Improving Performance of Direct Photosynthetic/Metabolic Micro Bio Fuel Cell (DPBFC) by Gene Manipulation of Bacteria

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Abstract

In our laboratory, we have developed mediator-less direct photosynthetic/metabolic bio fuel cell (DPMFC) in which microparticles of polyaniline were adopted as electrodes to draw electrons from bacteria. We selected purple photosynthetic bacteria (PPBs) which is activated by organic compounds as well as emitting hydrogen in photosynthetic and metabolic processes for improving the electron generation efficiency of PPB by applying gene manipulation to inhibit hydrogen emission. Our hypothesis is that more electrons will be stored inside PPB if hydrogen emission is inhibited. As a result, the power density of Gene manipulated bacteria became 10μW/cm^2, that is higher than the normal one.

1 Introduction

The fuel cell is expected to be a new energy technology that can offer an effective solution to energy and environmental problems. Furthermore, portable equipment is becoming increasingly sophisticated and will require the miniaturization of high-output power sources in the future. To realize such power sources has received the greatest attention, fuel cells have been developed of which the direct methanol fuel cell (DMFC) is the most watched. However, it requires rare metals and uses methanol, a toxic material. To solve these problems, bio fuel cells that use electrons released from biocatalystic reactions have been developed.

Lin et al. have developed a micro-photosynthetic/metabolic bio fuel cell that is composed of cyanobacteria and that could make electricity by photosynthesizing and metabolizing[1][2]. This bio fuel cell requires mediators such as methylene blue to extract electrons from cyanobacteria. However, in this cell, bacteria are killed once mediators attack them; furthermore, such mediators lose their function rapidly when exposed to light. To overcome these problems, we have developed a novel mediatorless direct photosynthetic/metabolic bio fuel cell (DPBFC) in which microparticles of polyaniline were adopted as electrodes to draw electrons directly from bacteria. 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 still be increased at least ten-fold. To realize this, the electron generation efficiency of the bacteria must be improved. We selected PPBs because they have special characteristic such as being activated by organic compounds as well as emitting hydrogen in photosynthetic and metabolic processes. We improved the electron generation efficiency of PPB by applying gene manipulation technology to hydrogen emission. Our hypothesis is that more electrons will be stored inside PPB if hydrogen emission is inhibited. The objective of this research is to evaluate the usefulness of gene manipulation in increasing what output.

2 Biology of Purple Photosynthetic Bacteria

2.1 Biological Characteristics of PPB

In this research, we used three types of PPB; accumulation-type PPB, emission-type PPB, the biological character of which was changed by gene manipulation, and normal-type PPB. All these types of PPB are anaerobic and heterotrophic. They absorb organic compounds in photosynthetic and metabolic processes. The PPBs were cultivated by anaerobic culture in PYS medium which contains organic compounds under light.

2.1.1 Biological Characteristics of Normal-Type PPB

Fig.1 shows the photosynthetic and metabolic mechanisms of the normal-type PPB. Normal-type PPB absorbed organic compounds for the production of the energy-storing molecule ATP (adenosine triphosphate), which is synthesized from ADP (adenosine diphosphate). Normal-type PPB has a nitrogen-fixing enzyme (nif) that uses ATP energy, inevitably releasing H2 from this reaction.

$$N_2 + 8H^+ + 8e^- + 16ATP + 16H_2O \rightarrow 2NH_3 + H_2 + 16ADP + 16Pi \quad (1.1)$$

Some H_2 released is taken up into normal-type PPB cell using the hydrogen-uptake enzyme (hup), which provides reducing power in the form of electrons.

$$H_2 + NAD^+ \leftrightarrow H^+ + NADH \quad (1.2)$$

Therefore, the removal of these enzymes enables us to control electron generation capacity. However, these enzymes have susceptibility to oxygen.

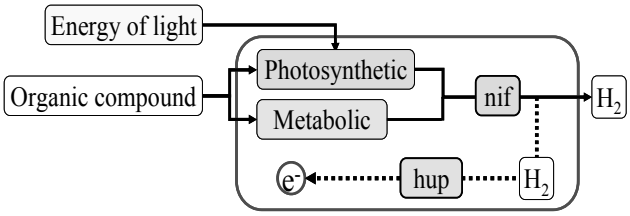

Fig.1. Photosynthetic and Metabolic Mechanisms of Normal-Type PPB

2.1.2 Biological Characteristics of Accumulation-Type PPB
Fig.2 shows the photosynthetic and metabolic mechanisms of the accumulation-type PPB. This PPB was developed by removing the gene nif from normal-type PPB. Hydrogen emission is inhibited as a result of nif removal; therefore, more electrons will be stored these bacteria than normal-type PPB.

