Figure 1B) was used to compare bacterial growth, electricity (potential and current) production and substrate consumption according to the differences of biocatalyst, electrodes and substrates. The modified electrodes with metal ions (Fe^{3+} and Mn^{4+}) and the normal (unmodified) graphite electrode were used. Surface area of both unmodified and Mn^{4+} anode was 150 cm^2 and the surface area of Fe^{3+} cathode was 100 cm^2, respectively. One side of Fe^{3+} cathode was exposed and another side coated with porcelain membrane (2mm thickness) was contacted with anolyte (bacterial culture) but not contacted with bacterial cell. The proton can move through micro-pore of porcelain membrane from anolyte to surface of cathode at where proton was oxidized to water by reaction with electron moved from anode and oxygen molecule from atmosphere.

Results and Discussion

The two-compartment fuel cell system (Fig. 1A) with normal graphite electrodes and electron mediators has been exclusively used befor Park and Zeikus [9, 10] developed the single-compartment bacterial fuel cell. The two-compartment fuel cell has some problems for application to real bioreactor because of continuous aeration of catholyte, continous addition of electron mediator to anolyte, continuous addition of buffer to cathode compartment for maintenance of catholyte volume and structural complexity. However, the single-compartment fuel cell (Fig. 1B) with the modified electrodes is simple and practical because the electron mediator, aeration of catholyte and maintenance of catholyte volume are not absolutely required. For this research, the single-compartment fuel cell with Mn^{4+} anode, normal anode and Fe^{3+} cathode was used. The potential difference between anode and cathode is electron-driving force that was increased by modification of anode and cathode with Mn^{4+} and Fe^{3+}, respectively. As shown in cyclic voltammograms of figure 2 and 3, the *Eh* (half redox potential) difference between graphite-Mn(IV) anode of which *Eh* is 0.35 volt vs. NHE and graphite-Fe(III) cathode of which *Eh* is 0.693 volt vs. NHE was about 0.34 volt. In open-circuited bacterial fuel cell electricity potential is proportional to *Eh* difference between anode and cathode, and electron density on anode surface which is determined by electrode-affinity to bacterial cell. In close-circuited bacterial fuel cell electricity potential must be lower than the potential in open-circuited fuel cell because the electron density on anode surface is quickly dissipated through cathode to oxygen but the dissipation of electron density can be controlled by using an external resistance between anode and cathode [5].

The two-compartment bacterial has two problems, one is to spend much more energy for aeration of cathode compartment than electrical energy produced from fuel cell system and another are to have two electron barriers which are cytoplasmic membrane of biocatalysts and cation-selective membrane of bacterial fuel cell. Only bacterial fuel cell without cathode compartment can be a fuel cell being application to a real system, for example, wastewater treatment system, space station, submarine and separation places such as islands to which electricity is difficult to leach. By using the Mn^{4+} anode, Fe^{3+} cathode and electrochemical system without cathode compartment we composed a single-compartment bacterial fuel cell. As shown in

