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USING SHEWANELLA ONEIDENSIS MR1 AS A BIOCATALYST IN A MICROSCALE MICROBIAL FUEL CELL

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ABSTRACT

Microbial fuel cell (MFC) technology is a promising area in the field of renewable energy because of their capability to use the energy contained in wastewater, which has been previously an untapped source of power. Microscale MFCs are desirable for their small footprints, relatively high power density, fast start-up, and environmentally-friendly process. Microbial fuel cells employ microorganisms as the biocatalysts instead of metal catalysts, which are widely applied in conventional fuel cells. MFCs are capable of generating electricity as long as nutrition is provided. Miniature MFCs have faster power generation recovery than macroscale MFCs. Additionally, since power generation density is affected by the surface-to-volume ratio, miniature MFCs can facilitate higher power density. We have designed and fabricated a microscale microbial fuel cell with a volume of 4 µL in a polydimethylsiloxane (PDMS) chamber. The anode and cathode chambers were separated by a proton exchange membrane. Carbon cloth was used for both the anode and the cathode. *Shewanella Oneidensis MR-1* was chosen to be the electrogenic bacteria and was inoculated into the anode chamber. We employed Ferricyanide as the catholyte and introduced it into the cathode chamber with a constant flow rate of approximately 50 µL/hr. We used trypticase soy broth as the bacterial nutrition and added it into the anode chamber approximately every 15 hours once current dropped to base current. Using our miniature MFC, we were able to generate a maximum current of 4.62 µA.

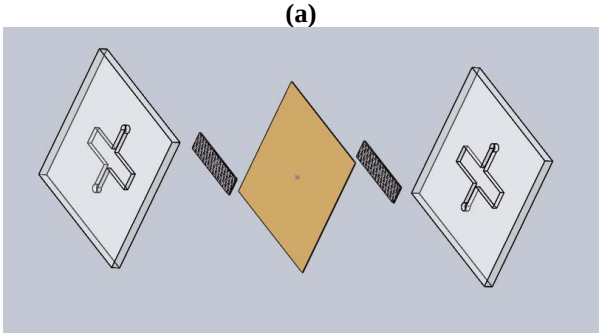
INTRODUCTION

Microbial fuel cell is a developing technology which utilizes the chemical energy stored inside microorganisms to generate electricity (*1-5*). This type of fuel cell is under the category of biofuel cell, which is separated from conventional microfluidic fuel cells because of the different choice of catalyst. Most microbial fuel cells apply pure bacteria (*6-9*) or algae (*10*) as the electrogenic microbes, however, there are numbers of experiments that use multiple culture, and obtain similar results. Microscale microbial fuel cell have a great potential in substituting conventional batteries for portable devices (*11*), such as cell phones (*12*), laptops (*12, 13*), biosensors (*14, 15*) and clinical applications (*16*). Wastewater management is another promising area for microbial fuel cell technology and is an example of where multiple culture fuel cells could be used. A large amount of energy is contained in wastewater; microbial fuel cell technology allows us to exploit the energy in these biomasses and convert it into electricity (*17*). Microbial fuel cells are also desired as an environmental-friendly power source, which operates with less pollution to the environment than fossil fuels (*18, 19*). Most microbial fuel cell designs employ Polydimethylsiloxane (PDMS) microfluidic frames where bacteria is inoculated in the anode and chemical solution flows through the cathode continuously at a certain speed and is separated from the anode by a proton exchange membrane (PEM) (*20*). Bacteria in the anode oxidize the nutrients through metabolism. The protons transfer through the PEM, and the

electrons run through connecting wires to complete the circuit and consequently generate electricity.

Shewanella, *Pseudomonas*, and *Proteobactor* families are the most widely employed bacteria for lab-scale microbial fuel cells (*21*). Multiple cultures are commonly used for wastewater applications (*21-23*). Although most MFCs prefer their fuel to have low-molecular weight, Niessen et al. presented a fuel cell that operated on starch with *Clostridium butyricum* and *Clostridium beijerinckii* as the biocatalysts (*17*).

