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Short communication

Electricity generation from acetate and glucose by sedimentary bacterium attached to electrode in microbial-anode fuel cells

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Abstract

Microbial-anode fuel cells (MAFCs) with high electron recovery (>50%) from acetate and glucose have been constructed in this study. By inoculating fresh sedimentary microorganisms into anaerobic anode compartments, a stable current (~0.42 mA for acetate-fed MAFCs; ~0.35 mA for glucose-fed MAFCs) is generated from the oxidation of the added organic matter until its concentration decreases to a low level. SEM micrographs indicate that thick biofilms of microbial communities (coccoid cells with a diameter of ~0.5 μm in acetate-fed MAFCs; rod-shaped cells with a length of 2.0–4.0 μm and a width of 0.5–0.7 μm in glucose-fed MAFCs) completely cover the anode electrodes. These anodophillic biofilms are thought to be responsible for the current generation, and make these microbial-anode fuel cells exhibit good performance even when the growth medium is replaced by a salt buffer without any growth factor. In comparison with those microbial fuel cells that require the addition of artificial electron transfer-mediating compounds, the findings in this study imply a potential way to develop excellent mediator-less MAFCs for electricity generation from organic matter by using substrate-induced anodophillic microbial species.

Keywords: Microbial-anode fuel cell; Anodophillic bacteria; Electron recovery; Acetate; Glucose

1. Introduction

The need for less dependence on fossil fuels (e.g. oil and coal) and the use of renewable fuels requires the development of alternative sources such as waste biomass for environmental benefits and alternative global energy supplies. Microbial fuel cells (MFCs) provide new opportunities for the sustainable production of energy from biodegradable and reduced compounds, and thus, have attracted substantial research efforts to develop different devices for generating electricity and removing wastes [1–5].

In a microbial-anode fuel cell, microbial oxidation of organic matter occurs in the anodic compartment where the anode serves as the sole electron acceptor for anaerobic respiration. The electron transfer to the anode can be controlled by soluble electron mediators, by components associated with the bacterial cell wall, or by both mechanisms. Based on electron transfer mechanisms, three kinds of microbial-anode fuel cells (symbolized as A, B and C) have been reported. In microbial fuel cell A, artificial redox mediators, such as potassium ferric cyanide, thionine, or neutral red are added to the anode chambers to enhance the rate of electron transfer [6–11]. In fuel cell B, natural mediators can be released by some special microbial species added to the anode chambers [12–14]. Finally, direct electron transfers to solid electrodes have also been observed to occur in anode compartments of fuel cells C by bacterium colonizing the surface of the electrodes [15–17]. In microbial-anode fuel cell (MAFC) C, direct electron transfer processes are speculated to occur through respiratory enzymes (i.e., cytochromes). However, the very recent discovery that extracellular electron transfer can be performed via highly conductive pili, serving as biological nanowires, offers a new way to understand electron transfer between microorganisms and electrodes [18]. Generally speaking, microbial fuel cells that do not need the addition of an artificial mediator to enhance the rate of electron transfer are called mediator-less microbial fuel cells in literature, although a microbially generated electron mediator is involved in the electron transfer in the microbial fuel cell B.

Compared with the other fuel cells including enzymatic biofuel cells [19] and direct methanol fuel cells [20], microbial

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fuel cells may have a wider range of fuel sources (e.g. complex organic matter in waste water), although the level of achieved power densities is not high yet. Moreover, pure enzymes do not need to be used to construct electrodes. A previous study showed that microbial fuel cells C (i.e., MAFCs containing anodophillic bacteria) have advantages over other types of MAFCs in wastewater treatment and power generation from organic matter due to higher electron recovery and their longevity [21]. Moreover, microbial fuel cells catalyzed by anodophilic bacteria may be simply recharged just by replacing the anodic medium. Up to now, some anodophillic iron reducing bacteria in the family Geobacteraceae have been identified as associated with power generation in sediment fuel cells by the analysis of 16S rRNA [4,22,23], however, the bio-diversity of microbial communities in the anode compartment of microbial fuel cells will provide more opportunities for electricity production from the oxidation of different substrates. Whether the change of substrates induces a shift in the microbial communities on the surface of the anode of microbial fuel cells, and how this shift affects the MAFCs performance should be explored in order to understand the characteristics of electron transfer in microbial-anode fuel cells. In this preparative study, we construct two types of mediator-less fuel cell by inoculating with fresh water sedimentary microorganisms, and find that substrate-induced anodophillic bacteria exhibit excellent abilities to deliver electrons.

