Microbial Fuel Cells Using Purple Photosynthetic Bacteria with Dry-Surface Biofilms

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We fabricated a microbial fuel cell (MFC) which was composed of three parts: a piece of towel paper for membrane, an anode electrode with photosynthetic bacteria biofilm and a cathode electrode coated potassium ferricyanide. The MFC could generate electricity with 20 µl water adding to the dry biofilm anode for activation. We measured repeatedly electricity generation every week using the MFC. This paper studies three points: (1) the dry-surface biofilms of Purple photosynthetic bacteria can generate electricity when activated by water; (2) the bacteria can survive in the condition of dry-surface biofilms for several weeks; (3) carbon nanotube (CNT) improves the performance of the electrodes. As a result, the MFC generated the maximum power density and current density of 2.90 µW/cm² and 24.1 µA/cm², respectively.

Keywords: purple photosynthetic bacteria, dry-surface biofilms, water-activated, carbon nanotube, potassium ferricyanide.

(Received: 24 July 2018, Revised: 25 December 2018)

1. Introduction

Nowadays, there are many kinds of battery, for example: dry-cell batteries, photovoltaic batteries, biobatteries and so on. Biobatteries have been recently paying attention because they are environment-friendly [1]. MFC is a type of biobatteries, which use living microorganisms to produce electricity from biofuels [2]–[4]. An MFC typically consists of anodic and cathodic compartments separated by a proton exchange membrane. The power output of MFCs depends on the distribution of bacterial cells in the anode electrode. To obtain stable output, researchers usually use the anodes with pre-loaded bacterial biofilms.

Recently, photosynthetic bacteria have been used in MFCs, which enable power self-sufficiency [5], [6]. In this type of MFC, the light and dark reactions can operate independently by using the co-culture of photosynthetic bacteria and heterotrophic bacteria [7]. These bacteria are also used for producing hydrogen gas from acetate (typically, purple non-sulfur bacteria) [8]. These purple non-sulfur bacteria are isolated from soil and water and they can grow as aerobes if oxygen is present to generate energy by respiration or as photoautotrophic organisms in the presence of light with the production of hydrogen by cyclic photophosphorylation.

Photosynthetic bacteria-based MFCs produce bioelectricity based on the exploitation of biocatalytic reactions of the photosynthetic microorganisms [2], [9]–[11]. During photosynthesis, the microorganisms capture solar energy to convert carbon dioxide and water into oxygen and carbohydrates, which will subsequently be used for their respiratory reaction, re-generating carbon dioxide and water. During those reactions, electrons are released through extracellular electron transfer pathways and flow to the cathode through the external electrical circuit. Simultaneously, protons are also released and diffused from the anodic compartment to the cathodic compartment through the proton exchange membrane. At the cathodic compartment, they re-combine with electrons and oxygen to re-form H₂O. Ideally, through the aforementioned processes, photosynthetic MFCs can continuously generate electricity from solar energy without the addition of organic matter. Requiring only sunlight, water, and carbon dioxide to operate, photosynthetic MFCs offer advantages over potentially competing sustainable power sources such as heterotrophic MFCs. This system resembles the Earth’s natural ecosystem, where living organisms work in conjunction with the nonliving components to offer self-sustainable and self-maintainable features [5].

Small, light-weight, and portable power source devices have attracted widespread attention in recent years [4], [12]. In this work, we created a portable MFC that established a new capability in photosynthetic bacteria, which is the dry-surface biofilms. To the best of our knowledge, this is the first research that has made efforts to generate electricity from the dry-surface biofilm of purple photosynthetic bacteria.

