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"Living electrode" as a long-lived photoconverter for biophotolysis of water

(hydrogen production/thermophilic blue-green algae)

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ABSTRACT **Living blue-green algae (*Mastigocladus laminosus*), immobilized on an SnO_2 optically transparent electrode with calcium alginate, functioned as an anodic photoelectrode on continuous illumination for periods of time adequate for use in a conventional electrochemical cell. This "living electrode" shows promise of use as a long-lived photoconverter of solar radiant energy to electric energy and as a suitable replacement for unstable chloroplast systems.**

In a previous paper (1) we reported that chloroplast electrodes, upon illumination, could function to generate an anodic photocurrent with concomitant oxidation of water molecules. Photoconverters of this type have been suggested as an electron-donating system to hydrogenase in production of hydrogen through water photolysis (2), especially for schemes using a two-stage system (3). However, from a practical standpoint, there are major difficulties in such an approach. Chloroplast preparations from sources such as spinach and higher plants have variable activity and readily deteriorate upon warming and aging. Hence, there is a serious limitation in using chloroplast preparations as practical photoconverters.

Alternatively, we have considered the use of intact thermophilic algal cells. In general, chloroplast organelles in plant cells are stabilized by cellular dynamic processes. Therefore, the problems of stability and longevity that occur with chloroplast photoconverters would be avoided. In this article, we report the favorable outcome of such experiments on a strain of thermophilic blue-green alga, *Mastigocladus laminosus*.

MATERIALS AND METHODS

The strain of thermophilic alga, isolated from Matsue hot springs, was easily cultured under laboratory conditions. The culture medium was as follows: 1 liter of hot spring water as a basic culture medium, 0.2 g of KNO_3, 0.05 g of KH_2PO_4, 0.05 g of $MgSO_4$, and 1 mg of $FeSO_4$ (at pH 8.0). A culture vessel (capacity, 1 liter) was illuminated from both sides by fluorescent lamps (Mitsubishi FL-20SPG) at 2000 lux. Bath temperature was kept at 47 ± 2°C. Aeration (1.5 liter/min per liter of culture medium) was continued throughout the culture. No living contaminants were found in the culture medium. Fig. 1 shows the growth curve of the algae under the conditions used. The algae are filamentous and often aggregate as balls so that they could be easily collected by simple filtration with layers of nylon cloth. We disrupted the algae balls with the aid of a soft brush and prepared a homogeneous suspension. The suspension cells were washed once by centrifugation for use.

This alga was identified as *Mastigocladus laminosus*, based upon pigment analysis and microscopic morphological observation. We can assay both photosystem I (PS-I) and photosystem II (PS-II) activities by the use of the *intact* algal cells, a unique circumstance as compared to the general experience with blue-green algae such as *Plectonema* sp. and *Anacystis* sp. or green algae such as *Chlorella* sp. Electrons are available from the intact cells directly to electron carriers such as 2,6-dichlorophenol indophenol (DPIP) and methyl viologen. On the other hand, no NADPH accumulation can be detected by the routine assay procedure with intact cells.

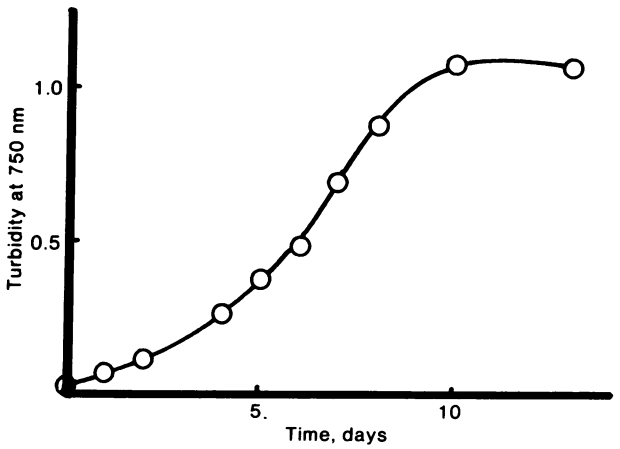

FIG. 1. Growth curve of the thermophilic blue-green alga.