2. Materials and methods

2.1. Sedimentary inoculum and anodic growth media

Fresh water sediments used for inoculating microbial fuel cells were collected from a pond located on the campus of Yangzhou University. This pond, with an area of about 3×10^3 m^2 and an average depth of 1.5 m, is polluted by domestic waster resulting in a COD range of 20–30 mg l^{-1} in the water column. The brown, flocculent surface sediments were taken by a method of pumping with a PVC tube connected to a vacuum-pump. The sediments were transported to the laboratory within 30 min and were homogenized by shaking in 500-ml Erlenmeyer flasks under a stream of N_2. For each anode chamber inoculation, a 10 ml subsample of this sediment inoculum was transferred to the anode chamber which contained 180 ml of growth media. The growth medium was prepared using the following constituents (in grams per liter of deionized water): $NaHCO_3$, 2.5; $CaCl_2{\cdot}2H_2O$, 0.1; KCl, 0.1; NH_4Cl, 1.5; $NaH_2PO_4{\cdot}H_2O$, 0.6; NaCl, 0.1; $MgCl_2{\cdot}6H_2O$, 0.1; $MgSO_4{\cdot}7H_2O$, 0.1; $MnCl_2{\cdot}4H_2O$, 0.005; $NaMoO_4{\cdot}2H_2O$, 0.001;yeast extract 0.05. The medium was adjusted to pH 7.0, and was flushed with N_2 to remove oxygen before autoclaving in sealed bottles.

2.2. MAFCs construction and operation

Two-bottled fuel cells were constructed with 80-mm-outside-diameter glass bottles and a 22-mm-outside-diameter pinch clamp assembly as sketched in Fig. 1. The top of each bottle was sealed with a glass dome attached to a ground glass fitting, and the junction was sealed with silicone grease and thick

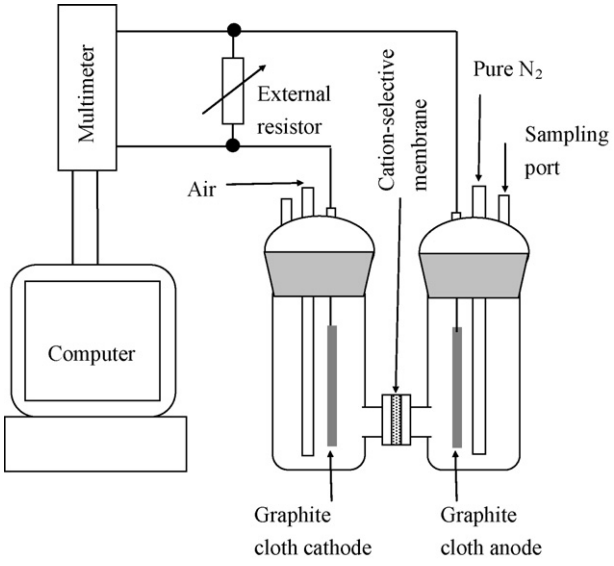

Fig. 1. Schematic of the experimental set-up. Organic substrate is oxidized in the anodic compartment and electrons were transferred via an external resistor to the cathode. The voltage output was measured continuously by a high impedance multimeter.

adhesive tape. Three ports were opened in each glass dome for purging, sampling and introducing electrodes. Electrodes were introduced from the top by feeding a wire through the ports, and the gaps between the wires and glass ports were sealed with epoxy resin. The volume of each chamber with the electrode was approximately 180 ml, except for a 70-ml headspace. The chambers were separated with a cation-selective membrane (GEFC-101; Golden Energy Fuel Cell Co. Ltd., Beijing). The electrodes for fuel cells were 5 cm × 6 cm graphite cloth (grade G10; Xinxing Carbon Co. Ltd., Shanghai). Prior to use, the electrodes were washed in 1N HCl to remove possible metal contamination. Connections were made with a watertight copper wire and the junctions were sealed with epoxy resin. The anode chamber (where bacteria were to be grown and used to donate electrons to the anode) was sterilized, flushed with pure N_2 and filled with anaerobic growth medium. The cathode chamber was filled with 180 ml electrolyte solution containing 50 mM $K_3Fe(CN)_6$ and 100 mM KH_2PO_4 (pH adjusted to 7.0 with 1N NaOH), flushed with air that was passed through a 0.22-μm-pore-size filter. Experiments were conducted at a constant temperature (30 °C), and one set-up with no inoculum was also operated in parallel for a control.

