The Factors Affecting Biofilm Formation in the Mediatorless Microbial Fuel Cell

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The mediatorless microbial fuel cell (MFC) was constructed with *Geobacter metallireducens* and the factors affecting biofilm formation and structure were studied using sodium acetate as substrate. The process of biofilm formation was clearly observed by SEM. Current in MFC was mainly generated by the bacteria attached on the anode surface. Experiments showed that the adhesion of *Geobacter metallireducens* on the anode primarily depended on the chemical bond, rather than the electrostatic force between the bacteria and the anode. Shear force changed the structure of the biofilm, and consequently affected the current generation. The electricity could resume while the open circuit was connected, which demonstrated that the MFC could endure temporary intermittence. In addition, the substrate concentration and the anode surface area were in proportion to electricity.

Key words:
Mediatorless microbial fuel cell, *Geobacter metallireducens*, biofilm, current, anode surface

Introduction

A microbial fuel cell (MFC) is a kind of energy transit equipment, which converts chemical energy directly to electricity. According to electron transfer from bacteria to the anode, MFC can be classified into two types: indirect (mediators) MFC,[1,2] in which electron shuttles or mediators are added into the anode chamber constantly, and direct (mediatorless) MFC,[3–5] in which no mediators are added. Since most of the mediators are expensive and toxic, MFCs employing mediators have not been studied widely.

Recently, great attention has been paid to mediatorless MFC, and its research has been primarily concentrated on two aspects. One is the bacteria which are able to produce electricity in the absence of mediators. The microbes used in mediatorless MFC include axenic cultures, such as *Geobacter metallireducens,*[3] *Geobacter sulfurreducens* and *Rhodoferax ferrireducen*,[4] and mixed cultures like marine sediments[6] and wastewater. The second aspect is the parameters related to the performance of MFC, such as its design, and the materials used in the MFC. For example, MFCs should be constructed to have an internal resistance as low as possible, and to have an optimal external resistance to obtain the highest power output.[7] Attempts have been made to replace or eliminate the Proton Exchange Membrane (PEM) due to its high cost.[8,9] In the cathode chamber, different aeration rates could cause different electricity output,[10,11] and it could increase the maximum power subsequently to replace dissolved oxygen by using ferricyanide.[12] Further, a higher current was observed as a larger anode surface was available for bacteria growth.[4] Among all the factors, the biofilm formed on the electrode is foremost to the electricity generation. It has been shown that the electrogenesis of MFC primarily resulted from the microbes attached on the surface of anode. However, how the biofilm is formed and how it influence the MFC is rarely mentioned in previous studies.

This study discussed the factors affecting the biofilm of a MFC. We first observed the biofilm formation process by SEM; second, whether the chemical bond or the electrostatic force mainly influenced the biofilm formation was discussed; the effects of stirring and breaking the circuit on the biofilm were also investigated.

Materials and methods

Cultures and medium

The pure culture used in the experiment was *G. metallireducens* (DSMZ 7210) which was purchased from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen). Growth medium contained the following components (per li-

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ter): a nutrient medium (0.1 g of KCl, 0.25 g of NH_4Cl, 0.6 g of $NaH_2PO_4 \cdot H_2O$), 6.8 g of sodium acetate, 10.7 g of poorly crystalline Fe(III) oxide,[13,14] 2.5 g of $NaHCO_3$, 10 mL of vitamin mix, and 10 mL of trace mineral mix. The growth medium in electrode-containing chambers was almost the same as that mentioned above, except that the former did not contain 10.7 g of poorly crystalline Fe(III) oxide and was amended with 2.9 g of NaCl to minimize differences in osmolarity between the fumarate medium[15] and the electrode growth medium. Sodium acetate was added separately to the medium as the substrate. The bacteria were transferred three times in a medium containing 40 mmol L^{-1} fumarate as the electron acceptor prior to inoculation into anode chambers (inoculation concentration 10 %).

