Photosynthetic bio-fuel cells using immobilized cyanobacterium *Anabaena variabilis* M-3

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**Introduction**

A photosynthetic reaction begins with charge separation in the reaction centers producing reduced equivalents. In a photosynthetic bio-fuel cell, such reductants in micro-algae are captured by electron transfer mediators and the electrons are transferred to an electrode to produce electricity as shown in Fig. 1. Similarly, the reducing power generated from oxidative degradation of reserved carbohydrate is also converted into current in the bio-fuel cells running in the dark. We have studied two bio-fuel cells using the marine cyanobacterium *Synechoccus* sp. (UTEX2380) and the freshwater cyanobacterium *Anabaena variabilis* M-2 with 2-hydroxy-1,4-naphthoquinone (HNQ) as a mediator [1, 2]. In a previous study [1], we found that endogenous glycogen in algal cells decreased during discharge not only in the dark but also under illumination and that these outputs were higher for larger glycogen contents. Therefore, the recovery of the

![Figure 1. Schematic diagram of a photosynthetic bio-fuel cell. Fe(III): ferricyanide; Fe(II): ferrocyanide.](image-url)
glycogen content to initial levels might extend the lifetime and improve the current output of the bio-fuel cells.

In this study, we examined the performance of the bio-fuel cells using immobilized *A. variabilis* M-3 which was removed from the bio-fuel cells at regular intervals and was cultured with light and CO₂ to supply endogenous glycogen. Cells of *A. variabilis* were immobilized within the beads of calcium alginate to facilitate the manipulation. The bio-fuel cells were run in the dark, since the current output in the dark was almost the same as that under illumination. We compared the performance of the bio-fuel cells using immobilized *Anabaena* cells with that using freely suspended cells.

**Experimental**

Cells of *Anabaena variabilis* M-3, from the algal collection of Tokyo University, were grown at 30 °C in modified detmer medium [3] 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 harvested by centrifugation (3000 rpm, 20 min) were suspended in 50 mM Tricine–KOH buffer (pH 8.0) at a chlorophyll concentration of 180-300 mg/ml.

**Immobilization of Anabaena**

Five ml of *Anabaena* suspension was thoroughly mixed with 5 ml of 50 mM Tricine–KOH buffer (pH 8.0) containing 1.5% (w/v) sodium alginate. The mixture was extruded as drops into Tricine-KOH buffer containing 30 mM CaCl₂ solution 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 is shown in Fig. 2. The anode solution (40 ml) contained freely suspended or immobilized *Anabaena* cells (total of 940 mg Chlorophyll), 1 mM HNQ and 30 mM CaCl₂ in 50 mM Tricine–KOH buffer (pH 8.0), and the cathode solution (40 ml) contained 0.12 M potassium ferricyanide in the same buffer. 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 100–150 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 Tricine–KOH buffer 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 [4]. 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. [5]. 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 continuously running bio-fuel cells

The bio-fuel cells were left in open circuit for 20-30 min after the addition of HNQ and the voltage of these bio-fuel cells increased and reached ca. 0.8 V during this period as shown in Fig. 3. The increase in voltage follows the variation in potential at the anode governed by the Nernst equation:

$$E = E^0 + \frac{RT}{nF} \ln \frac{C_O}{C_R}$$

where $E^0$ is the formal potential of the electrode, $F$ is Faraday's constant, and $C_O$ and $C_R$ are the concentrations of the oxidized and reduced substances, respectively. In the bio-fuel cell using freely suspended Anabaena cells (abbreviated as suspended bio-fuel cell), the voltage rose as soon as HNQ was added, and it reached a steady state in 5 minutes. On the other hand, there was a lag of 2 minutes in voltage rise in the bio-fuel cell using immobilized Anabaena (abbreviated as immobilized bio-fuel cell), and the rise was slower than that in the suspended bio-fuel cell due to the diffusional limitation of HNQ.

A typical example of the voltage, current-time profiles of the bio-fuel cells using freely suspended and immobilized Anabaena cells with HNQ is shown in Fig. 4, where the bio-fuel cells continuously ran in the dark under a 1 kΩ load. Since the rate-determining step is oxidation of HNQ at the electrode under the condition of a
Figure 3. Behavior of open circuit voltage. HNQ was added at t=0. Solid line: immobilized bio-fuel cell; dashed line: suspended bio-fuel cell.