figure 4, one side of Fe^{2+} cathode was exposed to air and another side is coated with porcelain membrane. Figure 4 is a proposal to explain operational mechanism of single-compartment bacterial fuel cell. The porcelain membrane protects cathode from contact with bacterial cell, by which Fe^{3+} can be only reduced to Fe^{2+} coupled to oxidation of Mn^{2+} of anode but not biochemically reduced coupled to oxidation of bacterial reducing power. Figure 5 was result obtained from test with single-compartment bacterial fuel cell for comparison between Mn^{4+} anode and normal anode. In the test with growing cell of *E. coli*, the bacterial growth, electricity (potential and current) production and substrate consumption were more activated in bacterial fuel cell with Mn^{4+} anode than that with normal anode. It is possible that *E. coli* may actively grow by using Mn^{4+} as an electron acceptor instead of oxygen under anaerobic condition, and Mn^{4+} can be reduced to Mn^{2+} coupled oxidation of bacterial reducing power and can be re-oxidized to Mn^{4+} coupled to reduction of Fe^{3+} to Fe^{2+} of cathode which is spontaneously re-oxidized to Fe^{3+} by contact with oxygen on atmosphere. In bacterial fuel cell with Mn^{4+} anode and Fe^{3+} cathode electrons can be spontaneously transferred by potential gradient from Mn^{4+} anode (*Eh* = 0.35 volt vs. NHE) to Fe^{3+} cathode (*Eh* = 0.693 volt vs. NHE), which is an electron driving force for production of electricity in single-compartment bacterial fuel cell. Actually, *E.coli* was reported to reduce Mn^{4+} under anaerobic growth condition [11], which is confirmed by difference of current production (Figure 5B), potential difference (Fig. 5C) and glucose consumption (Fig 5D) in bacterial fuel cells with different electrodes- Mn^{4+} anode and normal anode. Because bacterial cell functions as biocatalyst for production of reducing power, amounts of biomass are one of key factors for effective operation of bacterial fuel cell.

When used the resting cells of *E. coli* electricity production (figure 6A and 6B) and glucose consumption (figure 6B) was about 5 times and 1.5-2.0 times of that by growing cell, respectively. In wastewater treatment plant, the anaerobic digestive reactor has to be operated for a long time because the bacterial metabolism is dependant on only fermentation and methanogenic respiration. The sludge digestive system is absolutely required for decreasing quantity of sludge produced from aerobic system but the reaction was too slow not to be effective for treatment of sludge in short period. When applied the anaerobic sewage sludge itself with 20g/L glucose and 100 mM phophate buffer to bacterial fuel cell system, substrate consumption was increased to about 3 times at 80 hr as shown in figure 7. In this system, the external resistance was not used to test how much maximal electricity can be produced from bacterial fuel cell using the anaerobic sewage sludge itself. For 80 hr cultivation, the current and potential was increased to about 3.5 mA and 0.58 volt in bacterial fuel cell with Mn^{4+} anode but 0.4 mA and 0.42 volt in bacterial fuel cell with normal anode, respectively. For obtaining regular electricity from the close-circuited bacterial fuel cell, the external resistance has to be used but the bacterial metabolism coupling with redox-electrodes has to be inhibited. The growing bacterial cell has to produce free energy (ATP) and reducing power such as NADH, however, some of free energy or reducing power has to be consumed to remove toxicity or inhibitory factors from environment by which the bacterial growth can be repressed. The consumption of reducing power for making electricity also may be another one of inhibitory factors. For normal growth in bacterial fuel cell with Mn^{4+} anode, bacteria have to produce more reducing power (free energy) than in bacterial fuel cell with normal anode. This is reason why *E. coli* and anaerobic bacterial consortium in bacterial fuel cell with Mn^{4+} anode and Fe^{3+} cathode consume more substrate than in bacterial fuel cell with normal anode at same incubation time as shown in figure 5, 6 and 7.

Acknowledgment

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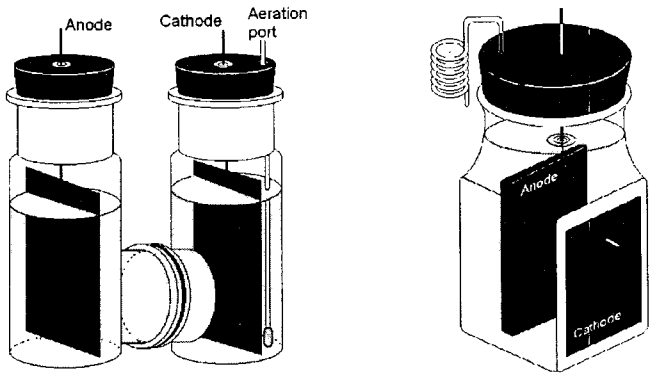

Fig. 1. Comparison between real structures of two-compartments (A) and single-compartment (B) bacterial fuel cell. The aeration of catholyte is absolutely required for operation of two-compartments system but not required for single-compartment system. A porcelain membrane was used as a septum between anode and cathode in both one and two-compartment systems, and the septum was completely attached to inside of cathode in one compartment system. In single-compartment system, cathode is located between air and bacterial culture of which another functions is anolyte, and the proton can move through the septum and cathode to outside of cathode at which proton reacts with electron from anode and oxygen from air to make water.

