Note

Production of Molecular Hydrogen by a Semi-continuous Outdoor Culture of *Rhodopseudomonas sphaeroides*

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As reported previously,¹⁾ we carried out an outdoor batch culture for hydrogen production using a selected strain, *Rhodopseudomonas sphaeroides* B5, which was isolated in the Bangkok area, Thailand.²⁾ In the batch culture, the rate of hydrogen production decreased in the later period of the incubation. Therefore, we performed a semi-continuous culture in which a part of the culture medium was replaced with fresh medium at suitable

intervals. First, we used strain B5 under laboratory conditions. It was found that the rate of hydrogen production in a semi-continuous culture decreased with time. Therefore, we examined another thermostable photosynthetic bacterium, *Rhodopseudomonas sphaeroides* B6, which was also isolated in the Bangkok area.³⁾ We found that strain B6 was more stable than B5 with respect to the rate of hydrogen production. In this paper, we report the semi-continuous culturing of strain B6 for hydrogen production under outdoor conditions.

The semi-continuous culture was conducted in a polyacrylate culture vessel, which was similar to the one described before¹⁾ except for the size (light receiving area, 34 cm × 30 cm; 5 cm in width). The vessel contained 6 liters of culture medium. The culture was started with the modified basal medium of Ormerod *et al.*,⁴⁾ from which *p*-aminobenzoic acid was omitted and to which 25 mM DL-lactate and 5 mM L-glutamate were added.

A flow meter (wet type, model WK-0.5; Shinagawa Co., Ltd., Tokyo) was connected to the gas outlet of the culture vessel, with which the rates of and total gas evolution were measured. The evolved gas was analyzed for hydrogen and carbon dioxide with a Yanagimoto G1800 gas Chromatograph as described previously.¹⁾ The amount of hydrogen produced was expressed as its volume under the standard conditions. Bacterial growth was followed by measuring the absorbance at 660 nm ($A_{660\text{ nm}}$) with a

FIG. 1. Time Courses of Hydrogen Production, Lactate Consumption and Cell Density in a Semi-continuous Outdoor Culture.

A: Daily variation in hydrogen production (●) and consumption of lactate (○).

B: Time courses of total hydrogen production (●) and cell density (○). Arrows indicate the times of replacement of the spent medium with fresh medium containing 60 mM DL-lactate and 6 mM L-glutamate (↓), or 150 mM DL-lactate and 30 mM L-glutamate (↑).

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TABLE I. PRODUCTION OF MOLECULAR HYDROGEN BY A SEMI-CONTINUOUS OUTDOOR CULTURE OF *Rhodopseudomonas sphaeroides* B6

Duration of culture (days)	Total amount of lactate supplied (mol)	Remaining lactate (mol)	Wasted lactate (mol)	Lactate consumed (mol)	Hydrogen evolved (liters)	Conversion efficiency (%)
46.3	1.643	0.050	0.099	1.494	125.4	62.5

The highest ambient temperature was about 34°C and the minimum about 13°C.

Hitachi photoelectric colorimeter FPW-4. Lactate was determined colorimetrically by the method of Barker and Summerson.⁵⁾

The semi-continuous outdoor culture was conducted from May 25 to July 10, 1982. The culture vessel was set so that its light receiving plane faced the south and was inclined at an angle of 15°. The replacement of a part of the culture medium (1 liter) with the same volume of fresh medium was performed at 9 p.m. throughout the culture. Two kinds of fresh media were used. One contained 60 mM DL-lactate and 6 mM L-glutamate and the other 150 mM DL-lactate and 30 mM L-glutamate.

Figure 1A shows the daily variation in hydrogen production and consumption of lactate during the culture. Hydrogen production by the outdoor culture was found to be dependent on the illumination intensity and the temperature of the medium.¹⁾ It was again found in the continuous culture that in fine weather (16 days) a large amount of hydrogen was produced, at the rate of 3.5~5.1 liters/day, but in cloudy weather (22 days) the rate decreased to 2.0~3.15 liters/day, and in rainy weather the rate was only 0.3~1.8 liters/day. Figure 1B shows the time courses of the total hydrogen production and changes in cell density during the culture. The cell density increased to a value of approximately 1.4 ($A_{660\text{ nm}}$) with the repeated replacement of the culture medium with fresh medium containing the higher concentrations of lactate and glutamate at the early stage of the culture. The addition of the less concentrated fresh medium after this stage was still sufficient to maintain the cell density at this level. The hydrogen production appeared to be saturated under these conditions, because no significant increase in the production was observed when the more concentrated medium was added at the middle of the culture. The pH of the culture medium remained almost constant (7.0 to 7.3) throughout the incubation. Throughout the culture, the

evolved gas was composed of about 75 to 78% of hydrogen, the remaining gas being carbons dioxide. Table I is a summary of the outdoor semi-continuous culture.

We reported earlier that the maximum amount of hydrogen produced by an outdoor batch culture was about 20 liters/day/33 liter culture.¹⁾ In addition, hydrogen production was observed only for 4 days during incubation for 25 days. This value corresponds to about 3.64 liters/day/6 liter culture. In the present experiment, we had 16 fine days during the culture period of 47 days. The average hydrogen production on these fine days was 3.9 liters/day/6 liter culture. It was concluded that a semi-continuous culture was much better than a batch culture with respect to hydrogen production. To our knowledge, this is the first report of a semi-continuous outdoor culture of a photosynthetic bacterium for hydrogen production.

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