(a)



(b)

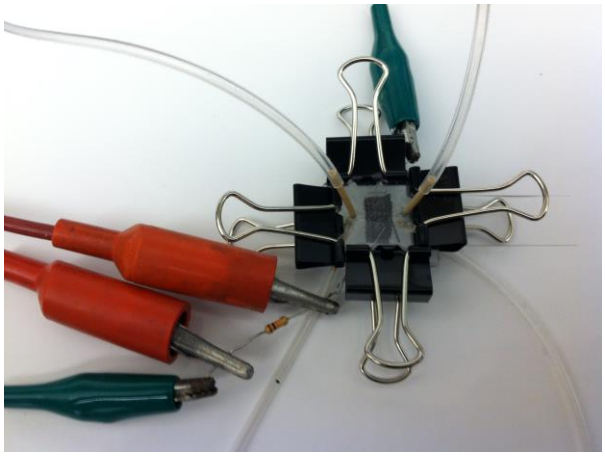


Figure 1: (a) A schematic of the MFC representing carbon cloth, PEM, and chambers. (b) A photograph of the MFC assembly.

Microbial fuel cells are desirable for their mild operating conditions, relatively high power density, and long running period without recharging (*24-26*). This technology has a considerably lower cost than that of enzymatic fuel cells, as the process required to purify enzymes is not needed. The miniaturization of microbial fuel cells is a newly developed field for this technology and the high surface-to-volume ratio leads to higher power density, shorter start time, and faster power generation recovery after refilling (*6*, *16*). The reason behinds all of these advantages is the higher surface-to-volume ratio decreases the distance from the fluid to electron surface, which improves the charge transport efficiency (*16*). This development of microbial fuel cell technology has been inspired by the concepts and technology from chemical and biological fields (*26-28*).

In this experiment, *Shewanella Oneidensis MR-1* has been chosen to be the electrogenic bacteria, and carbon cloth has been employed as the electrode. Detailed description and results will be discussed in later sections. The limitation to widely utilize microbial fuel cell technology is that they can not generate sufficient amount of power density to supply those potential applications as a power source (*29*). We have presented some experiments based on a group of microbial fuel cells in series and in parallel, in order to obtain a higher power density than a single fuel cell.

MATERIALS AND METHODS

2.1. Materials and chemicals

The materials and chemicals used in these experiments include 184 silicone elastomer kit (Dow Corning, Midland, MI), Nafion® membrane (Nafion® 115, Ion Power, New Castle, DE), carbon cloth (Fuel Cell Earth, Stoneham, MA) , titanium wire, 0.25mm, (Alfa Aesar, Ward Hill, MA), polyethylene tubing (Dow Corning, Midland, MI), trypticase soy broth (Sigma Aldrich, St. Louis, MO), and phosphate buffered saline (Sigma Aldrich, St. Louis, MO).

2.2. Bacteria and culturing process

Shewanella Oneidensis MR-1 was chosen to be the bacteria used in this experiment because of its wide utilization in MFC experiments. The original bacteria strain was subcultured aerobically in trypticase soy broth (TSB) under room temperature (~20°C) for 24 hours before being stored in a refrigerator at a temperature of roughly 2°C for future use. In order to keep the MR-1 strain under the best quality, it is recommended that the bacteria should be subcultured every week following the former instructions. The bacteria was soaked into liquid TSB medium and put into a syringe before experiment.

2.3. Fabrication of PDMS chamber

The mold used to fabricate PDMS chamber was made following the method presented by Grimes et al. (*30*). The features of the MFC chamber was printed onto a Shrinky Dinks sheet 3 times by a laser jet printer and put into an oven at 160°C for 8 minutes. A post heat of 7 minutes at the same temperature was applied to the mold in order to create a flatter print surface. The material used to make the PDMS chamber was the mixture of Sylgard 184 silicone elastomer and curing agent (Dow Corning, Midland, MI) with a mass ratio of 10:1. The mixture was poured onto the mold, and then allowed bubbles to come out for

approximately 5 minutes before heating. The PDMS chamber was then cured at 110°C for 15 minutes. The final product had an area of 10×4 mm and a height of 100 μm. Holes were punched through the PDMS chamber at appropriate locations for connecting tubes.