The circuit was usually operated under a fixed load of 1000 Ω, except during current–voltage analysis when the electrical resistance was set using a variable resistor box. The voltage across the known resistance was continuously measured by using a high impedance multimeter (input impedance >3000 MΩ; resolution 0.1 mV; accuracy ±0.04%) with a data acquisition system (UT803, UNI-Trend Group Ltd., Guangdong). Current (I) was calculated using a resistance (R) and the voltage (V) according to $I = V/R$. Power (P) was calculated according to $P = IV$. Power was normalized by the cross-sectional area (projected) of the anode. For current–voltage analysis, microbial fuel cells were allowed to equilibrate at open circuit for ∼2 h until the open

circuit potential was stable. The resistance between the electrodes was lowered stepwise, pausing at each resistance setting for about 5 min.

2.3. Scanning electron microscope (SEM)

Bacteria on the electrodes were examined using a scanning electron microscope. Electrodes were removed from the electrode chambers, rinsed with a sterile medium, and immersed in 5% formaldehyde overnight to fix the samples. Then, the samples were dehydrated stepwise in a graded series of water/ethanol solutions (25, 50, 70, 85, 95, 100%), and then dried. Electrode samples were mounted onto copper specimen mounts with contact adhesive. The samples were then sputter coated in a Polaron E-5100 Sputter coater by using a gold–palladium target and observed in a Philips XL-30ESEM scanning electron microscope. The SEM images were captured digitally.

2.4. Chemical analysis

Fatty acids were analyzed by a gas chromatograph (Agilent, 6890) equipped with a flame ionization detector and a 30 m × 0.32 m × 0.5 μm DB-FFAP fused silica capillary column as described by Liu and Logan. [2]. Glucose was measured by using the phenolsulfuric acid method [24]. COD was measured according to standard methods. Gas chromatography was used for determination of the CH_4 contents in the headspace of the electrode chambers [25].

3. Results and discussion

3.1. Bacteria growth and electricity generation

To initiate bacteria growth on graphite electrodes, sterile anaerobic chambers (180 ml) containing a graphite electrode were inoculated with 10 ml sediment inoculum that had been homogenized under a stream of N_2. The anode chambers were continuously flushed with pure N_2 for 2 h during which the external circuit was not connected. Then, the anode was connected via a 1000 Ω fixed resistor to the cathode. Acetate (20 mM, pH adjusted to 7.0) and glucose (10 mM, pH adjusted to 7.0) were provided as the electron donor, respectively, and no electron acceptors other than the electrode were present. In Fig. 2, the current–time curves show a typical lag phase of 3–5 days followed by a exponentially increasing phase in the initial period. These findings suggest that the number of bacteria cells in anodic chamber is a controlling factor for electricity generation. By comparison, the electrical current increase for glucose-fed MAFCs is observed to occur typically in 2–5 days later than the acetate-fed MAFC (Fig. 2), which implies a diversity in bacterial species induced by different substrate feeding (as illustrated by the SEM observations below).

When the current production began to decrease, the growth medium and sediment in anode chambers were removed. However, the current production was restored to the maximum level similar to previous levels as soon as fresh anaerobic corresponding growth medium was added. Higher substrate concentration

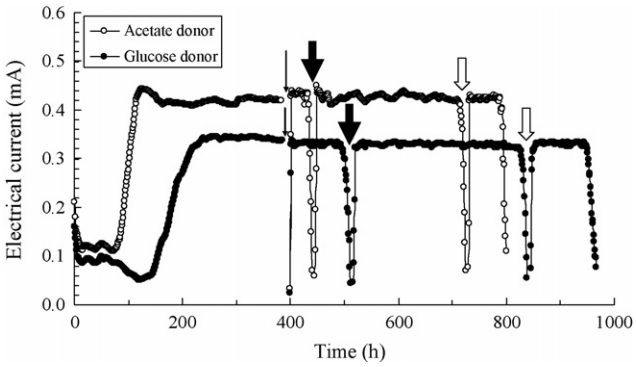

Fig. 2. Representative electrical current generation in mediator-less microbial fuel cells initiated by fresh sedimentary bacterium. The first media replacement (indicated by the thin arrows) was finished with fresh growth media containing 0.5 mM of electron donor, and the second replacement (indicated by the wide black arrows) was also finished with fresh growth media containing more substrates (5 mM acetate; 2 mM glucose, respectively). The wide blank arrows indicate the media replacement with sterile, anaerobic salt buffer containing electron donor (1 mM acetate; 1 mM glucose, respectively).

