Improving Performance of Direct Photosynthetic/Metabolic Bio-Fuel Cell (DPBFC) Using Gene Manipulated Bacteria

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Abstract

In our laboratory, we have developed mediator-less direct photosynthetic/metabolic bio fuel cell (DPBFC) in which microparticles of polyaniline were adopted as electrodes to get electrons from bacteria. In this paper, we selected purple photosynthetic bacteria (PPB) as new fuel sources which is activated by organic compounds and emits hydrogen in photosynthetic and metabolic processes. To improve the electron generation efficiency of PPB, gene manipulation is performed so as to suppress hydrogen emission. We assumed that if hydrogen emission is suppressed, more electrons will be stored inside PPB. As a result, the power density of gene manipulated bacteria became higher than the normal one.

Keywords: Photosynthetic, Metabolic, Micro Bio Fuel Cell, Genetic manipulation, Bacteria

1 - INTRODUCTION

The fuel cell is expected to be a new energy technology to solve energy and environmental problems. Furthermore, miniature and high-output power fuel cell is required for sophisticated portable equipment in the future. To realize this, direct methanol fuel cell (DMFC) has been developed. However, it requires rare metals and uses methanol that is known as a toxic material. To solve these problems, bio fuel cells that use living organism as a fuel source have been developed.

Lin et al. have developed a micro- photosynthetic/metabolic bio fuel cell that is composed of cyanobacteria and could generate electricity by photosynthesizing and metabolizing [1], [2]. This bio fuel cell requires mediators such as methylene blue to extract electrons from cyanobacteria. However, this methylene blue is not biocompatible, furthermore, loses its function rapidly when exposed in light. To overcome these problems, we have developed a novel mediator-less direct photosynthetic/metabolic bio fuel cell (DPBFC) in which microparticles of polyaniline were adopted as electrodes to extract electrons directly from bacteria[3],[4]. Our bio fuel cell resulted in an increase output by more than ten-fold that of Lin et al. Nevertheless, the output is still low and must be increased at least ten-fold. So, electron generation efficiency of the bacteria itself must be improved as well as the battery system efficiency.

In the present paper, we replaced the fuel source from cyanobacteria to purple photosynthetic bacteria (PPB) as a fuel source because PPB possesses such special characteristics as to be activated by organic compounds and emitted hydrogen in photosynthetic and metabolic processes. If this hydrogen emission is suppressed in anyway, more electrons will be stored inside of PPB, hence more electricity will be drawn out. To realize this, we applied gene manipulation technology so as to suppress hydrogen emission and to improve electron generation efficiency of PPB.

2 - BIOLOGY OF PURPLE PHOTOSYNTHETIC BACTERIA

2.1 - Biological Characteristics of PPB

In the present research, the electric generation performance of three types of PPB were tested, namely, a normal type, an accumulation-type, and an emission-type respectively. All these types of PPB are anaerobic and heterotrophic and they absorb organic compounds in both of photosynthetic and metabolic processes. They were cultivated by anaerobic culture in PYS medium which contains organic compounds under light.

Genes of normal PPB were manipulated so as to take off either of nitrogen-fixing enzyme (nif) or hydrogen-uptake enzyme (hup) in Fig.1 in order to suppress or stimulate hydrogen emission of bacteria.

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Biological Characteristics of Normal-Type PPB – Figure 1 shows the photosynthetic and metabolic mechanisms for normal-type PPB. As already explained in the previous paper [3],[4], cyanobacteria needs no nutrient to live, on the other hand, this PPB needs organic compounds which are absorbed inside of bacteria both in photosynthetic and metabolic process to produce an energy-storing molecule ATP (adensine triphosphate) that is synthesized from ADP (adenosine diphosphate). A nitrogen-fixing enzyme (nif) involved in PPB receives ATP energy and releases hydrogen as shown in Eq. (1).

\[
N_2 + 8H^+ + 8e^- + 16ATP + 16H_2O \rightarrow 2NH_3 + H_2 + 16ADP + 16Pi \tag{1}
\]

A part of released hydrogen is taken in a cell assisted by hydrogen-uptake enzyme (hup), and generates electron again using NAD\(^+\) (nicotinamide adenine dinucleotide) as shown in Eq. (2).

\[
H_2 + NAD^+ \leftrightarrow 2H^+ + 2e^- + NADH \tag{2}
\]

Taking these photosynthetic and metabolic processes of normal-type PPB into account, there comes an idea to suppress or stimulate nif or hup to control generation of hydrogen and electron.