2.4. Assembly and operation of MFC device

The MFC device was assembled by putting a Nafion® 115 proton exchange membrane (PEM) in the middle of two carbon cloth electrodes (Fuel Cell Earth, Stoneham, MA) in each side of the chambers. The two pieces of carbon cloth were cut to 10×4 mm, which is the same as the chamber area. Two carbon cloth electrodes were connected to two titanium wires with a diameter of 0.25 mm (Alfa Aesar, Ward Hill, MA) separately and connected in series to a 10kΩ resistor. Binder clips were used to hold the assembly together.

The assembled device was autoclaved at 120 °C for 15 minutes before the experiment in order to keep the inside of the fuel cell sterile. *Shewanella Oneidensis MR-1* was injected into the anode chamber by a syringe. The TSB medium was used as the anolyte and injected into the chamber when the read of electricity dropped to the base line. Ferricyanide (50mM K3Fe(CN)6 in a 100 mM pH 7.4 phosphate buffered saline) was used as the catholyte and pumped into the cathode chamber continuously with a rate of 50 μ L using a syringe pump.

2.5. Measurements and calculations

Voltage across the resistor was recorded continuously using Labview. Current was calculated based on Ohm's Law I = V/R, and plotted as a function of time.

RESULTS AND DISCUSSION

3.1. Current generations

Liquid culture of *Shewanella Oneidensis MR-1* had been put inside an incubator of 37°C for 10 hours before the inoculation in order to guarantee the bacteria was fully grown, and was then inoculated into the anode fuel cell chamber through a syringe. Two syringes were connected to the fuel cell under a UV hood to avoid contamination of other microorganisms. Voltage across the 10 kΩ external resistor was taken as a function of time. Labview was set to record data every 10 minutes. Current output before the inoculation of bacteria was observed to be 0.01424 μA, which was the baseline current for this microbial fuel cell. The current went up immediately after inoculation, and reached a value of 0.7308μA. Although the current output dropped at some time points, the overall trend increased for 13 hours after inoculation (Figure 2). The local maximum of this cycle was obtained to be 1.8928 μA, and was observed 360 minutes (6 hours) after inoculation. The current then dropped to 0.2623 μA 19 hours after the local maximum value was observed. After the local minimum value was achieved, current went up to 0.8429 μA, then started decreasing gradually, and finally stabilized around 0.6 μA for the rest of this cycle. For the cycle after inoculation, the total time length was about 50 hours, the current had been above 1μA for approximately 10 hours and above 0.5 μA for 33 hours. The current had been higher than 0.5 μA for 66% of the entire cycle length. The first refill was conducted fifty hours after inoculation, and the current output increased gradually to 1.2483 μA - which was also the highest current value in this cycle - and was obtained 30 minutes after the refill of TSB medium. The current once again dropped and reached its minimum value 9.5 hours after refill. Another local maximum was achieved roughly 13 hours after a second refill at a value of 0.4471 μA. The second refill of nutrition, which was also the last refill of this experiment, was made 24 hours after the first refill. The current spiked upwards, and achieved the highest value of 4.62 μA for this experiment. The current dropped faster in this cycle than it in the two former cycles, and stayed below 0.1 μA 7.5 hours after refill.

The results shows that this microbial fuel cell design is a quite promising technology for power generation, as the current output lasted for more than three days with only two refills conducted during this time period. The first cycle had the best output among the three of them, since the current stayed above 0.5 μA for 66% of the entire cycle length. The bacteria seemed to be less vibrant after the first cycle, and the amount of current generation went down as time passed by. Additionally, the power output was very stable and relatively high during the first cycle. Although the peak value was achieved in the last cycle, it had poor stability and dropped quickly afterwards. Multiple local maximums were observed in each cycle, which may be caused by the complicated nutrition contained in TSB medium. A medium of simpler nutrition contents may have produced only one spike during a single cycle, but the cycle lifetime might have also been smaller because less nutrition would be contained within the medium.