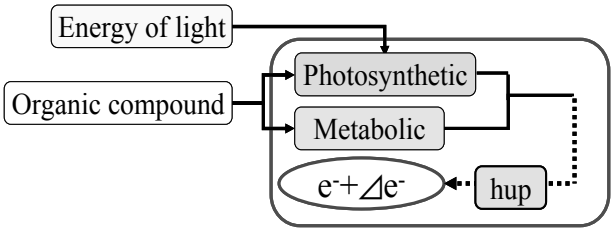

Fig.2. Photosynthetic and Metabolic Mechanisms of Accumulation-Type PPB

2.1.3 Biological Characteristics of Emission-Type PPB
Fig.3 shows the photosynthetic and metabolic mechanisms of the emission-type PPB. This PPB was developed by removing the gene *hup* from normal-type PPB. The accumulation of electrons is inhibited as a result of *hup* removal, and hydrogen emission is enhanced by nif.

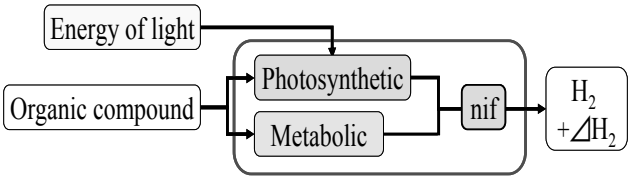

Fig.3. Photosynthetic and Metabolic Mechanisms of Emission-Type PPB

2.2 Biological Characteristic change by Gene Manipulation of PPB

Table 1 shows the biological charactaristic change of PPB. The accumulation-type PPB stores more extractabel electrons in the cell, therefore, the DPBFC performance which used the accumulation-type PPB will be expected to improve. Futhermore, the direct supplementation organic compounds to PPB can improve the performance, because all types of PPB become activated.

Table 1. Biological Characteristics of PPB

Type of PPB	Hydrogen Emission	Electron Accumulate	Effect of organic compound
Normal-Type	○	○	○
Accumulation-Type	×	◎	○
Emission-Type	◎	×	○

3 Working Principle of DPBFC

3.1 Principle of Electric Generation

Fig.4 shows a schematic of the 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.

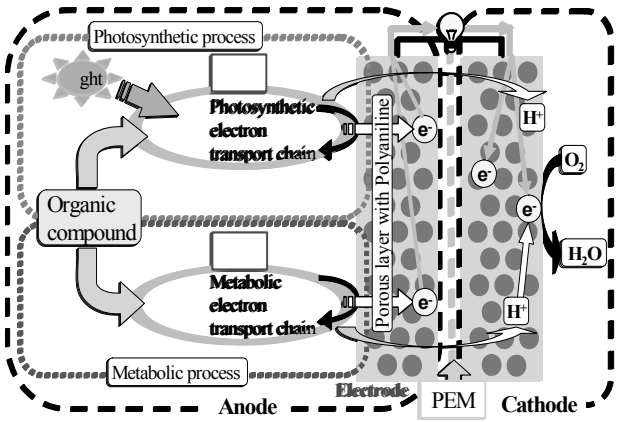

Fig.4. Working Principle of DPBFC

3.2 Electron Extraction Mechanism from PPB Cell

Fig.5 shows the electron extraction mechanism from PPB cell. The cell of PPB is composed of cytoplasm, periplast, periplasm space and cell wall from the inside. The thickness of periplast and cell wall becomes several nanometer (5~10nm) when the diameter of the cell is assumed to be 1μm. Electron which was raised in photosynthetic and metabolic processes was transported on electron transport chain in periplast.

The electron transfer mechanisim between polyaniline and PPB cell are two processes. One is that electrons in the periplast were extracted by polyaniline's redox potential through cell wall, and the other is that, polyaniline nano sized particle sticks into the cell walls and contact to periplast directly. These reactions enable to draw electrons more directly, therefore, the DPBFC performance was improved more than ten-fold compared to the performance of using mediators[3].