![Figure 1 – Photosynthetic and Metabolic Mechanisms of Normal-Type PPB.](image1)

Biological Characteristics of Accumulation-Type PPB – Figure 2 shows the electron generation mechanism of accumulation-type PPB in which more electrons are expected to be stored inside of cell because an emission of hydrogen is suppressed as a result of having removed nif.

![Figure 2 – Electron Generation Mechanism of Accumulation-type PPB.](image2)

Biological Characteristics of Emission-Type PPB – Figure 3 shows the hydrogen emission mechanism of emission-type PPB in which more hydrogen are emitted because an accumulation of electrons is suppressed as a results of having removed hup.

![Figure 3 – Hydrogen Emission Mechanism of Emission-type PPB.](image3)

Table 1 summarizes these characteristics.

<table>
<thead>
<tr>
<th>Type of PPB</th>
<th>Hydrogen Emission</th>
<th>Electron Accumulate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal-Type</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>Accumulation-Type</td>
<td>×</td>
<td>○</td>
</tr>
<tr>
<td>Emission-Type</td>
<td>○</td>
<td>×</td>
</tr>
</tbody>
</table>

2.2. Processing of Gene Manipulation

Accumulation and emission-type PPB were developed by a site-directed gene defect. First, required gene was cloned via polymerase chain reaction (PCR) method. Partial gene of nif or hup was eliminated using restriction enzyme, and this defective gene was implanted into plasmid. Then, this plasmid was introduced into normal bacteria and cultivated. Finally, gene mutant bacteria (accumulation and emission-type PPB) were separated from normal one.

3. WORKING PRINCIPLE OF DPBFC

3.1. Principle of Electric Generation

Figure 4 shows a schematic of working principle of DPBFC. DPBFC has an anode chamber (left side) and a cathode chamber (right side) separated by a proton exchange membrane (PEM). Electrons are extracted from the electron transport chain of PPB, which is in contact with an electrode consisting of polyaniline, thus generating electricity.

![Figure 4 – Working Principle of DPBFC.](image4)

3.2. Electron Extraction Mechanism from PPB Cell

Figure 5 shows the electron extraction mechanism from PPB cell. The cell of PPB is composed of cytoplasm, periplast, periplasm space and cell wall. The thickness of periplast and cell wall are set at 5~10 nanometer while the diameter of cell is assumed to be 1μm. Electrons which will be generated during both photosynthetic and metabolic processes are transported to an electron transport chain in periplast.

The electron transfer between polyaniline and PPB cell is considered by two steps. First, the cell walls stick to nano
sized particle of polyaniline and contact to periplast directly, secondly, the electrons in periplast are extracted by polyaniline’s redox potential through the cell wall. Therefore, it is expected that the battery performance will be improved much compared to those of using mediators [3].

**4.2 - Processing of MEA**

MEA was fabricated using PEM and a carbon fiber electrode. Nafion117, used as PEM material, was treated with H2O2 (3-5 wt. %) at 80°C for 1 hour, then boiled in deionized water for 1 hour. Carbon fiber, which has an excellent electric characteristic and a steric structure, was used as the catalyst carrier. Polyaniline solution was placed on carbon paper or carbon cloth, and dried. Then, two electrodes were hot-pressed into one piece with a pretreated Nafion117 membrane to at 150°C, 50 kg/cm2 for 3 minutes to complete the MEA.

**4.3 - Structure of Medium Replaceable Type DPBFC**

Figure 7 shows the structure of medium replaceable type DPBFC. The whole structure is similar to the prototype DPBFC; chamber was made by aluminum alloy. This DPBFC has two ports for inserting fresh medium solution into anode chamber. Anode chamber was separated into PPB chamber (under) and medium chamber (top) by an optically transparent membrane filter (pore diameter: 5µm). PPB was filtered by membrane filter, therefore only old medium could be replaced. The reactive area is 4.8 cm².

