Electrochemical Analysis of *Shewanella sp.* Behavior In Microbial Fuel Cells

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Abstract
A cube two-chamber microbial fuel cell cultivated with *Shewanella sp.* was investigated and analyzed in terms of voltage and current generation behavior, maximum attainable power, electrochemical activity, and operational considerations from the beginning. The results indicated that with a certain architecture of the MFC utilized in this study, 80% of the maximum power is achievable when the MFC reached a stable pattern of substrate consumption. 84.6 mW/m^2 of power density was obtained at the resistance of 229 Ω. The configuration of the MFC allowed one of the lowest internal resistances to the time as 141 Ω.
Keywords: Microbial fuel cell, *Shewanella sp.*, MFC internal resistance, cyclic voltammetry

1. Introduction
At present, sustainable energy sources free of environmental pollution is the main concern of human being and is the focal point of sustainable development and circulation economy. The microbial fuel cell is one of the new technologies that directly address this concern [1]. Microbial fuel cells (MFCs) represent a particular case of fuel cells in which the direct conversion of organic matter into electricity is due to the action of microorganisms such as bacteria [2,3]. Producing electricity from carbohydrates and other organic compounds with MFCs has a history of 100 years old [4]. An MFC consists of three major components: an anaerobic anode chamber, an aerobic cathode chamber and a cation exchange membrane in between. Anode chamber is the microbial powerhouse in which the growth occurs. It provides all the necessary condition for the growth of the suitable microorganism. Electrons and protons are produced in the anode through metabolic reactions. Cathode chamber is the compartment in which electrons and protons are consumed. The cathode electrode is platinum

coated graphite which catalyses the reaction between oxygen molecules, and electrons and protons to produce water. Alternatively, compounds such as ferricyanide can be used, which acts as the electron acceptor. Cation exchange membrane acts as a partitioning between the anode and cathode chambers [5]. A schematic of the MFC is presented in Figure 1.

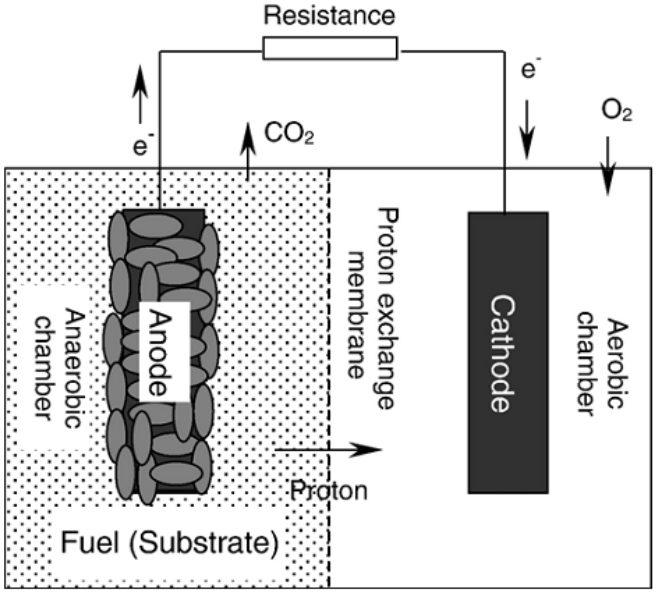

Fig . 1. Schematic diagram of a typical two-chamber microbial fuel cell. [6]

The appeal of this novel technology comes from the wide range of potential applications, including the possibility of gaining energy from wastewaters. Hydrogen production, sulphide removal, and biosensors for organic content are other applications of MFCs [2]. This technology is advantageous over the traditional technologies from many aspects, such as the divers sources of fuel, high energy efficiency, mild operating conditions, strong

biocompatibility, no pollution, and so on [1]. The MFCs have drawn considerable attention from microbiological, environmental, and energy communities due to their capabilities in producing electrical current via the anaerobic oxidation of organic compounds using specific bacteria (e.g., *Shewanella putrefaciens*, *Geobacter sulfurreducens*, etc.) as catalysts in the absence of a mediator [7].

In this study, electrochemical behavior of a pure culture MFC cultivated by *Shewanella sp.* was investigated. The maximum power density of 84.6 mW/m^2 was achieved at 229 Ω resistance that accounts for a remarkable achievement compared to published works with the same features [8,9].

