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Water Biophotolysis System Using Cyanobacterial Electrode

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By using living cyanobacterial electrode as a working electrode, hydrogen production was performed through water-biophotolysis with two-stage, three-electrode apparatus. Electrically reduced methyl viologen in the cathode vessel worked as a substrate of hydrogenase to evolve hydrogen gas in the presence of both phenazine methosulfate and NADH under concomitant supply of electric current.

Hydrogen production *via* water-photolysis is still an important project for future. Over the years, a variety of approaches have been attempted for biophotolysis of water. Since the first demonstration by Benemann *et al*,[1] both chloroplast and hydrogenase preparations have been often employed as biomaterials. In 1979, Yagi and Ochiai proposed a promissing setup for hydrogen production by using "two-stage apparatus",[2] which could protect the hydrogenase from poisonous oxygen evolved by chloroplast electrode. Alternatively, we have used "thermostable living chloroplast" in place of labile chloroplast preparations.[3] Living photosystems are stabilized by cellular dynamic processes. They have always "active oxygen removing system" *in vivo*, such as ascorbate peroxidase, glutathione peroxidase and catalase systems. In 1983, we reported that *Phormidium lapideum*, a strain of thermophilic cyanobacteria, immobilized on SnO_2 optically transparent electrode can work as a working electrode to generate steady current on illumination.[4] This result prompted us to perform a hydrogen production system by water biophotolysis using the living cyanobacterial electrode and hydrogenase preparations. Here, we wish to report successful result of the biophotolysis to get hydrogen gas.

Figure 1 shows a schematic diagram of the apparatus used for biophotolysis. Based upon the data so far obtained about cyanobacterial electrode,[3-5] optimal anodic condition was settled as described in the legend. Cyanobacterial electrode using *Phormidium lapideum* as a photobioreactor was freshly prepared and used with an illumination area of 80 cm^2. Excitingly, the output capacity of the photocurrent increased by 100-fold through the drying of the living electrode. Moreover, the photocurrent yield was more than doubled by the addition of Vitamin K_3 (2-methyl 1,4-naphthoquinone, 10 μM in final concentration) into the anode vessel.[4] In the cathode system, glassy carbon electrode was selected based upon

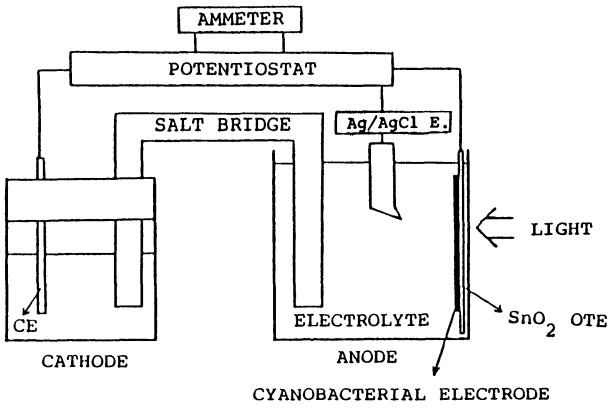

Fig.1. Water biophotolysis system.

Potentiostat: +0.8 V vs. Ag/AgCl electrode
CE: Glassy carbon electrode
Electrolyte: H_3BO_3-KCl-Na_2CO_3 (pH 8.0, 45 °C) plus Vitamin K_3
Light intensity: 250 J/m^2

Table 1. Hydrogen evolution from electrically reduced methyl viologen by addition of various additives

	Hydrogenase	MV(red)[a]	Additives	H_2↑
1.	-	+	$Na_2S_2O_4$	-
2.	+	-	$Na_2S_2O_4$	-
3.	+	+	-	-
4.	+	+	$Na_2S_2O_4$	+
5.	+	+	$Na_2S_2O_4$(ox)[b]	+
6.	+	+	$Na_2S_2O_5$	+
7.	+	+	Na_2SO_3	-
8.	+	+	Na_2SO_4	-

a) Electrically reduced. b) O_2-bubbled.
Each additives: 15 mM in final concentration.

comparative study on the electrode materials (data not shown). Hydrogenase was prepared from Rhodospirillum rubrum G-9 according to the report by Kakuno et al.[6] and used with reduced methyl viologen as substrate to produce hydrogen gas. The hydrogenase preparations are stable for use in the presence of 1 M NaCl. Gas phase of the cathode vessel was replaced by argon gas and analyzed at intervals for hydrogen content with gas chromatography (Hitachi GLC 164).

However, we were surprised to find the difficulty that electrically reduced methyl viologen is not capable of substrate of the hydrogenase preparations(3 in Table 1) though $Na_2S_2O_4$-reduced one can work with the same hydrogenase.[7] In this case, methyl viologen was reduced with the cathodic electrode of glassy carbon settled in the conventional three electrode cell system, the electrolyte solution in which was 100 mM Tris-HCl buffer, pH 8.0. Table 1 shows the unique property of electrically reduced methyl viologen. On air-bubbling into the solution, $Na_2S_2O_4$ was oxidized to be unable to reduce methyl viologen. This oxidized $Na_2S_2O_4$ solution, however, is capable of hydrogen production with electrically reduced methyl viologen. Moreover, hydrogen gas was evolved on addition of $Na_2S_2O_5$ which never reduce methyl viologen.[7] Figure 2 illustrates the courses of hydrogen evolution as a function of time after the addition of $Na_2S_2O_4$ and $Na_2S_2O_5$, respectively, to the electrically reduced methyl viologen solution containing the hydrogenase preparations. These results suggest that each reagent dissociating SO_2^- anion could reactivate the hydrogenase that was deactivated to be incapable of hydrogen production with electrically reduced methyl viologen as illustrated below. With this in mind, we considered that it is necessary to pretreat the hydrogenase with any reagents in place of SO_2^- anion for the water-splitting hydrogen production using the living electrode.