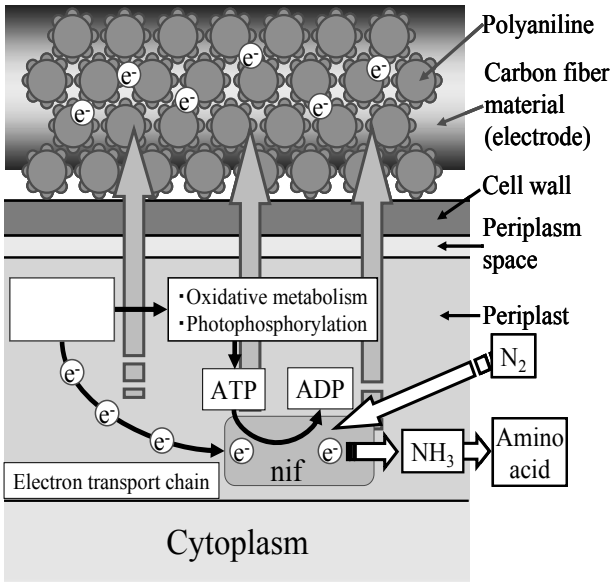

Fig.5. Electron Extraction Mechanism from PPB Cell

4 Structure of DPBFC

Fig.6 shows the structure of the prototype DPBFC. An MEA (membrane electrode assembly) was fabricated using Nafion117 (DuPont Co. Ltd.) and carbon paper (0.19 mm or 0.11 mm thick) or carbon cloth (0.33 mm thick). To collect current, MEA was sandwiched between two copper electrodes (0.3 mm thick) manufactured by electric discharge machining. To make space for anolyte and catholyte injections, an O-ring of internal diameter 18 mm and 1.5 mm thick was used. Finally, the fuel cell was sandwiched between layers of acrylic, which is an optically transparent material. The DPBFC was 40 mm×40 mm with a reactive area of 2.54 cm^2.

5 Processing of Prototype MEA

MEA was fabricated using PEM and a carbon fiber electrode. Nafion117, used as PEM, was treated with H_2O_2 (3-5 wt. %) at 80℃ for 1 hour, and 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 in to one piece with a pretreated Nafion117 membrane to at 150℃, 50 kg/cm2 for 3 minutes complete the MEA.

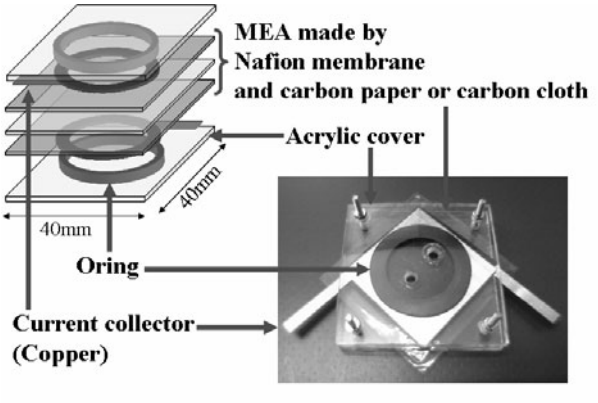

Fig.6. Structure of Prototype DPBFC

6 Factors Determining DPBFC Performance

Fig.7 shows a schematic of the two factors determining DPBFC performance; PPB status and DPBFC structure. To confirm the effects of PPB status, we used two evaluations, and to confirm those of DPBFC structure, we used the evaluation shown in Fig.7.