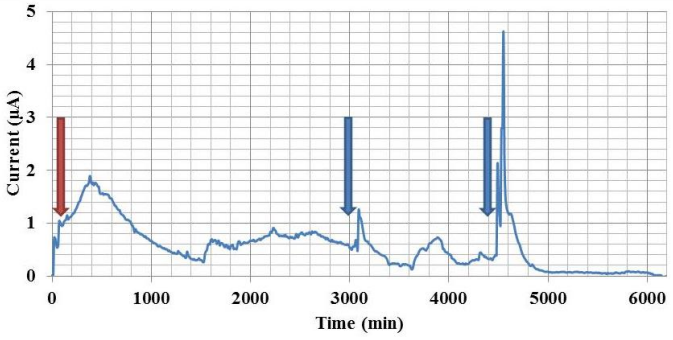


Figure 2: This plot shows the current generations as a function of time, with red arrows indicating inoculation and blue arrows indicating refill of TSB medium.

3.2. Polarization curve and power output

A polarization curve was generated for this microbial fuel cell design. The open circuit voltage was measured to be 0.12 V. The fuel cell was then connected to an external resistance of 15 kΩ, which was decreased to 1 kΩ gradually, and the voltage across the external resistance was measured during this experiment. The polarization curve was presented in Figure 3, and the power output as a function of volumetric current was shown in Figure 4.

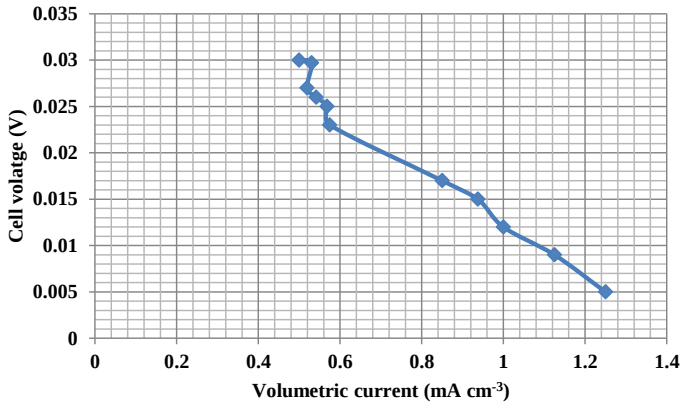


Figure 3: Polarization curve: this graph indicates the change of cell voltage with decreasing external resistance.

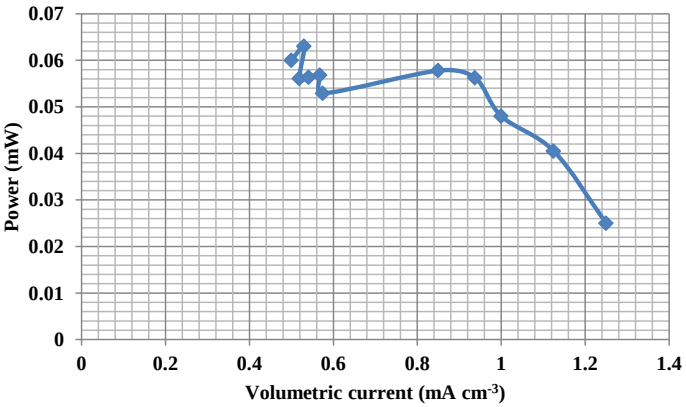


Figure 4: This graph indicates the change of power with decreasing external resistance, as a function of volumetric current.

3.3 Comparing with other MFCs

Using this microfluidic MFC design, we were able to create a maximum current of 0.005 mA cm^{-2} and a maximum volumetric power density of 15.7 W m^{-3}. However, MFC designs with larger volume could produce a higher power density compared to this design. Niessen et al., 2004(*17*) reported a MFC with an anode volume of 100000 µL, which was operated on starch and produced a maximum current density of 1.3 mA cm^{-2}. A 350000 µL single chamber MFC introduced by Ishii et al., 2012(*31*) had a maximum current density of 1.12 mA cm^{-2}. One reason could be that in batch-fed MFC operations, the microorganisms have access to less amount of nutrition than MFCs of larger volumes with continuous operation. Improvement of power density is needed for small volume MFCs to be applied into more areas.

CONCLUSIONS

A microfluidic microbial fuel cell with a chamber volume of 4 µL produced a peak current of 4.62 µA and a peak current density of 1155.75 A m^{-3} during 100 hours of operation. This MFC design was proved to be capable of generating power for a relatively long time with only two refills. Since we were able to employ a less expensive fabrication process than other fuel cells, this MFC has potential to be mass produced and supplied to the market. The miniaturization of MFCs improves the power density and makes this technology more suitable for smaller devices.

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