PS-I activity was assayed by the method of Epel and Neimann (4) with the intact cell suspension, as we did for chloroplast suspensions. The assay mixture contained the following: 7.6 ml of 50 mM potassium phosphate buffer (pH 8.0), 0.5 ml of 100 mM ascorbate/5 mM DPIP, 0.5 ml of 2 mM methyl viologen, 0.1 ml of 300 μM 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea (DCMU), 0.1 ml of 100 mM NaN_3, and 1.2 ml of intact cells (equivalent to 40 μg of chlorophyll), in a total volume of 10 ml. PS-II activity was assayed by determining the photoreduction of DPIP in accordance with the method of Katoh and San Pietro (5), slightly modified. The assay mixture contained 4.0 ml of 50 mM potassium phosphate buffer (pH 7.0), 0.5 ml of 1 mM DPIP, and 0.5 ml of intact cells, in a total volume of 5.0 ml. The assay mixture for overall activity was as follows (4): 7.7 ml of 50 mM potassium phosphate buffer (pH 8.0), 0.5 ml of 2 mM methyl viologen, 0.5 ml of 20 mM NH_4Cl, 0.1 ml of 100 mM NaN_3, and 1.2 ml of intact cells, in a total volume of 10 ml. Throughout the assay procedures, illumination of 20,000 lux and a temperature of 40°C were maintained. For chlorophyll determination, the intact cells were ground with a Potter–Teflon homogenizer and treated with 80% acetone. The chlorophyll a/acetone extract was determined spectrophotometrically by the method of MacKinney (6).

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Abbreviations: PS-I, photosystem I; PS-II, photosystem II; DPIP, 2,6-dichlorophenol indophenol; PVA polyvinyl alcohol; DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea.

Polyvinyl Alcohol (PVA)/Algae Electrode. The fresh algae (about 2 g, equivalent to 1 mg of chlorophyll a) were mixed with 50 mM potassium phosphate buffer (pH 8.0) containing 20% PVA-117 (average degree of polymerization 1750, degree of saponification 98.5% or more) and 1% bovine serum albumin. The mixture was spread uniformly on the surface of an SnO_2 optically transparent electrode glass plate and dried under reduced pressure on P_2O_5. There was no need to keep the algae sample cold. The PVA/alga film deposited on the SnO_2 electrode, around 25 μm in thickness, was covered and fixed with a monolayer of nylon cloth. This arrangement functioned as a working electrode upon illumination in the conventional three-electrode system (7). A platinum plate and a saturated calomel electrode served as the counter electrode and the reference electrode, respectively. As the light source, a projector lamp was used in combination with a Toshiba glass filter to eliminate photoresponse of the SnO_2. Illumination was adjusted to 50,000 lux. Electrolyte solution used in the experiments was Tris/NaCl buffer (50 mM Tris·HCl/10 mM NaCl, pH 8.0). Potentials of various kinds were applied to the working electrode against the standard calomel electrode, by use of a potentiostat, and photocurrent on illumination was recorded.

The recovery of PS-I activity of the algae film thus prepared was almost quantitative; that of PS-II was as much as 50% of the activity observed with intact cells. The PVA/algae electrode retained the initial activities of the photosystems for an adequate time when kept in a refrigerator, as observed with chloroplast electrodes; in 8 weeks of storage at 4°C, as much as 100% of PS-I activity and 85% of PS-II activity were retained.

Algae Electrode Immobilized with Calcium Alginate. We suspended the cells of the algae in a 5% sodium alginate solution of hot spring water. The suspension was spread on the surface of SnO_2 optically transparent electrode and the electrode plate was dipped into 50 mM $CaCl_2$ solution (buffered at pH 8.0) for 5 min. The electrode was washed with the hot spring water. Thus we obtained the living algae electrode, $\approx$250 μm in thickness. The PS-I activity of the immobilized algae was 50% of that seen with intact cells; the PS-II activity was 70%. These values were variable and depended on the permeability of the reagents such as DPIP or methyl viologen to calcium alginate as immobilizing material.

RESULTS AND DISCUSSION

Table 1 summarizes the photochemical activities of the algae, with and without DCMU. The values shown indicate that the photochemical activities of the intact algae are comparable to activities of the chloroplast preparation from higher plants. The ability to take up electrons from the intact cells suggests the availability of the intact alga as a "live photoconverter." On addition of DCMU, the full photosynthetic activity was not completely inhibited, in contrast with the chloroplast preparation, showing that electron donors other than water molecules, such as NADH or reduced substances formed from the catabolic chain, are also available as electron donors to the PS-I in the intact cells of the algae.

Table 1. Photosystem activity of the thermophilic blue-green alga in intact cells

Reaction	Specific activity*
Overall reaction†	832
+DCMU	112
PS-I	446
PS-II	332
+DCMU	0
$NADP^+$ reduction‡	0

* Electron (microequivalent per mg of chlorophyll per hr).
† $H_2O \rightarrow$ methyl viologen.
‡ $H_2O \rightarrow NADP^+$.