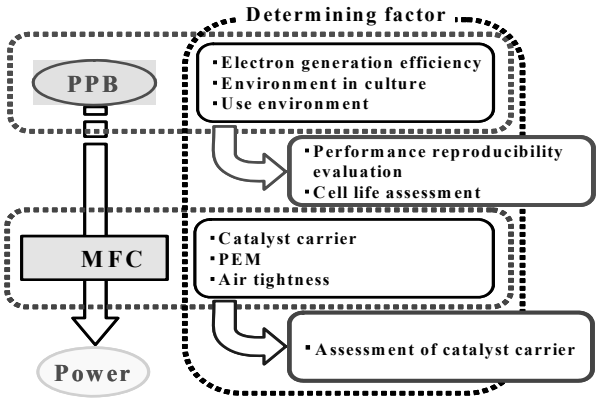

Fig.7. Schematic of Factors Determining DPBFC Performance

7 Experimental Methods

DPBFC performance was evaluated on the basis of electric power, which was measured by connecting resistors, from 10 Ω to 1 kΩ, to DPBFC. The voltage of the resistor was measured with a data logger (Kyowa Co. Ltd. PCD320A) and current was measured with a digital multimeter (Advantest Co. Ltd. AD7461A). PPB cultured for 3 days was used in all the experiments.

7.1 Performance Reproducibility Evaluation

Gene manipulation in PPBs influences the stability of their DPBFC performance. Therefore, to confirm the stability of DPBFC performance after gene manipulation, electric power was measured five times under the same conditions. Carbon cloth was placed on as the catalyst carrier, and the amount of polyaniline used was 0.3 ml.

7.2 Cell Lifespan Assessment

If the lifespan of PPBs is extended, allowing for the storage of more electrons PPBs, the cell life of DPBFC will be extended. To determine the cell life extension after gene manipulation, open circuit voltages, electric power as determined by connecting 100 Ω resistors and the pH of PPB culture before and after the experiment were measured. Carbon paper (0.11 mm thick) was placed on as the catalyst carrier, and the amount of polyaniline used was 0.4 ml.

7.3 Assessment of Catalyst Carrier

Table 2 shows the experimental conditions. A changing in the structure of the catalyst carrier can increase the area of contact between PPB and polyaniline; thus, this contact area was varied. To determine the optimal structure of the catalyst carrier in DPBFC, the electric power was measured while changing the structure of the catalyst carrier. The amount of polyaniline used was 0.4 ml.

8 EXPERIMENTAL RESULTS & DISCUSSION

8.1 Result of Performance Reproducibility Evaluation

Table 3. shows the experimental result of the performance reproducibility evaluation. Gene manipulation did not clearly increase electric power, and the electric power observed was unstable. This may result from nif and hup's susceptibility to oxygen. The PPBs were exposed to air; therefore, the performance of nif and hup were suppressed because of their susceptibility to oxygen. Furthermore, MEA performance was determined by evaluating the states of the polyaniline coating, joint of Nafion117 and catalyst carrier. It was difficult to measure these states quantitatively. Therefore, a strict management of PPB cultures, an airtight DPBFC structure and quantitative processing of MEA are necessary to improve and achieve performance reproducibility.

Table 2. Experimental Conditions of Assessment of Catalyst Carrier

Experimental number	Anolyte (Type of bacteria)	Catalyst carrier
No.1	Normal	Carbon paper 0.19mm thick
No.2	Accumulation	Carbon paper 0.19mm thick
No.3	Emission	Carbon paper 0.19mm thick
No.4	Normal	Carbon cloth
No.5	Accumulation	Carbon cloth
No.6	Emission	Carbon cloth
No.7	Normal	Carbon paper 0.11mm thick
No.8	Accumulation	Carbon paper 0.11mm thick
No.9	Emission	Carbon paper 0.11mm thick

Table 3. Result of Performance Reproducibility Evaluation

	Power density[μ W/cm^2]					
Experimental number	1	2	3	4	5	Average
Normal	7.8	5.3	3.5	5.4	2.2	4.9
Accumulation	7.6	8.1	4.0	4.5	2.8	5.4
Emission	6.6	4.5	4.6	3.1	4.6	4.7

8.2 Results of Cell Lifespan Assessment

Fig.8 shows the open-circuit voltage performance of DPBFC, and Fig.9 shows the electric power performance of DPBFC. From time course of electric power, the cell lifespan was about 2 hours. Although it was forecast that the cell lifespan of the accumulation-type PPB would be longest, the cell lifespan of the normal and emission-types were actually longest. This was a result of nif and hup's susceptibility to oxygen. With a decrease in pH, a proton permeability decrease due to Nafion117 drying, and an ion electron imbalance between the anode and cathode chambers occurred. Therefore, it is important to determine a method of injecting H_2O, which was generated in the cathode chamber, into Nafion117 of the MEA, and developing a more airtight structure of DPBFC to protect nif and hup from oxygen.