2. Method

2.1. Experimental setup

The experimental setup is shown in Fig. 1. We measured the voltages between the anode and cathode with a data acquisition system (National Instrument, USB-6211), and recorded every 30 seconds interval via a customized LabView interface.
Cathode electrodes (10 mm × 10 mm) were prepared by the hydrophilic carbon sheets, potassium ferricyanide (Wako, Japan), purified water, and CNT as follows: 2 ml of 0.75 M potassium ferricyanide and 20 ml CNT solution were mixed at 1500 rpm for 24 h by a magnetic stirrer. After that, the hydrophilic carbon sheets were dipped into this compound liquid for 1 min and then dried at 50 °C for 20 min. A paper-based proton exchange membrane (PEM) was made by a piece of towel paper (15 mm × 15 mm × 0.1 mm) (Monotaro, Japan), which was made hydrophobic by coating hydrophobic solution followed by drying process.

2.2. MFC design and materials

The construction of the fabricated MFC is shown in Fig. 2 (a). The electrodes (10 mm × 10 mm) were prepared by hydrophilic carbon sheets (0.2 mm thickness). They were dipped into CNT solution (N7006L, KJ specialty Paper, Japan) for 1 min, and dried at 50 °C for 20 min. After drying, the electrodes were hydrophobic due to CNT coating. To improve hydrophilicity of the electrodes, we conducted ozone treatment for 10 h by an ozone killer (Filgen, UV253E). In contrast to conventional methods of cleaning, such as the use of solvents or aggressive chemicals, ozone cleaning leaves no residue [13], [14].

An external resistor connected between the anode and cathode. The current through this resistor is calculated using Ohm’s law.

**Fig. 1** Measurement setup.

**Fig. 2** Diagram of the constructed MFC (from the top: cathode-side silicone rubber, cathode, paper-based PEM, anode, and anode-side silicone rubber) (a). Photo image of the fabricated MFC (b).

**Fig. 3** SEM images of the surfaces of biofilm anodes made of carbon sheet coated CNT with ozone treatment (a), carbon sheet coated CNT without ozone treatment (b), and carbon sheet only (c).
The battery case was made of two pieces of silicone rubber (15 mm × 15 mm × 2 mm). On the anode side the silicone rubber case was drilled a hole (5 mm × 5 mm) for injecting water to the anode. A photo image of the assembled MFC is shown in Fig. 2 (b). Two paper clips were used to clamp the MFC.

2.3. Biofilm formation

Purple photosynthetic bacteria were inoculated in a special culture solution (Culture 1), which contained purple photosynthetic bacteria 1 ml, PSB medium 0.1 ml and purified water 8.9 ml. This incubation was conducted at 30 °C under lamp light illumination for 2 weeks. To make biofilms, the electrodes were put into the inoculated solution for 4 days. After that, they were dried at room temperature for 2 days. The dried biofilm electrodes were stored in zip lock plastic bags. The surface morphology images of the anode electrodes with the attached bacterial biofilms were observed by a scanning electron microscopy (SEM).

3. Results

3.1. SEM images

We observed the surface of the photosynthetic bacteria biofilm formed on the anode electrode, as shown in Fig. 3. The thicker biofilm limits the oxygen diffusion and MFC performance, which suggests that the power generation as well as biomass production is favored up to a certain thickness of photosynthetic biofilm on the anode. The charge-balancing cation transport from the anode to the cathode compartment may influence the increase of power generation [11]. The size of bacterial cells is 2~3 μm. In addition, the number of bacterial cells was different from each electrode type. From Fig. 3, we can check the concentration of bacterial cells on the surface of these anodes. It seems to have more cell concentration on the surface of the CNT-coated electrodes (Fig. 3 (a) and (b)) than the carbon sheet only electrode (Fig. 3 (c)).

3.2. MFC performance

In this paper, we focused on photosynthetic bacteria applied to paper-based MFCs. We expected that the dry-surface biofilm could be activated by an adequate amount of water and generating electricity even after storing for several weeks. We also verified the influence of CNT and ozone treatment on the output of the MFC. CNT improves electrode’s surface area and ozone treatment increases hydrophilicity of the anode electrode.

Fig. 4 displays the comparison of the performance of the anodes made of carbon sheet, carbon sheet coated CNT without and with ozone treatment. The MFCs were discharged by a 5 kΩ resistor and the discharging voltage was recorded after adding a drop of water to the dried biofilm anodes. Obviously, the anodes made of carbon sheet coated CNT generated much higher output than the anode made of carbon sheet.