in growth medium resulted in a longer current production interval (Fig. 2). The rate of electron flow through the circuit was limited by different types of resistances such as the external resistor, electron transfer between solution and electrode surface and mass transfer in solution. Chemical analysis indicates that the decrease in current generation in Fig. 2 was caused by substrate limitation. The decrease in current from maximum levels to baseline typically spans about 10 h. Considering the suspended bacteria cells in solution have been removed when the growth medium is changed, quick recharge (spans typically 1–1.5 h in Fig. 2) to its original charged state after the replacement of fresh growth medium containing plenitudinous substrates could be attributed to the bacteria cells attached to the anode.

To determine if this power production is affected by the soluble medium compounds, the growth medium in the anode chambers was removed under sterile, anaerobic conditions. Chambers were refilled with a sterile, anaerobic buffer that did not contain NH_4Cl, $MnCl_2$, $NaMoO_4$, $MgSO_4$ and yeast extract, in order to remove any soluble compounds and limit further growth of cells. When the organic substrates, acetate and glucose are again added into the corresponding initiated anode chambers, electrical current production rapidly went up to a maximum and stabilized at levels similar to those observed before the medium was replaced (Fig. 2). In the control set-up (with no inoculum), the observed current at a 1000 Ω fixed resistance was no more than 0.03 mA throughout the experiment.

3.2. Current–power profile

When the electrical current production became stable (∼0.42 mA, or ∼0.014 mA cm^{-2} for acetate-fed MAFCs; ∼0.35 mA, or ∼0.011 mA cm^{-2} for glucose-fed MAFCs) (Fig. 2), data were collected to determine the voltage and power generation sustained across a range of current densities obtained by varying the resistance between the electrodes (Fig. 3). Limiting factors for power output generally varied with the current

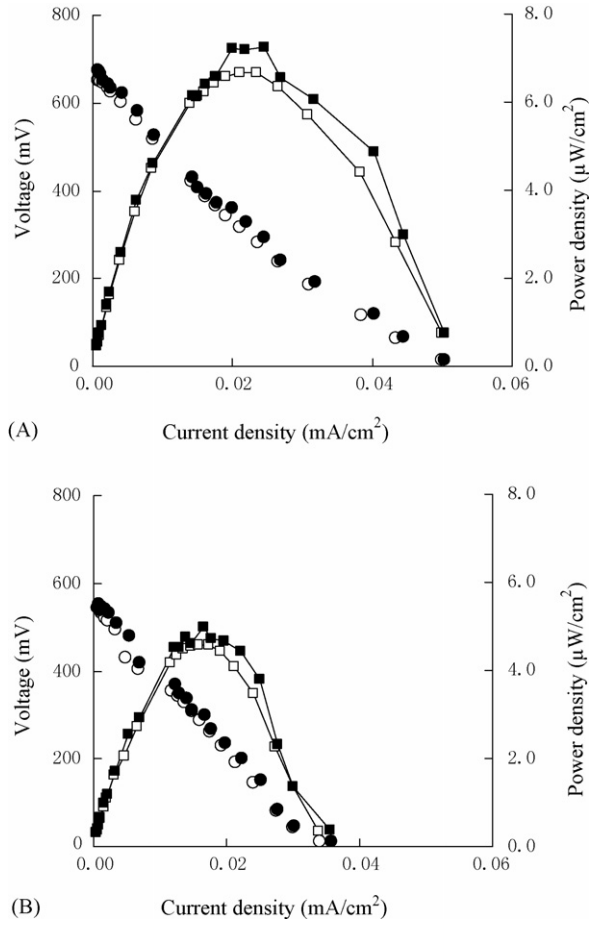

Fig. 3. Current density–voltage (○, ●) and current density–power density (□, ■) relationships for acetate-fed (A) and glucose-fed (B) microbial fuel cells. Open symbols represent the results before the growth mediums were first replaced. Closed symbols represent results after the medium was first replaced with a fresh growth medium.