2. Experimental

2.1 MFC architecture

MFC studied in this work was a cubic two-chamber reactor made of acrylic sheet. The chambers were separated from each other using a cation exchange membrane (CMI7000S, Membranes International Inc.) with the surface area of 32 cm^2. The total volume of each part was approximately 200 ml. Electrodes (anode and cathode) were rectangular carbon felts (PANEX35, Zoltek) with the same surface area equal to 54 cm^2 and electrode spacing of 2.5

cm. Cathode surface was coated with platinum as catalyst (10% Pt, Volcun XC-72, Premetek) for reaction in cathode chamber. Aeration was carried out using a small air pump, and a handmade sparger was set up in the cathode compartment for uniform distribution of air in catholyte. A flow of nitrogen gas (>99% purity) was bubbled into the anolyte and exhausted from head space to keep the anode chamber anaerobic. Electrodes were connected together using stainless steel wires, tightly attached to the electrodes, and a 10 KΩ potentiometer to set the resistance. An image of the MFC is shown in Figure 2.

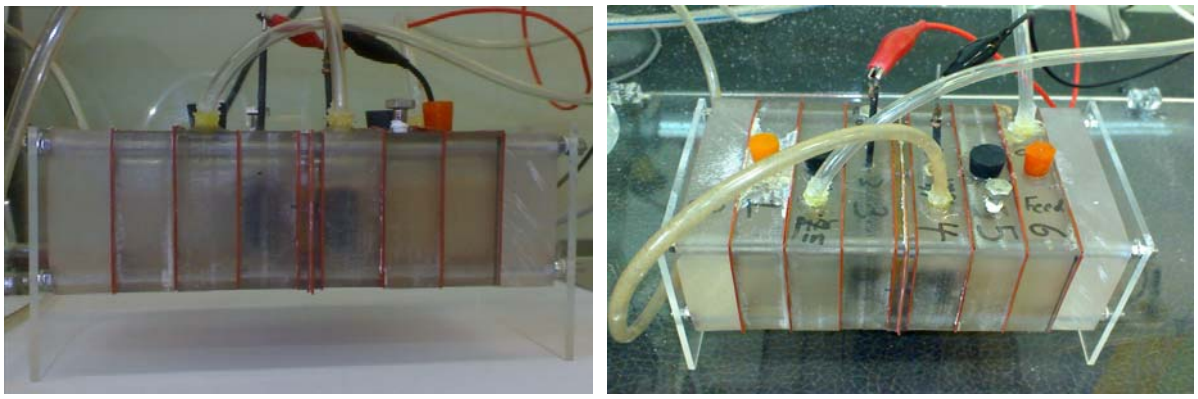

Fig.2. Overview of the MFC used in this study (left: front view, right: top view).

2.2 Medium and inoculum

Shewanella sp. was purchased from Persian Type Culture Collection (PTCC 1711). Before inoculation, cells were grown in Tryptone Soya Broth (TSB) growth medium for about 70 hours at $30\,°C$ (shaking at 140 rpm, aerobically). The MFC medium consisted of the following components [10]: PIPES buffer, 15.1 g/l; sodium hydroxide, 3 g/l; ammonium chloride, 1.5 g/l; potassium chloride, 0.1 g/l; potassium phosphate, 0.6 g/l; sodium chloride, 5.8 g/l; L-glutamic acid, 1.75 mg/l; L-arginine, 2 mg/l; minerals solution, 10 ml. PH of the solution was adjusted to 7 with HCL or NaOH, as required, and autoclaved. Lactic acid 90% was used as carbon source with 18 mM concentration in final solution. Anode and cathode chambers were filled with 150 ml of the solution each. For inoculation of the MFC, 22.5 ml of TSB solution (15% v/v) was centrifuged (4000 rpm, 10 min) and cells were separated and added to anode chamber.

2.3 Data acquisition and start up

For data acquisition, an analog to digital converter card (ADC10016, TNM electronics) was used. LabView software (version 10.0, National Instruments) was installed on a local computer and was connected to the A/D card as hardware. Voltage and current of the system were recorded on-line and power, external resistance, current density, and power density were calculated based upon. All experiments were carried out at $30 \pm 1°C$ inside an incubator.

3. Results and Discussion

3.1 Voltage generation

Once the MFC was inoculated current generation was observed. However, because of the lack of biofilm on the anode surface the current and voltage data was quite unstable. For the first six days, the MFC was periodically fed with inoculant and fresh medium until the cell voltage adopted a certain pattern. Figure 3 shows the cell voltage trend on and after day 6. As soon as substrate was added to the anode chamber (no inoculant and fresh medium after day 6), cell voltage sharply increased and then decreased gradually. Substrate depleted in approximately 2.5 days demonstrating a smoother voltage drop after six days than initial days of operation. As seen in Figure 3, in the next cycle of substrate (lactic acid) addition on day 8, the voltage did not drop rapidly indicating the stability of current and voltage. Current and voltage stability was a good sign of forming biofilm formation on the anode surface. Mature biofilms tend to a sustainable current as long as the substrate is available.