MFC construction and operation

The MFC was constructed by polymethacrylate plastic, and the electrode compartments, which were separated by a cation exchange membrane (Nafion-117) sealed between silicone rubber gaskets, was about 100 mL capacity measuring 63 × 63 × 30 mm. The graphite electrodes (50 × 50 × 5 mm) had an approximate geometric surface area of 50 cm^2. Copper wires were attached to the electrodes and all exposed metal surfaces were sealed with waterproof rubberized fabric. The cathode chamber was continuously sparged with sterile air, the anode and cathode chambers were stirred with magnetic stir bars. *G. metallireducens* was cultured with acetate as an electron donor and fumarate as an electron acceptor; culture was inoculated in the anode chamber after four growth cycles. Before inoculation, the anode chamber was sparged with a slow stream of N_2–CO_2 (80 : 20) to remove oxygen.

Reactions in MFC:

Anode:

$$C_2H_4O_2 + 2H_2O \xrightarrow{\text{Microbe}} 2CO_2 + 8e^- + 8H^+$$

Cathode:

$$4H^+ + 4e^- + O_2 \longrightarrow 2H_2O$$

Total Reaction:

$$C_2H_4O_2 + 2O_2 \xrightarrow{\text{Microbe}} 2CO_2 + 2H_2O$$

Calculation and analyses

Voltage (V) was measured using a data acquisition system (AD8201H, Ribohua Co., China). The external resistance (R) was fixed, and the current (I) (mA) could be calculated as follows:

$$I = V/R$$

Scanning electron micrograph (SEM) was taken after the enriched electrode samples were sputter-coated with gold. Protein concentration of bacteria attached to the electrode was measured by the Bradford method.

