PHOTOSYNTHETIC BIO-FUEL CELLS USING CYANOBACTERIA

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ABSTRACT

Effects of glycogen and glucose on the current outputs have been studied in photosynthetic bio-fuel cells using two kinds of cyanobacteria, *Anabaena variabilis* M-3 and *Synechocystis* sp. M-203, and 2-hydroxy-1,4-naphthoquinone. In the bio-fuel cell using *Anabaena variabilis* M-3, by repeating discharge-culture operation, the lifetime of the bio-fuel cell extended to net 80 hours, although the current output was sustained only for 10 hours under the condition of continuously running. In the case using *Synechocystis* sp. M-203, the current outputs both in the light and dark substantially increased by the addition of glucose to the anode solutions, indicating that glucose was transported into *Synechocystis* cells and was consumed to produce electricity.

KEYWORDS

Bio-fuel cells; mediator; photosynthesis; cyanobacteria; glycogen content; effect of glucose.

INTRODUCTION

In recent years, energy conversion of light energy into electricity using microalgae and its photosynthetic components have been widely studied, since photosynthetic process is a sophisticated reaction with high efficiency. In the first step of photosynthesis, charge translocation on electron transport chain is occurred producing reduced equivalents. In a photosynthetic bio-fuel cell using cyanobacteria, such reduced equivalents are conveyed to an electrode to produce electric current by a catalytic action of cyanobacteria and redox mediators. Substantial electric current is also produced in the dark. In this case, reducing power from endogenous reserves (mainly glycogen) are similarly converted into electrical energy. The performance of the bio-fuel cells depends on the amount of endogenous glycogen accumulated within cyanobacteria by photosynthesis (Yagishita et al., 1993). Endogenous glycogen is an electron source in the bio-fuel cells running in the dark. The current outputs under illumination were higher for larger glycogen contents and endogenous glycogen was also decreased under illumination as well as in the dark, although the current output is derived from electrons generated via oxidation of water.

In this study, we examined the effects of glycogen and its monomer, glucose on the performance of the bio-fuel cells containing two kinds of cyanobacteria, *Anabaena variabilis* M-3 and *Synechocystis* sp. M-203, and 2-hydroxy-1,4-naphthoquinone (HNQ) as a mediator. *Anabaena variabilis* M-3 was removed from the bio-fuel cell at intervals of 10 hours and was cultured with light and CO₂ to produce endogenous glycogen. Cells of *Anabaena variabilis* M-3 were immobilized within the beads of calcium alginate to facilitate the manipulation. In the case using *Synechocystis* sp. M-203, endogenous glycogen might be supplied by the addition of glucose to the anode solution of the bio-fuel cell, since *Synechocystis* sp. M-203, which is identified with *Synechocystis* sp. PCC6803, has glucose transport system, and therefore this strain can grow chemoheterotrophically and photoheterotrophically (Rippka et al., 1979). We examined the behavior of the output currents from the bio-fuel cells containing *Synechocystis* sp. M-203, when glucose was added to the anode solution.
EXPERIMENTAL

Growth Condition

Cells of *Anabaena variabilis* M-3 and *Synechocystis* sp. M-203 were from the algal collection of University of Tokyo. The media of *Anabaena variabilis* and *Synechocystis* sp. were modified Detmer medium (Watanabe, 1960) and *Synechocystis* medium (Eley, 1988), respectively. They were grown at 30 °C under aeration with air containing 3% (v/v) CO₂. Continuous illumination was provided by a fluorescent lamp at 27 μE m⁻² s⁻¹. Cells of *Anabaena variabilis* and *Synechocystis* sp. harvested by centrifugation (4000 rpm, 10 min) were suspended in 50 mM Tricine-KOH buffer (pH 8.0) and 50 mM phosphate buffer (pH 8.0), respectively.

Immobilization of *Anabaena variabilis*

Five ml of *Anabaena variabilis* suspension was thoroughly mixed with 5 ml of 50 mM Tricine-KOH buffer (pH 8.0) containing 3% (w/v) sodium alginate. The mixture was extruded as drops into Tricine-KOH buffer containing 30 mM CaCl₂ using a thin injection needle. The drops formed into beads (about 2 mm diameter) upon contact with CaCl₂ solution. The beads were washed once with Tricine-KOH buffer containing CaCl₂ and replaced in the same buffer. They were allowed to stand in it for 1 hour for complete hardening and were then ready for use.