Figure 4. Typical example of voltage, current-time profiles of Immobilized (solid line) and suspended (dashed line) bio-fuel cells.
1 kΩ load, immobilization of the Anabaena cells did not have any significant effect on the output of the bio-fuel cells. A current output of more than 0.5 mA was sustained for about 10 hours in both the suspended and immobilized bio-fuel cells. Immobilized Anabaena cells sustained the activity of oxygen evolution after discharge and kepted green color. On the other hand, we observed effusion of dye from freely suspended cells to the anode solution. The dye had maximum absorption at about 620 nm, which corresponds with that of an antenna dye, phycocyanin. Although the suspended cells were placed on the culture medium gel plate after discharge, they did not grow. Therefore, immobilization of Anabaena cells is needed to extend the lifetime of the bio-fuel cell by the recovery of endogenous glycogen content to initial level.

Repeated discharge-culture operation
To supply endogenous glycogen, immobilized A. variabilis was removed from the bio-fuel cells at intervals of 10 hours and then cultured for 10 hours. Table 1 shows the amount of glycogen content at each phase in a single cycle of the discharge-culture operation. After discharge for 10 hours, the glycogen content decreased 4.5 mg. During a subsequent culture period, the glycogen content increased by 7.3 mg, indicating that the glycogen content level has significantly recovered during this culture period. Figure 5 shows the voltage-time profile of the bio-fuel cell run in a repeated discharge-culture operation. Outputs of higher than that obtained during the first discharge were observed up to the 8th discharge. This is due to the culture in which endogenous glycogen was excessively provided as shown in Table 1. On and after the 9th discharge, the current output after loading sharply dropped. At the 13th discharge, the output was significantly reduced and the effusion of dye to the anode solution was observed.

Table 1
<table>
<thead>
<tr>
<th></th>
<th>Initial /mg glycogen</th>
<th>After discharge /mg glycogen</th>
<th>Coulombic output /C</th>
<th>After culture /mg glycogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immobilized cells</td>
<td>10.9</td>
<td>6.4</td>
<td>19</td>
<td>13.7</td>
</tr>
<tr>
<td>Suspended cells</td>
<td>10.9</td>
<td>5.5</td>
<td>21</td>
<td>—*</td>
</tr>
</tbody>
</table>

*The effusion of dye was observed in 2 hours of culture and the amount of glycogen content after culture was negligible.
Figure 5. Current vs. time curve of immobilized bio-fuel cell running in repeated discharge-culture operation at intervals of 10 hours. The wide lines on the abscissa represent the periods of culturing.

**Conversion efficiency**

We calculated the coulombic output theoretically available from endogenous glycogen by its complete oxidation to CO₂ using the following equation;

\[
\text{Glycogen} \rightarrow \text{C}_6\text{H}_{12}\text{O}_6 + 6\text{H}_2\text{O} \rightarrow 6\text{CO}_2 + 24\text{H}^+ + 24\text{e}^- \quad (2)
\]

The amount of glucose is obtained by multiplying that of glycogen by 1.1. The electrons of 24 mol are generated per consumption of 1 mol glucose. The coulombic yield is obtained by multiplying the amount of electrons by Faraday constant. For the immobilized bio-fuel cell, as shown in Table 1, the loss of glycogen was 4.5 mg, which corresponds to a theoretically available coulombic yield of 64 C, during discharge of 19 C. The coulombic efficiency was 30%. For freely suspended cells, the glycogen content decreased 5.4 mg, corresponding to the theoretically available 79 C during discharge of 21 C. The coulombic efficiency in this case was 27%. Therefore, the coulombic efficiencies of the both cases were almost the same. The energy efficiency of light energy into electric energy was ca. 0.3% during the repeated discharge-culture operation.

**Conclusions**

In the repeated discharge-culture operation at intervals of 10 hours, the current outputs of more than 0.58 mA was sustained for a net 80 hours (i.e., 8 cycles). Immobilization of *Anabaena variabilis* M-3 did not improve the duration of the current output and the coulombic efficiency, but it leaves *Anabaena* cells
intact after discharge. Also it allowed the glycogen content to recover from depletion due to discharge, although suspended *A. variabilis* did not grow after discharge. With further improvement, it will allow *Anabaena* cells to sustain a repeated discharge-culture operation over long time period.

**References**