Results and discussion

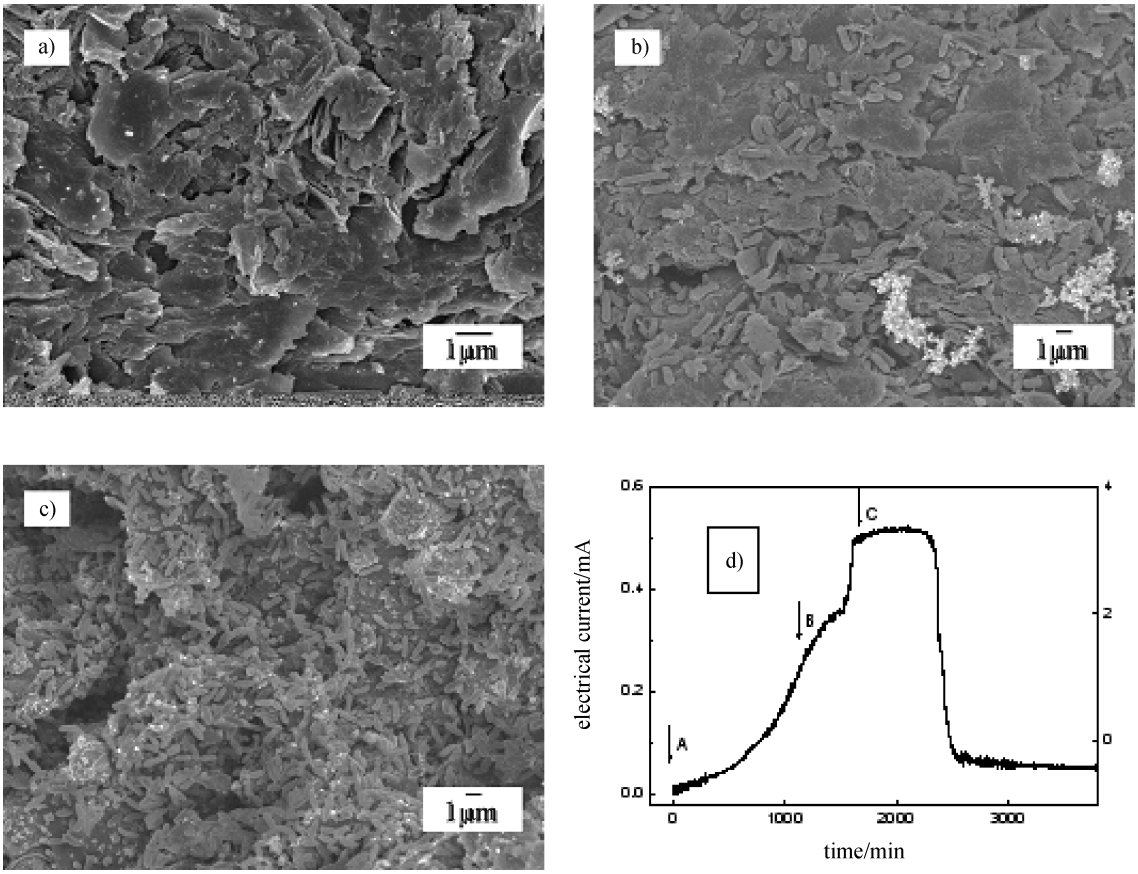
Biofilm formation

Biofilm formation began with transferring *G. metallireducens* inoculum into the anode chamber, and was coupled with current change. Fig. 1 showed the SEM images of anode surface which presented the adsorption in different period of *G. metallireducens* growth. The number of bacteria attached on the anode continuously increased until the current became stable, which implied that the biofilm was maturely formed. The biomass on the electrodes at different growth period was assayed. The attached biomass on the anode ranged from 6.84 ± 0.68 mg m^{-2} to 1368 ± 197 mg m^{-2}.

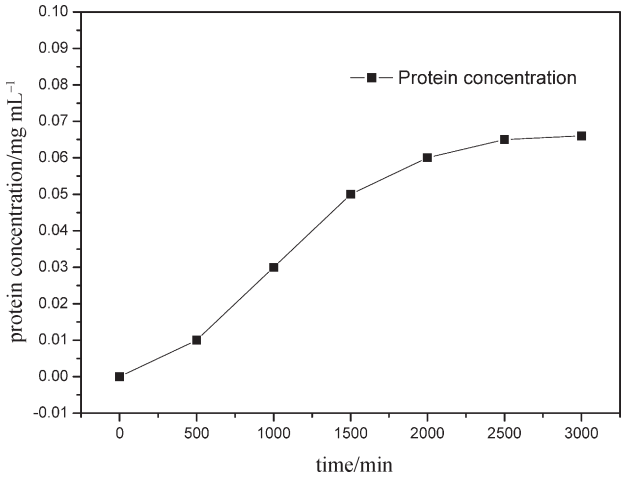
Experiments showed that the electricity production of direct microbial fuel cell mainly depended on the biofilm adsorbed on the anode surface. Therefore, the formation of biofilm was crucial to MFC performance. The current and the substrate consumption were monitored when the biofilm was being formed on the electrode. 3 mmol L^{-1} sodium acetate was injected into the anode, which were inoculated with 10 % cell suspension. The protein on the anode surface was measured and the results were shown in Fig. 2.

Fig. 3 showed that the current increased 4 hours after inoculation, which indicated that the biofilm was beginning to form. Then the current increased at a rate of 0.028 mA h^{-1} until it rose to 0.51 mA, after which it kept steady for more than 10 hours. The time for current stabilization is equal to the time for maturation of the biofilm formed by *G. metallireducens*.

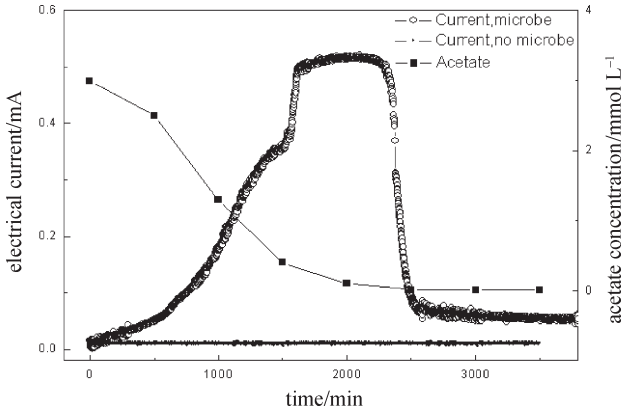
The biofilm formation went through the process as follows: reversible adsorption – irreversible adsorption – multiple colony formation and gradual expansion – mature biofilm – aged and dissociated biofilm.[16,17] At the beginning of formation, the interaction between microbes, as well as microbes and the interface, helped enrich sufficient numbers of *G. metallireducens*, as they attached with each other through the extracellular secretion to form the biofilm. In the meantime more and more metabolites accumulated in the anode chamber, and the density of suspended cells was increased. A month later, the protein concentration in the anode solution could reach to 60 mg L^{-1}, and the majority of the suspension cells came from the aged and dissoci-