Fuel Cells

The construction of the fuel cells was similar to that described previously (Yagishita, 1993). The anode solution (40 ml) of the bio-fuel cell containing immobilized *Anabaena variabilis* contained immobilized *Anabaena* cells (total of 940 mg Chlorophyll), 1 mM 2-hydroxy-1,4-naphthoquinone (HNQ) and 30 mM CaCl₂ in 50 mM Tricine-KOH buffer (pH 8.0), and in the case of *Synechocystis* sp. it contained *Synechocystis* cells (total of 470 mg Chlorophyll) and 1 mM HNQ in 50 mM phosphate buffer (pH 8.0). Both cathode solutions (40 ml) contained 0.12 M potassium ferricyanide in the same buffer, respectively. Both electrodes were carbon cloth (anode: 8 cm²; cathode: 16 cm²). The fuel cells were run at 30 °C. The anode solutions were agitated by bubbling with N₂ at a flow rate of 200 ml/min. The beads of immobilized *Anabaena* cells were removed from the fuel cell compartments and were washed with Tricine-KOH buffer containing 30 mM CaCl₂ until HNQ was completely removed. They were then placed in culture medium (except for K₂HPO₄) containing 30 mM CaCl₂ and cultured with continuous illumination using a xenon lamp (100 W/m²) and air containing 3% (v/v) CO₂.

Determination of Chlorophyll Content and Glycogen Content

Chlorophyll was extracted from the *Anabaena* cells with methanol and determined spectrophotometrically using the absorbance at 666 nm (Ogawa and Shibata, 1965). The glycogen content of the algal cells was obtained by multiplying the amount of glucose content by 0.9, which was determined by the method of Dubois et al. (1956). The beads of immobilized *Anabaena* cells were washed with 50 mM phosphate buffer (pH 8.0) containing 0.9% NaCl several times until the alginate was thoroughly removed.

RESULTS and DISCUSSION

Performance of the Bio-fuel Cell Using Immobilized *Anabaena variabilis* M-3

A typical example of the voltage, current-time profiles of the bio-fuel cells using immobilized *Anabaena* cells and HNQ is shown in Fig. 1. Since there was not almost difference between the current outputs in the light and dark, the bio-fuel cells continuously ran in the dark under a 1 kΩ load. A current output of more than 0.5 mA was sustained for about 10 hours. Initial glycogen content was 10.6 mg. After discharge of 10 hours, the glycogen content decreased 4.5 mg. During a subsequent culture period for 10 hours, the glycogen content increased by 7.3 mg, indicating that the glycogen content level has significantly recovered during this
culture period. By repeating this discharge-culture operation, outputs higher than that obtained during the first discharge of 10 hours were observed up to the 8th discharge. After the 8th discharge the current output

![Graph showing voltage and current vs. time curve for bio-fuel cell using immobilized Anabaena variabilis M-3.](image)

**Fig. 1.** Voltage, current vs. time curve of the bio-fuel cell using immobilized *Anabaena variabilis* M-3

![Graph showing effect of glucose on current output in bio-fuel cells using Synechocystis sp. M-203.](image)

**Fig. 2.** Effect of glucose on the current output in the bio-fuel cells using *Synechocystis* sp. M-203
after loading sharply dropped and was significantly reduced at the 13th discharge. Immobilized *Anabaena* cells were cultured in culture medium without K$_2$HPO$_4$, since phosphate ion combines with calcium ion, which sustains gelation of calcium alginate, and precipitates. Phosphate ion is a major component of the culture medium and *Anabaena variabilis* M-3 is difficult to grow up without phosphate ion. It is thought that the lack of phosphate ion results from the decrease in the current output after the 9th discharge.

**Effect of Glucose on the Performance of the Bio-fuel Cell Using *Synechocystis* sp. M-203**

Figure 2 shows the behavior of the current outputs in the bio-fuel cells using *Synechocystis* sp. M-203 when glucose was added to the anode solution of the bio-fuel cells. The bio-fuel cell was illuminated by a 650W projector lamp at 6 µE s$^{-1}$ m$^{-2}$ under the condition of light. In the bio-fuel cells using *Synechocystis* sp. M-203, current output was lower under illumination than that in the dark. By the addition of 0.5 mg glucose the current outputs under illumination rapidly increased from 0.2 mA to more than 0.3 mA, indicating that glucose was transported into *Synechocystis* cells and was quickly consumed to produce electricity. After reaching the peak, the current output gradually decreased. In the dark the current output also substantially increased by the addition of glucose and then was sustained stably. From these results the bio-fuel cell using *Synechocystis* sp. M-203 is promising. Because culture of *Synechocystis* sp. M-203 is not needed to supply endogenous glycogen and both the current outputs from oxidation of water and degradation of glucose added are piled up under illumination. In future, glucose will be replaced with waste water.

**REFERENCES**