$$Na_2S_2O_4 \rightleftharpoons 2Na^+ + S_2O_4^{--} \rightleftharpoons 2SO_2^-$$

$$Na_2S_2O_5 \rightleftharpoons 2Na^+ + S_2O_5^{--} \rightleftharpoons SO_2^- + SO_3^-$$

$$\text{Hydrogenase (inactive)} \xleftarrow{SO_2^-} \text{Hydrogenase (active)}$$

Fortunately, we found that the hydrogenase in the cathode vessel can be activated and stabilized only when both phenazine methosulfate and NADH are present in the cathode vessel under concomitant supply of electric current. This implies a downhill reaction from cathodic electrode through phenazine methosulfate to NAD^+ moiety. The resulting NADH moiety, especially nascent one, may be able to reactivate the hydrogenase protein. In 1962, one of us(H.O.) had found that O_2-sensitive oxygenase, metapyrocatechase, could be reactivated by the addition of a variety of reducing agents such as $FeSO_4$, $NaBH_4$, $Na_2S_2O_4$, SH-compounds, riboflavin plus NADH, respectively, especially under anaerobic conditions.[8] This process could be repeated, and the results indicated that deoxygenation from the surface of the enzyme molecule may be necessary for the reactivation reaction to reduce the oxygenase-bound iron. This is also the case for the hydrogenase reactivation. Based upon our careful experiments, the following reaction medium is found to be the best for the cathodic hydrogen production: hydrogenase in Tris-HCl buffer(0.1 M, pH 8.0), NaCl 1 M, methyl viologen(MV) 5 mM, phenazine methosulfate(PMS) 30 μM, NADH 10 mM, 8 ml in total.

Figure 3 is the results obtained by using 56 units of the hydrogenase. With

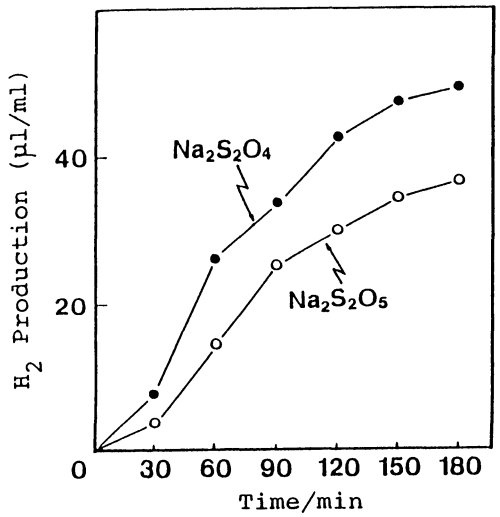

Fig.2. Time courses of H_2-evolution.
Reaction mixture contains hydrogenase(ca. 10 units), methyl viologen(reduced, 5 mM), $Na_2S_2O_4$ or $Na_2S_2O_5$(15 mM) in Tris-HCl buffer(pH 8.0, 0.1 M) with concurrent electron supply by potentiostat(-0.8 V vs. Ag/AgCl electrode).

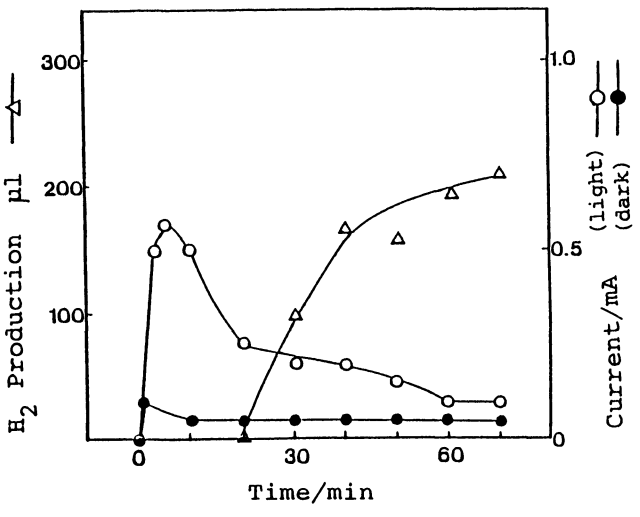

Fig.3. H_2-production by use of cyanobacterial electrode.

the onset of illumination to the cyanobacterial electrode (250 J/m^2) methyl viologen was reduced to show blue-green color and, indeed we observed the electric current from about 600 μA at first to 100 μA at final, as shown by white circle in the Fig.3. This is the case of hydrogen evolution from 30 min after the onset of the light. However, without illumination, no hydrogen production was observed in spite of concomitant supply of electric current *via* potentiostat. In fact, in this case, a little current(from about 100 μA to 50 μA) gave a little amount of reduced methyl viologen. As shown in Fig. 3., hydrogen evolution rate gradually decreased especially beyond 60 min. This depends upon the "reversible" inactivation of the living algal electrode, as demonstrated previously.[4] Thus, the conversion efficiency of 0.011% is obtained using the cyanobacterial electrode-hydrogenase system. In one experiment using a much amount of fresh hydrogenase(375 units) we found that hydrogen evolution occurs without phenazine methosulfate and NADH, though efficiency was very low. In the hydrogenase preparations used here may be present a small amount of active hydrogenase. Thus, the water biophotolysis was performed by using the living electrode. In order to get higher efficiency of the hydrogen production, hydrogenase should be immobilized and fixed on the cathodic electrode plate, if possible, together with polymeric(insoluble) methyl viologen.

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