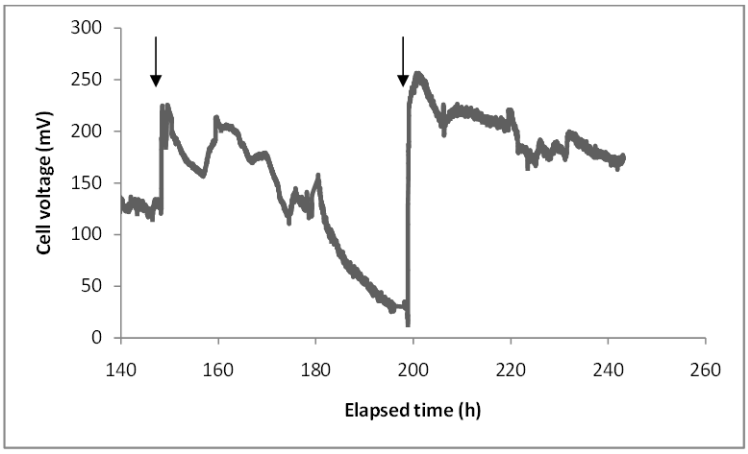

Fig. 3. Pattern of cell voltage from day 6 of cell operation. Arrows indicate substrate (lactate) addition periods on day 6 and day 8, respectively.

3.2 Cell Characterization

In electrical systems, current is a function of voltage. A polarization curve is used to characterize such relationship. This curve is also used to determine the internal resistance of the reactor design and consequently the maximum achievable power of the cell using open circuit voltage (OCV). For this purpose, the OCV of the cell was measured 586 mV. Beginning from 38 KΩ resistance to nearly 100 Ω, cell voltages and currents were recorded online. Plotting cell voltage against the current, polarization curve was produced (Fig. 4). The slope of linear portion of polarization curve represents the internal resistance of the MFC. In this figure the slope is approximately 141 Ω that accounts for a rather low internal resistance. This is due to the suitable electrode spacing. From polarization curve, power density curve was obtained (Figure 5). This curve illustrates power density (based on anode surface area) against current or current density. Examination of this curve indicates that the maximum power density 84.6 mW/m^2 occurs in 229 Ω external resistance. Thus, the maximum attainable power of the MFC can be calculated as follows:

$$P_{\max} = \frac{OCV^2 R_{ext}}{(R_{\text{int}} + R_{ext})^2} = \frac{(0.586V)^2 \times 229\Omega}{(141\Omega + 229\Omega)^2} \times \frac{10000}{54cm^2} \times 1000 = 106.4mW/m^2$$

knowing the maximum power density of 84.6 mW/m^2 obtained at 229 Ω and maximum attainable power density, the MFC is working at 80% of its nominal power.