**5 - EXPERIMENTAL METHODS**

The battery performance was evaluated by a generated electric power. It was calculated by an induced voltage and current of the battery. As the battery has its arbitrary potential voltage, a forced voltage is added to cancel it to zero, and swept down gradually always keeping zero. Meanwhile, the current between anode and cathode is measured by Solartron Co. Ltd. 1280Z. For all the experiments, PPB was cultured for 3 days, and these processes were done in an argon environment in order to avoid contact of PPB with oxygen.

**5.1 - Performance Reproducibility Evaluation**

To confirm the stability of DPBFC performance after gene manipulation, electric power was measured five times under the same conditions using a prototype DPBFC.
was used as the catalyst carrier, and the amount of volume of polyaniline was 0.4 ml.

5.2 - Cell Life Evaluation
To evaluate the cell life of DPBFC, electric power of accumulation-type PPB was measured as applied a constant voltage (0.44V: maximum power voltage) using a medium replaceable type DPBFC. First, only PPB was inserted into anode chamber. After a few hours, fresh medium was inserted into anode chamber to evaluate an effect of medium replacement. Carbon paper (0.11 mm thick) was placed on as the catalyst carrier and the amount of volume of polyaniline was 0.4 ml.

6 - EXPERIMENTAL RESULTS & DISCUSSION

6.1 - Result of Performance Reproducibility Evaluation
Table 2 shows the experimental result of the performance reproducibility evaluation. The accumulation-type PPB generated the highest electric power in all experiments and maximum power density became 18.3µW/cm² that is about up to 1.6 times of the normal one. From these, applying gene manipulation to PPB seems effective to increase output power. However, the amount of increase was far worse than expected, furthermore, the observed electric power was unstable. The reason may come from instability of MEA performance. MEA performance was determined by the states of the polyaniline coating and joint of Nafion117 and catalyst carrier. It was difficult to measure these states quantitatively. Therefore, quantitative analysis of MEA is necessary to realize performance stability.

Table 2 - Result of Performance Reproducibility Evaluation.

<table>
<thead>
<tr>
<th></th>
<th>Power density[µW/cm²]</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td>11.7</td>
<td>14.3</td>
<td>11.2</td>
<td>11.9</td>
<td>13.8</td>
<td>12.6</td>
</tr>
<tr>
<td>Accumulation</td>
<td></td>
<td>18.3</td>
<td>17.4</td>
<td>13.3</td>
<td>13.6</td>
<td>16.7</td>
<td>15.9</td>
</tr>
<tr>
<td>Emission</td>
<td></td>
<td>11.3</td>
<td>14.1</td>
<td>9.35</td>
<td>9.04</td>
<td>12.6</td>
<td>11.3</td>
</tr>
</tbody>
</table>

6.2 - Results of Cell Life Evaluation
Figure 8 shows the cell life performance of accumulation-type PPB. The electric power density decreased exponentially during Time 0 to 2, when the medium was changed to a fresh one. Then, the electric power density has recovered quickly and again began to decrease and reached to 2µW/cm² at Time 10. From this observation, it is known that the cell life is about 8 hours which was 4 times longer than the case of no medium replacement, the reason of which may come from an activation of fresh PPB.

In addition, it is required to mix PPB as much as possible because the dead or lower active PPB remains near to the anode. Therefore, it is desired to circulate PPB near the anode.

7 - CONCLUSIONS
Effectiveness of applying gene manipulation to PPB to increase output was verified from this research. Accumulation-type PPB stored more electrons in the cell, so DPBFC performance using this PPB was increased. It seems that this principle can be applied to other bio-fuel cell using bacteria. Furthermore, the cell life was extended by replacing the medium. From this, it may be possible to extend the cell life to the original lifetime of PPB (about 3 days) with the medium flowing systems in anode chamber.

Most keen issue for the current research is to verify and increase the effect of gene manipulation for the battery performance, and the prospects for future studies include the development of a medium flowing structure of DPBFC without supplying medium from outside.

REFERENCES