In addition, the effectiveness of ozone treatment was clearly observed. The anode with ozone treatment shows better response time and higher discharging voltage compared with the case without ozone treatment. This is attributed to the better hydrophilicity of the anode with ozone treatment. Therefore, more bacterial cells can be attached not only the outside but also the inside of the anode. Also, the better the hydrophilicity of the anode, the shorter time it takes to absorb added water to activate the MFC, as a result, the better response time can be obtained. Based on the experimental results shown in Fig. 4, we decided to use the carbon sheet coated CNT with ozone treatment as the anode.

Using the carbon sheet coated CNT with ozone treatment for the anode, we investigated the effect of storage time on the dry biofilm anodes. We conducted the experiment with the MFCs using the biofilm anodes stored for zero week (after drying), one week, two weeks, and three weeks. Output results are shown in Fig. 5. It shows that the output decreased after three-week storage. This attributed to the effect of the long-time storage on the deterioration of the dry-surface biofilms.

Fig. 4 Comparison of the performance of the MFCs with different anode electrode materials: carbon sheet (CS), carbon sheet coated CNT without and with ozone treatment.

Fig. 5 The voltage transition of the MFCs using stored biofilm anodes at different storage time from 0 to 3 weeks.
From this data, we are certain that long-term stored dry-surface biofilms of photosynthetic bacteria can be used for the paper-based MFC.

3.3. Power density measurement

Fig. 6 shows the diagram of the equivalent circuit of the measurement setup. The voltage value \( V \), the current value on the close circuit \( I \), internal resistance value of the MFC \( r \), and external resistance value \( R \) are indicated in Fig. 6. Calculation of these elements is as follows.

\[
V = (R + r)I. \tag{1}
\]

Then,

\[
I = \frac{V}{R+r}. \tag{2}
\]

Voltage drops on the external resistance \( V_R \) is

\[
V_R = \frac{R}{R+r}V. \tag{3}
\]

The consumed power on the external resistance \( P_R \) is

\[
P_R = I \cdot V_R = \left(\frac{V}{R+r}\right) \cdot \frac{R}{R+r}V = \frac{R}{(R+r)^2}V^2. \tag{4}
\]

\[
\frac{dP_R}{dR} = \frac{r-R}{(R+r)^3}V^2. \tag{5}
\]

From Eq. (5), the power density peak occurs when the external resistance value equals to the internal resistance value.

We measured the power density of the MFC by using various external resistors of 1 kΩ, 5 kΩ, 10 kΩ, 50 kΩ. Fig. 7 (a) displays the voltage transition discharged by these external resistors. The maximum output voltage and the power density are shown in Fig. 7 (b).

The power density was calculated based on the maximum output voltage and used external resistors.

![Diagram of the equivalent circuit of the MFC measurement setup.](image-url)

The maximum power density was obtained at 5 kΩ. Therefore, we could estimate that the internal resistance value of this MFC was also 5 kΩ. The maximum power density generated by the MFC was 2.90 µW/cm².

For comparison purpose, we also measured the power density in the case of using three different anode materials of carbon sheet, carbon sheet coated CNT without and with ozone treatment.

Fig. 8 shows the results of this experiment. The power density generated by the CNT-based anodes was an order of magnitude higher than that of the carbon sheet-based anode. Furthermore, comparing between the CNT-based anodes with and without ozone treatment, the maximum power density of the MFC using the CNT-based anode with ozone treatment was 2.90 µW/cm², while that of the MFC using the CNT-based anode without ozone treatment was only 0.90 µW/cm². It clearly shows the effectiveness of ozone treatment process on the performance of the MFC.
Three types of anodic material (carbon sheet, carbon sheet coated CNT without and with ozone treatment) have been tested to improve the performance of the MFC. The maximum power density of 2.90 µW/cm² and current density of 24.1 µA/cm² were obtained with the MFC using the anode made of carbon sheet coated CNT with ozone treatment.

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