flow through the circuit. For example, ohmic (transport of ionic species through the medium) and mass transfer (transport of donor or acceptor to the electrode surface) factors were predominant at higher rates of current flow while the rate of charge transfer at the electrode surface became the important limiting factor for power output at a lower rate of current flow. So, significant differences in the current–power relationships at low current flows would be observed after the medium was replaced if the electron mediators or bacteria in solution were responsible for electron transfer to the solid electrode. However, the current–power profile over a range of current densities observed after the replacement of the growth medium was almost identical to that observed before the suspended bacteria cells were removed (Fig. 3). All these observations indicate that it is mainly bacteria attached to electrode that were responsible for the electron transfer to the electrode surface, although there were also planktonic cells growing in solution before the replacement of growth medium.

The acetate-fed MAFC in this study generated a power density of $\sim$5.9 $\mu W\,cm^{-2}$ at a stable current density of $\sim$0.014 $mA\,cm^{-2}$, and the maximum power output is about 7.0 $\mu W\,cm^{-2}$ (Fig. 3A). These values are aproximately four times larger than that of experimental results with the pure strain, *Geobacter sulfurreducens*, attached to plain graphite in a previous report [23]. For the glucose-fed MAFC in the present study, a stable power output at $\sim$0.011 $mA\,cm^{-2}$ current density averaged 4.3 $\mu W\,cm^{-2}$, and the maximum power output was about 5.0 $\mu W\,cm^{-2}$ (Fig. 3B). This value is comparable to that of glucose-fed MAFC inoculated with pure train, *Pseudomonas aeruginosa*, reported by literature [26], but is much lower than the reported power of glucose-fed MAFC with mixed consortium repetitively enriched from anaerobic sludge [15].

3.3. Substrate oxidation and electron recovery

The decrease in concentration of substrate (e.g. acetate and glucose) was observed with electricity production by analyzing the samples taken from the anode chamber periodically. During the period of initial electricity production before the anodic medium and sediment were removed, methane was detected in the headspace of anode chamber with decreasing concentration. However, the methane concentration was less than the detection limit after the first replacement of the medium. These findings indicate that microorganisms in the sediment inoculum rather than bacteria attached to surface of electrode were responsible for the methane production. When the current production fell to a base rate, acetate acid and other volatile fatty acids were not detectable in the anodic chamber of the acetate-fed MAFC. However, in a glucose-fed MAFC, concentrations of acetic acid were measured in the anode chambers on average 0.12 ± 0.07 mM ($n = 3$), and no other fatty acids were detectable when glucose was oxidized over 95%.

Assuming that both acetate and glucose are completely oxidized to carbon dioxide (oxidation of 1 mol acetate theoretically produces 8 mol electrons, and oxidation of 1 mol glucose theoretically produces 24 mol electrons), recovery of electrons from the substrate oxidation was calculated by comparing the total charge through the circuit during the substrate pulse (Fig. 2) with the theoretical value from substrate oxidation. The overall electron recovery is a function of the substrate concentration both for acetate-fed and glucose-fed cells. Electron recovery for the acetate-fed cell decreased from 76 ± 12 ($n = 3$) to $57 \pm 7\%$ ($n = 3$) when the acetate concentration increased from 0.5 to 5 mM, and electron recovery for the glucose-fed cells decreased from 63 ± 11 ($n = 3$) to $51 \pm 9\%$ ($n = 3$) when the glucose concentration increased from 0.5 to 2 mM. Analysis of data collected from current production by addition of anaerobic substrate-containing buffer showed a similar result.