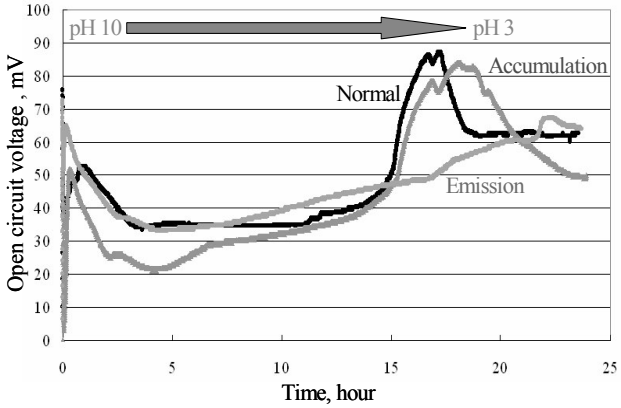

Fig.8. Open-Circuit Voltage Performance of DPBFC

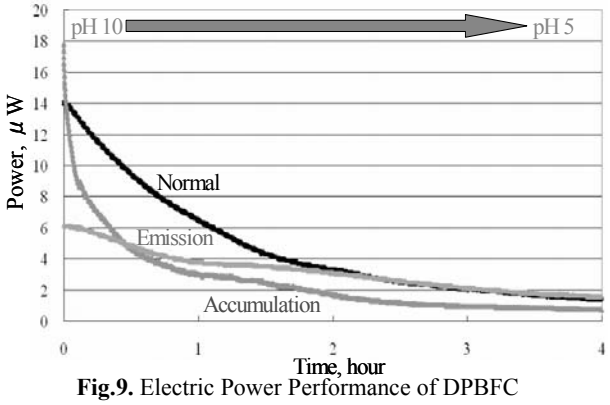

Fig.9. Electric Power Performance of DPBFC

8.3 Result of Assessment of Catalyst Carrier

Fig.10 and Table 4 show the experimental results of the assessment of the catalyst carrier. DPBFC performance was improved using a thin and highly porous material, namely, carbon paper (0.11 mm thick), to increase the area of contact between the PPBs, polyaniline and Nafion117, and the reaction between electrons and protons was promoted. Moreover, the accumulation-type PPB generated the highest electric power of all the catalyst carriers. Therefore, to generate high electric power, the accumulation- type PPB should be used.

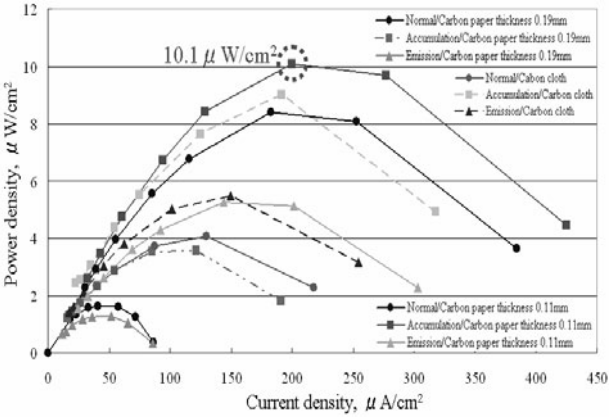

Fig.10. Results of Assessment of Catalyst Carrier

Table 4. Results of Assessment of Catalyst Carrier

	Power density[μ W/cm^2]		
	Carbon paper 0.19mm thick	Carbon cloth	Carbon paper 0.11mm thick
Normal	1.6	4.1	8.4
Accumulation	3.6	9.0	10
Emission	1.3	5.5	5.3

9 Conclusions

No clear improvement in DPBFC performance by applying gene manipulation in PPB was accomplished in this research. Nevertheless, it is highly possible that gene manipulation can improve the electric power output of accumulation-type PPB. The DPBFC performance was low because PPB can not stably produce electric power; therefore, it is necessary to improve PPB stability to develop a useful fuel.

Prospects for future studies include the direct supplementation organic compounds to PPB, and the development of an airtight and miniature structure of DPBFC to increase electric power output and improve PPB stability. Such improvements will allow DPBFC to output a high electric power. Furthermore, the development of a flexible structure DPBFC or medium reflux DPBFC will allow widespread application.

10 References

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