F i g . 1 – *SEM images of an electrode surface at different times after inoculation into the anode: a) at the beginning of inoculation; b) 20 h after inoculation; c) the time when voltage became constant; d) current generation during biofilm formation*



F i g . 2 – *Relationship between protein concentration on the anode surface and time (protein concentration in different periods was detected using Bradford)*



F i g . 3 – *Current generation and acetate consumption during biofilm formation on the electrode*

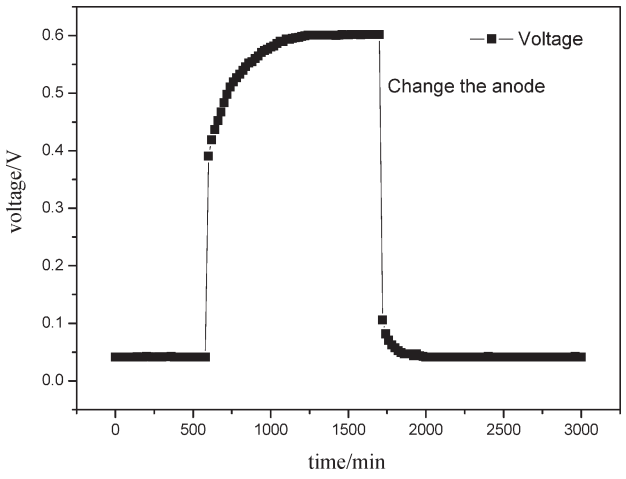
ated from biofilm. At this time the solution in the anode should be changed, and the metabolites, suspended dead bacteria were removed, which speeded up *G. metallireducens* metabolism, and increased the traveling rate of electron and proton in the mediatorless MFC.

The concentration of sodium acetate in the anode was measured as shown in Fig. 3. In the process of biofilm formation, the consumption of sodium acetate was the fastest, but after the biofilm was mature or the current was constant, the consumption rate was slower. This indicated that the microbes consumed a large amount of organic substrate during this process. The sodium acetate was used for *G. metallireducens'* metabolism to secrete

extracellular substance which was favorable for the building of the biofilm, and some of the electrons produced were released out of cell to generate electricity. At the beginning, *G. metallireducens* multiplied remarkably and secreted extracellular substances which helped the bacteria to adsorb on the anode surface. Once the current platform was set up, the current was still high even if the carbon and energy source were depleted. The current might be composed of two parts. One was that the sodium acetate had already been degraded into intermediate, which might be further decomposed and release electron. The other was that *G. metallireducens* resorted to endogenous respiration to generate a current.

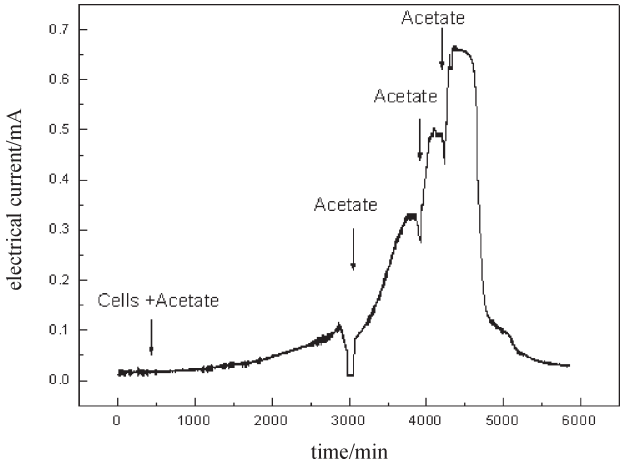
The background current of the MFC without *G. metallireducens* was the same all the time, and it was illustrated that the current totally relied on released electrons in the process of substrate oxidation by bacteria.