3.4. Bacterial diversity on two types of anodes

The species of bacteria colonizing on the electrode surface were not identified by 16S rRNA analysis in this study, but obvious differences in bacterial morphology of the two types of anodes was observed by the SEM at the end of current production. Nearly the whole full surface of the anode electrode was covered by bacteria. On the surface of the anode of the glucose-fed MAFC, the biofilm was mainly rod-shaped, 2.0–4.0 μm

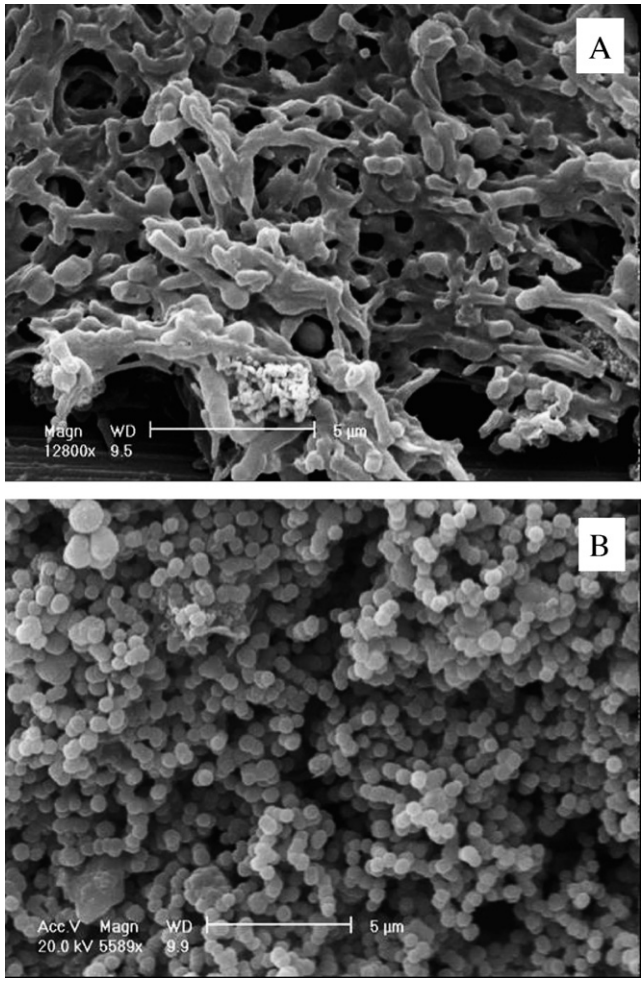

Fig. 4. SEM images of (A) glucose-induced and (B) acetate-induced bacteria morphology on the anode surface.

long and 0.5–0.7 μm wide bacteria cells combined with some organic matrix (Fig. 4A). However, the anode of the acetate-fed MAFC was heavily inhabited by microbial communities of homogeneous coccoid cells (∼0.5 μm diameter) consisting of a thick biofilm on the surface of the electrode (Fig. 4B). In a previous study [22], diversity in the microbial communities associated with anodes was observed with different aquatic sediments. In the present study, the difference in the anodophillic morphology is thought to be induced by the different substrate feeding (i.e., with acetate and glucose as the electron donor, respectively).

In previous studies on Maring and estuarine sediment microbial fuel cells, *Desulfuromonas acetoxidans*, *Desulfobulbaceae* and *Geothrix fermentans*, members of the proteobacterial families Geobacteraceae, are found to be rich on the surface of anode [4,5], but electron recovery of >50% from the oxidation of glucose by these species has not been observed yet, although the marine representative of the Geobacteraceae, *Desulfuromonas acetoxidans*, is observed to harvest >80% electron from oxidation of acetate as electricity [4]. In this study, >50% electron recovery from oxidation of both glucose and acetate was observed by these two types of anodophillic bacteria.

4. Summary

Microbial-anode fuel cells activated by bacteria attached to electrodes were obtained by inoculating fresh sedimentary microorganisms into the anode compartment. An acetate-fed MAFC and a glucose-fed MAFC in this study completely oxidized organic substrates with a quantitative transfer of electrons to the electrodes. On average, the acetate-fed MAFC had a higher current generation and electron recovery than the glucose-fed MAFCs at a fixed resistance (1000 Ω). Moreover, diversity in the morphology of the anodophillic bacteria was also observed, which is thought to be induced by different substrate-feeding. The results presented in this paper show that fresh sedimentary microorganisms can be used to initiate microbial fuel cells with good performance successfully. The findings in this study have demonstrated that direct electron transfer to anode by anodophillic bacteria occurs on the surface of the anode in a MAFC, however, if a natural electron mediator is involved in this electron transfer it is still a key problem. In ongoing studies, we are focusing our interests on this latter problem.

Acknowledgments

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