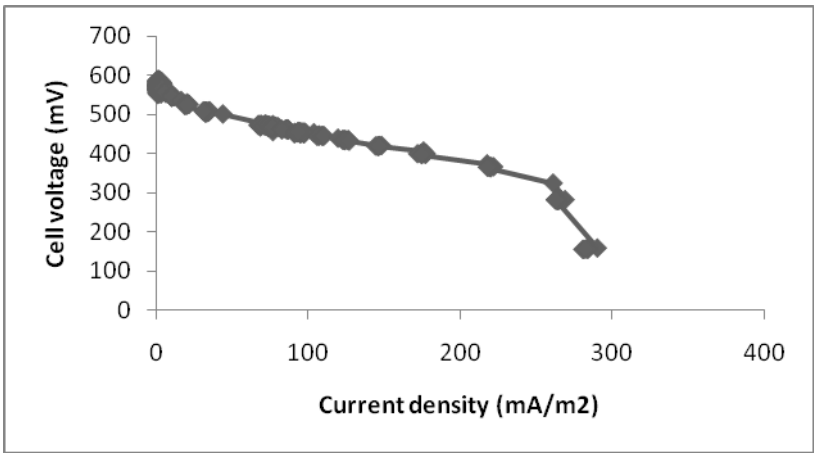

Fig. 4. Polarization curve

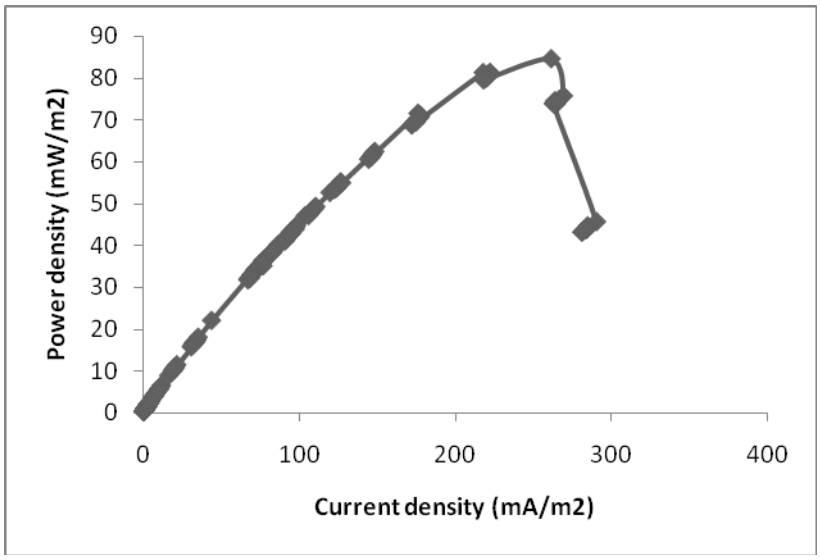

Fig. 5. Power density curve

3.3 Voltammetric analysis

Cyclic voltammetry was performed to investigate the electrochemical activity of the studied cell. This test is generally carried out on a range within which redox properties of different components of the medium can be detected. Every component with reversible oxidation/reduction property has a peak on both upper and lower curves. The test was conducted within the range of -1200 mV to 300 mV with a scan rate 1 mV/s using potentiostat (ACM instruments). Using anode as working electrode, counter electrode was a platinum wire, and Ag/AgCl electrode was used as reference. Before starting the test, MFC was operated as OCV for a few hours until a stable current was observed. The voltammogram (Figure 6) has two peaks at -760 mV and -60 mV, respectively. The peaks on both curves indicate that there are components in the anode compartment that regularly oxidized and reduced. This redox state is due to the presence of electrochemical active agents secreted by

Shewanella sp. The voltammogram of anode compartment before inoculation contains no peak (data not shown).

When the peaks on oxidation and reduction curves were connected together the line intersect abscissa (zero current) at -700 mV indicating the potential of a quasi-reversible oxidation/reduction reaction. Another reversible redox potential at -60 mV was observed that proved the presence of a component with the identical oxidation/reduction potential. Further investigation is required to detect the oxidized or reduced components. Overall, the voltammogram demonstrated that the MFC was electrochemically active and the flow of electron from bacterial cells to the anode electrode as terminal electron acceptor was running.

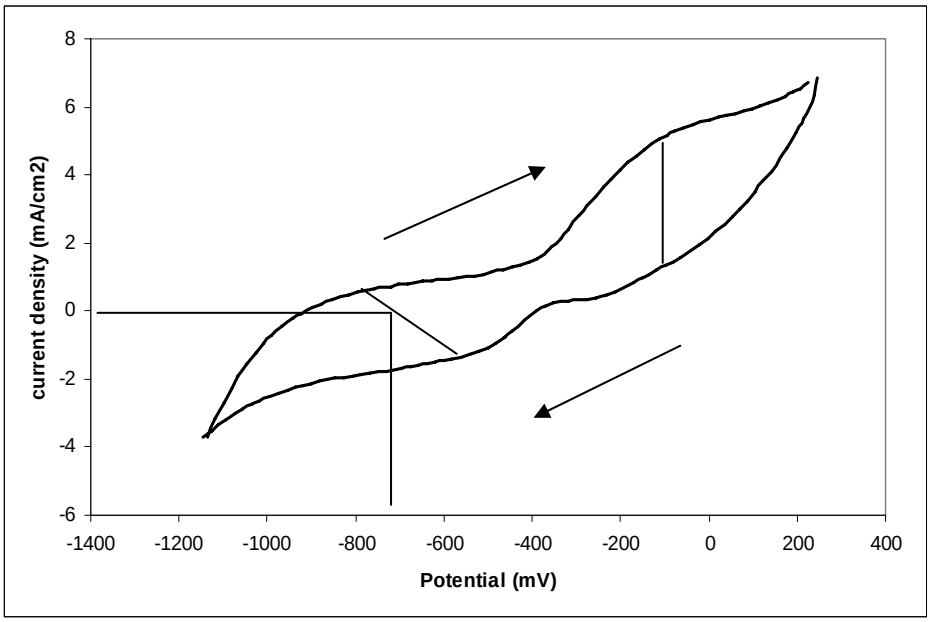

Fig. 6. Voltammogram of MFC. Arrows indicate the direction of the sweep. Upper curve is oxidation pattern and lower curve is reduction pattern.

4. Conclusions

The MFC studied in this research cultivated with *Shewanella* sp. grown on lactate as the sole carbon source generated the power density of 84.6 mW/m^2 at resistance of 229 Ω. This accounts for approximately 80% of the maximum power of the MFC. The MFC reached a stable situation after 5 days of operation; however, reaching a constant voltage and current requires more time and care. Voltammetric analysis revealed that the MFC was electrochemically active and the electron transfer process was perceived from the analysis.

Acknowledgements

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