Fig. 4 showed that voltage immediately declined from the platform to the background when the anode covered with the biofilm was changed by a new one. It indicated that the biofilm was mainly responsible for the current generation.



F i g . 4 – *Voltage of MFC when the anode was changed*

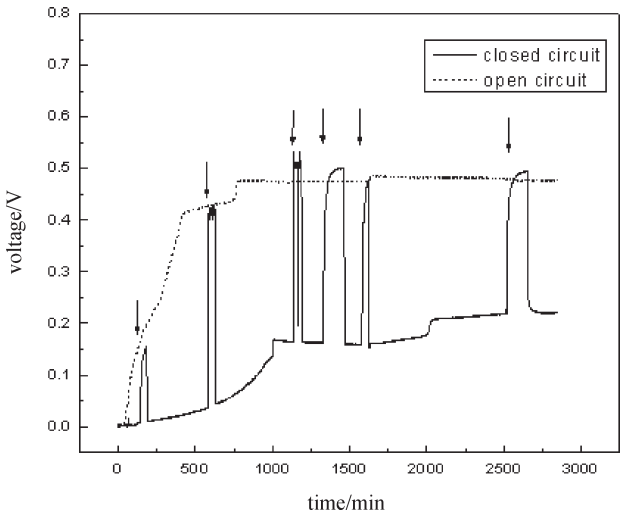
In addition, the biofilm was set up by adding 1 mmol L^{-1} sodium acetate gradually when 10 % inoculum of *G. metallireducens* was inoculated into the anode. The current was shown in Fig. 5. When sodium acetate was about to run out, the current declined; however, the current immediately resumed to the previous value when 1 mmol L^{-1} sodium acetate was added. The biofilm was established step by step, and became mature after repeating the above process several times.



F i g . 5 – *Current production by G. metallireducens when pulses of 1 mmol L^{-1} acetate were given at the indicated times*

The effect of the static force on the biofilm formation

In order to identify whether the static force played a role in biofilm formation, two MFCs were set up under the same conditions except that one was in an open circuit, and the other was in a closed circuit. The voltage of the two MFCs were measured as in Fig. 6.



F i g . 6 – *Time-voltage of the MFC with open and closed circuit (the closed circuit was disconnected as indicated by the arrows)*

The closed-circuit was periodically opened for 10 minutes to make a comparison with the open-circuit, in order to explore whether the potential difference between anode and cathode could affect the electron transferring and the biofilm formation. Fig. 6 showed that when the closed-circuit MFC was opened, its voltage was almost the same as that

of the open-circuit one at that same time (about 0.5 V), which indicated that whether the circuit was connected or not had no impact on the biofilm formation, and the speed of the formation was almost the same. As a result, the potential difference had no influence on the biofilm forming. The static force between bacteria and the anode or between bacteria was not caused by the potential difference but mainly the chemical bond. At first, the interaction between bacteria and the electrode, as well as that between bacteria themselves led to *G. metallireducens* aggregation. Once the amount of the gathering bacteria was enough, the biofilm would be formed through extracellular substances secreted by bacteria.[18,19]

The effect of stirring on the biofilm

When the current became stable, the stirring was stopped both in the anode and cathode chamber, and the current rapidly decreased from 0.89 mA to 0.82 mA (Fig. 7). When stirring was restarted, the current turned to the previous value. The reason was that stirring could accelerate the ion transfer and decrease the internal resistance, which led to the current increase. Shear force (F_d) due to stirring could cause dissociation in the biofilm. *G. metallireducens* growth relied on the penetration of the substrate, and the permeability of the substrate could be considered as a growth force (F_g). The test of stirring rate showed that the stable biofilm structure depended on how to balance the interaction between F_d and F_g, and the two forces could influence the distribution of organic substrate between the assimilation and dissimilation.[20–21] The high ratio of F_d/F_g caused by stirring could induce non-growth model energy metabolism of *G. metallireducens*, and strengthen the dissimilation as well as the current.