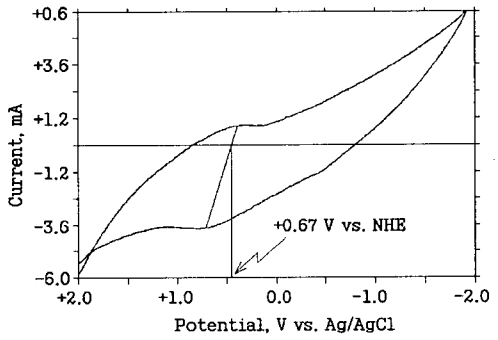

Fig. 2. Cyclovoltammogram of cathode modified with Ferric ion (Fe^{3+}) during successive 2 cycles following the introduction or the electrode into a phosphate buffer (50mM, pH 7.0). The scan rate was 10mV/s, the working electrode was the modified electrode with Fe(III) ion, the reference electrode was Ag/AgCl and the counter electrode was platinum wire. The oxidation potential is +0.58 volt (+0.78 volt vs. NHE), reduction potential is +0.47 volt (+0.67 volt vs. NHE) and the half redox potential (E½) is +0.493 volt which is same to 0.69 volt vs. NHE. The oxidation potential of 0.78 volt vs. NHE is exactly same to the theoretical redox potential of Fe(II).

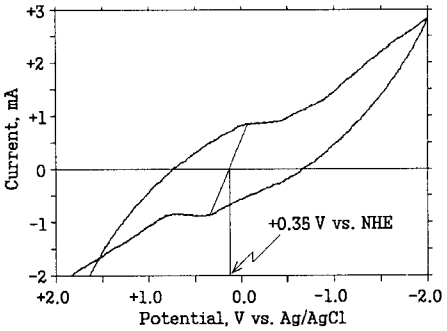

Fig. 3. Cyclovoltammogram of cathode modified with Mn-ion (Mn^{4+}) during successive 2 cycles following the introduction or the electrode into a phosphate buffer (50mM, pH 7.0). The scan rate was 10mV/s, the working electrode was the modified electrode with Mn(IV) ion, the reference electrode was Ag/AgCl and the counter electrode was platinum wire. The oxidation potential is +0.35 volt (0.55 volt vs. NHE), reduction potential is -0.03 volt (+0.17 volt vs. NHE) and the half redox potential (E½) is +0.15 volt which is same to 0.35 volt vs. NHE. The oxidation potential is nearly same to the theoretical redox potential of Mn(IV)/Mn(II) which is 0.595 volt.