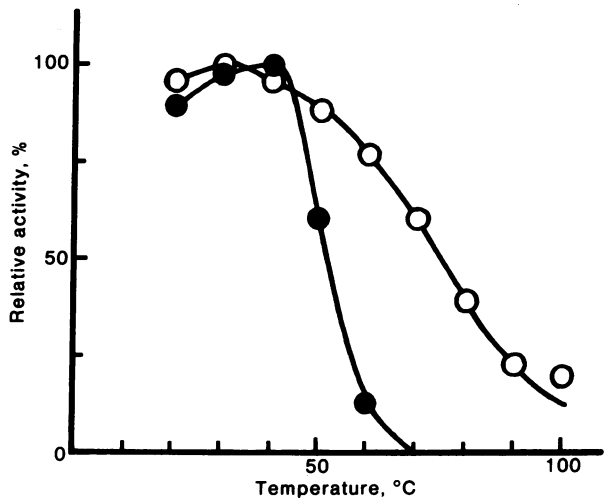

FIG. 2. Thermostability of the intact alga. ○, PS-I; ●, PS-II.

Fig. 2 illustrates the thermostability of the intact algae. The cell suspension in hot spring water was incubated at various temperatures for 10 min. After incubation, the temperature was adjusted to 40°C and the activities of PS-I and PS-II were assayed. PS-I was found to be active and stable in the range of 20°–60°C, and still active after heat treatment at 100°C for 10 min. The optimal temperature of PS-II was 40°C. The optimal pH for O_2 evolution was pH 8, which was the pH of the hot spring water (data not shown).

Fig. 3 shows a current/potential curve upon illumination of the PVA/algae film deposited on the SnO_2 electrode as the working electrode. Increasing output of the photocurrent was observed with increase of potential. The specific output value

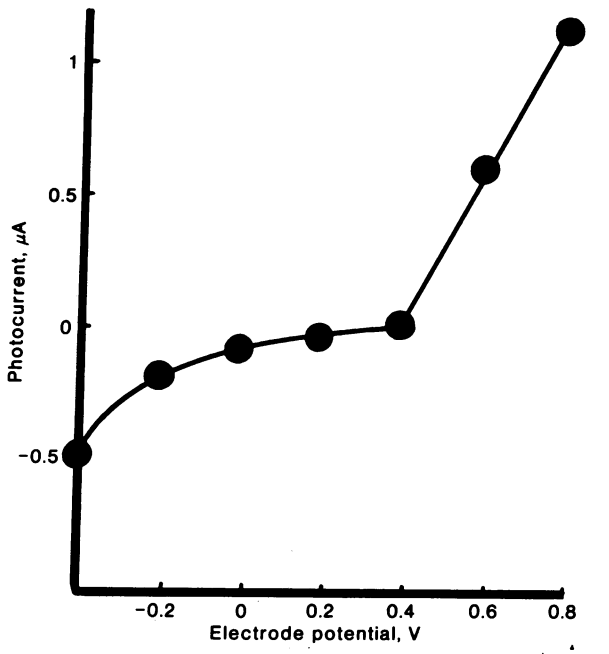

FIG. 3. Photocurrent–potential curve with the PVA/algae film deposited on an SnO_2 optically transparent electrode, with a Y-46 filter, which cuts off light of wavelength shorter than 460 nm. Photocurrent is shown as μA per 10 μg of chlorophyll per cm^2. The electrode potential is in V (against a standard calomel electrode).