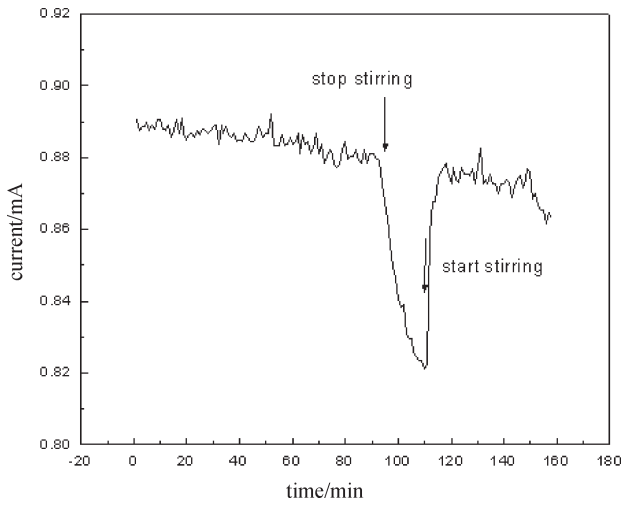


Fig. 7 – *Effect of stirring on the current production of the MFC*

The effect of temporary intermittence on the biofilm

The circuit between the anode and the cathode was turned off for 24 hours (Fig. 8), then it was connected and the current returned to the previous value. This suggested that the biofilm could withstand the unexpected interruption, which meant that the electron transfer could recover immediately as long as the biofilm had been formed.

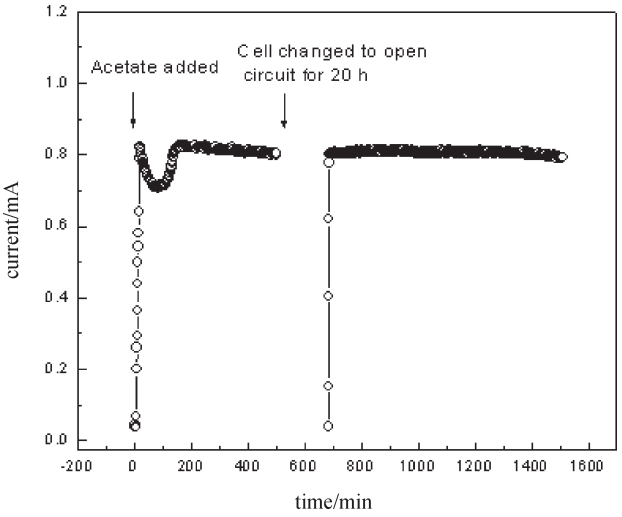


Fig. 8 – *Effect of temporary intermittent on current production*

In this study, the biofilm information and factors influencing the performance of MFC were macroscopically investigated. However, much of biofilm remained unknown, for example, whether *G. metallireducens* attached to the electrode formed one layer or multiple layers, how electron transferred in the biofilm, and how the bacteria interacted with each other. Therefore, more efforts and research are needed to have a thorough understanding of biofilm on electrode surface.

Conclusion

It was proved that current generation was mainly due to *G. metallireducens* attached to the electrode, rather than the suspended bacteria in the solution. The current of the MFC without bacteria maintained the background value all the time. The current could increase to a platform when the biofilm was mature. The way that the substrate was added had no effect on current or biofilm formation.

The concentration of protein on the surface of the anode increased with *G. metallireducens* inoculated in the anode chamber. The biofilm was being formed while the current increased. After the cur-

rent increased to the maximum value, it kept steady for more than 10 hours.

Whether the circuit was connected or not had no influence on the adsorption of bacteria. *G. metallireducens* adhesion to the electrode surface mainly depended on the chemical bond, rather than the potential between the anode and the cathode.

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List of symbols

F_d – shear force

F_g – growth force

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