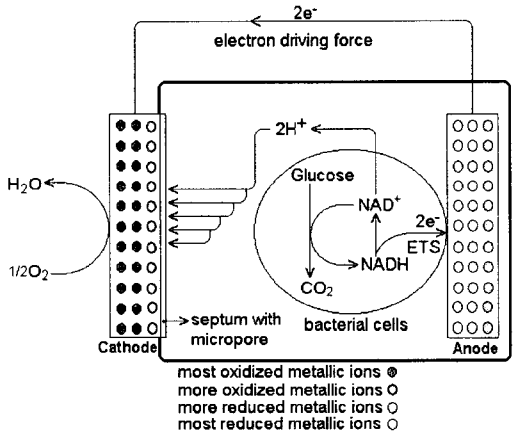

Fig. 4. Schematic structure of one compartment microbial fuel cell. The cathode and anode were made from the modified graphite with ferric ion (Fe^{3+}) and manganese ion (Mn^{4+}), respectively, and a septurm located between cathode aqnd anolyte was made from porcelain membrane to which cathode was completely attached. Both cathode and septum have micro-pores. Proton can move through micro-pores of septum and cathode to reach outside of cathode at which the proton and electron can react with oxygen to be oxidized to H2O. Catholyte, electron mediators and air bubbling for oxygenation of cathode can be excluded from microbial fuel cell. Cathode can be spontaneously oxidized by contact with oxygen in air. Electron driving force can be produced by potential differences between most oxidized ferric ion (Fe^{3+}) and most reduced manganese ion (Mn^{2+}).

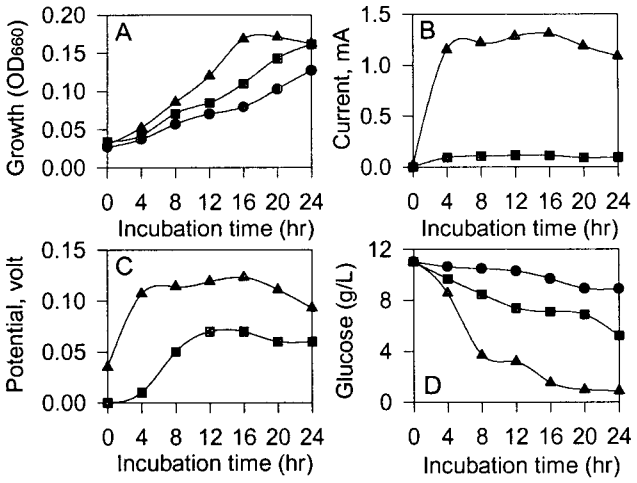

Fig. 5. Comparison of bacterial growth (A), current production (B), potential difference (C) and glucose consumption (D) in bacterial fuell cell with the normal carbon electrode (■) and the modified carbon electrode (▲) in single-compartment bacterial fuel cell. Growing cells of *E. coli* were used as a biocatalyst and cultivated in bacterial fuel cell without electrode (●), normal graphite anode (■) and Mn(IV)-graphite anode (▲). Fe(III)-graphite cathode was used for all bacterial fuel cells used in test. 5% (v/v) of 12 hr old culture of *E. coli* was used as an inoculum.

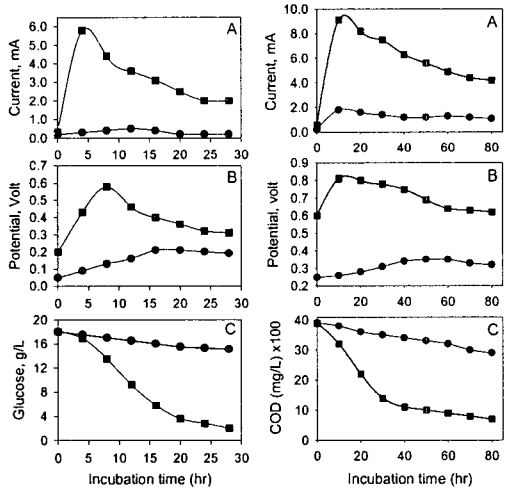

Fig. 6. [Left column] Comparison of current production (A), potential difference (B) and glucose consumption (C) in bacterial fuell cell with the normal carbon electrode (●) and the modified carbon electrode (■) in single-compartment bacterial fuel cell. Resting cells (OD_{660} 4.3) of *E. coli* were used as a biocatalyst. Fe(III)-graphite cathode was used for all bacterial fuel cells used in test.

Fig 7. [Right column] Comparison of current production (A), potential difference (B) and substrate consumption (C) in bacterial fuell cell with the normal carbon electrode (●) and the modified carbon electrode (■) in single-compartment bacterial fuel cell. Anaerobic bacterial consortium (OD_{660} 4.6) autogenously grown in sewage sludge was used as a biocatalyst. Fe(III)-graphite cathode was used for all bacterial fuel cells used in test.

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