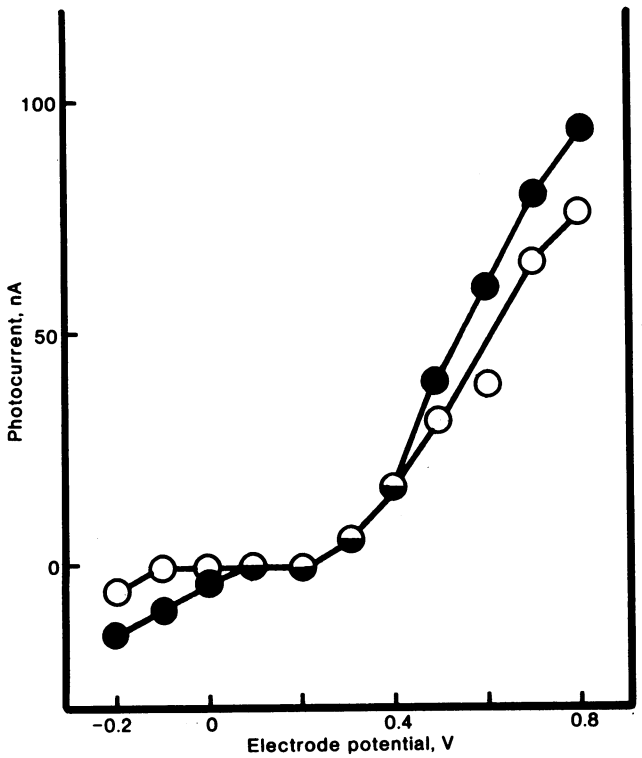

FIG. 4. Photocurrent–potential curves with calcium alginate/algae fixed on an SnO_2 optically transparent electrode, with (○) and without (●) a Y-46 filter. Photocurrent is shown as nA per 10 μg of chlorophyll per cm^2. The electrode potential is in V (against a standard calomel electrode).

on the basis of chlorophyll a concentration per cm^2 was similar to that of the chloroplast electrode reported previously (1). No decay of the photocurrent was observed for at least 30 min after repeated use of the film but some loss occurred after several days, indicating that the PVA-immobilized photosystems of the algae, especially PS-II, might be inactivated under experimental conditions.

Fig. 4 illustrates the photocurrent/potential curves of the living algae electrode immobilized with calcium alginate in the conventional electrochemical cell, with and without a Y-46 filter which cuts off wavelengths shorter than 460 nm. At a potential of 0.8 V against a standard calomel electrode, ≈80 nA was observed with the filter and ≈100 nA without the filter, on the basis of 10 μg of chlorophyll per cm^2. Thus, this electrode generated anodic photocurrent on illumination in a manner similar to the chloroplast electrode. It is likely that the low yield of photocurrent was due to imperfect contact of the algal cells with the surface of the SnO_2 electrode because the calcium alginate plate was 250 μm thick, that is, 10 times thicker than the PVA/algae film. The magnitude of the photocurrent is also dependent on the light intensity used, the density of the chlorophyll deposited on the SnO_2 electrode, the illuminated area, the nature of electrolyte solution, etc. We are attempting to determine the best conditions for photocurrent yield and longevity of activity.

Fig. 5 shows time courses of the photocurrent output at the calcium alginate/algae electrode illuminated continuously in the culture medium and the chlorophyll accumulation in the calcium alginate matrix. When the algae plate is illuminated in the culture medium, the algae remain alive and grow in the

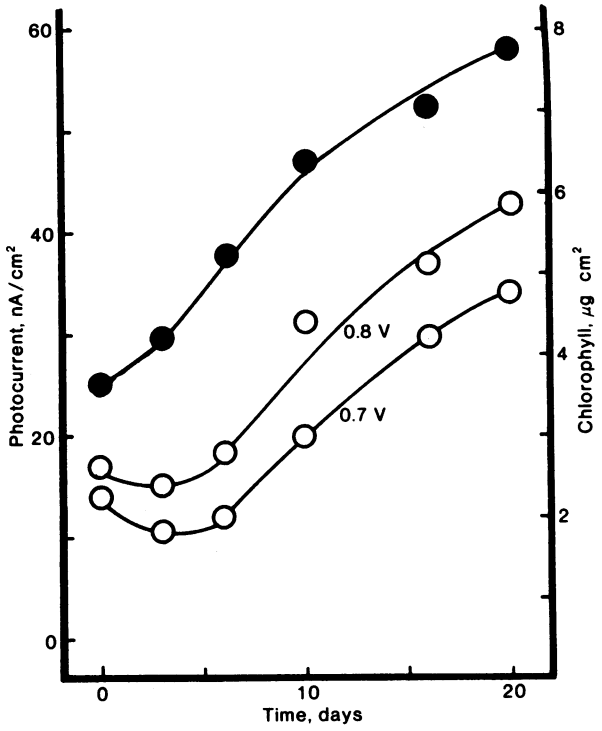

FIG. 5. Time courses of photocurrent output at the calcium alginate/algae electrode (against a standard calomel electrode) under continuous illumination and of chlorophyll accumulation in the calcium alginate matrix. ○, Photocurrent; ●, chlorophyll.

alginate matrix, as shown by the chlorophyll accumulation curve. In fact, the algae electrode is able to generate steady photocurrent upon continuous illumination for 20 days or more, indicating that the algae electrode could function as a long-lived photoconverter. We call an algae electrode of this kind a "living electrode."

The living electrode holds promise as an electron-donating system for the production of hydrogen by the use of hydrogenase coupled to the thylakoid photosystems, especially for the scheme using the two-stage system. However, it is still necessary to develop an immobilizing material that has higher electric conduction to achieve more efficient photoconversion.

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