Note

Production of Molecular Hydrogen by a Continuous Culture under Laboratory Conditions

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In a previous paper, we reported on the production of molecular hydrogen by a semi-continuous outdoor culture of *Rhodospseudomonas sphaeroides* B6,¹⁾ which was isolated in the Bangkok area, Thailand.²⁾ During the semi-continuous culture, a part of the culture medium was withdrawn and replaced by fresh medium from time to time. It is obvious that a true continuous culture, in which fresh medium is fed during the daytime, is preferable to a semi-continuous culture. However, the rate of feeding of the fresh medium is a problem in a continuous outdoor culture, because the rate of consumption of the electron donor, and therefore the rate of hydrogen production, varies depending on the strength of illumination and

temperature of the cultivation. To improve the efficiency of hydrogen production, we examined the conditions for a continuous culture including selection of bacterial strains and the effect of the illumination interval.

Rhodospseudomonas sphaeroides B6 was used throughout this work. As reported previously,¹⁾ this strain is more stable in a semi-continuous culture than *R. sphaeroides* B5, which was used for the outdoor batch culture.³⁾ As a basal medium, the modified Ormerod medium⁴⁾ was used, except that *p*-aminobenzoic acid was omitted. Two kinds of media were employed. Medium A was the basal medium with 25 mM DL-lactate and 5 mM L-glutamate added. Medium B contained 47 mM DL-lactate and 10 mM L-glutamate.

For the continuous culture under laboratory conditions, we used the apparatus for hydrogen production shown diagrammatically in Fig. 1. A glass bottle (light receiving area, 22 × 14 cm; width, 5 cm), containing 1,000 ml of culture medium, was connected to a medium reservoir and a receiver for the spent medium. Sampling of the medium was performed from the top of the bottle. The medium was stirred by a Teflon-coated magnetic bar (2.7 cm in length and 0.9 cm in diameter, and at about 200 rpm). After inoculation, the surface of the culture medium was sealed with liquid paraffin. The bottle was incubated in a glass water bath at 33°C under illumination (*ca.* 30 klux) by two 200 W incandescent bulbs. A wet-type flow meter (model WK-0.5; Shinagawa Co., Ltd., Tokyo) connected to the gas outlet of the bottle was used to measure the total and the rate of gas evolution. The evolved gas was analyzed for hydrogen and carbon dioxide with a Yanagimoto G1800-T gas chromatograph as described

FIG. 1. Apparatus for Continuous Culture for Hydrogen Production.

1) medium inlet; 2) medium outlet; 3) sampling needle; 4) gas outlet.

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previously.³⁾ The amount of hydrogen produced was expressed as its volume under standard conditions. Bacterial growth was followed by measuring the absorbancy at 660 nm (A_{660} nm) with a Hitachi photoelectric colorimeter, FPW-4. The concentrations of L-glutamate in the media were determined enzymatically with L-glutamate dehydrogenase,⁵⁾ and DL-lactate was measured colorimetrically by the method of Barker and Summerson.⁶⁾

The culture under uninterrupted illumination was started using medium A. After incubation for 48 hr, fresh medium (A) was supplied at the dilution rate of 0.0411 hr^{-1} . As shown in Fig. 2, the steady state of the culture was maintained at $A_{660} \text{ nm}$ approximately 1.0,

this value corresponded to 0.6 mg cell dry weight/ml of the culture. The concentration of lactate in the culture medium ranged between 3.8 and 6.2 mM, which corresponded to 84.8 to 75.2% consumption of the supplied lactate, respectively. Almost no L-glutamate was detected in the culture medium. The pH of the medium almost remained completely constant at 7.2 to 7.3 throughout the experiment. Hydrogen was produced at the rate of 70 to 86 ml/hr per culture (average, 1,874 ml/day). The composition of the evolved gas was 78% hydrogen and 22% carbon dioxide on average throughout the culture period. The average rate of hydrogen production was 125.8 ml/hr/g dry cells and the average conversion efficiency of consumed lactate to hydrogen was 68.8% of the theoretical

FIG. 2. Hydrogen Production by a Continuous Culture without Interruption of Illumination.

Rate of hydrogen production (●), cell density (○), concentrations of lactate (△), and L-glutamate (□).

FIG. 3. Hydrogen Production by a Continuous Culture under Illumination for 12 hr Intervals.

Cultures with and without stirring were started using medium B. After incubation for 96 hr, fresh medium (B) was supplied to both cultures at the dilution rate of 0.015 hr^{-1} . The hydrogen production results in the dark periods are omitted from the time courses in both cases.

A: With stirring. Stirring of the culture was stopped during the dark periods.

B: Without stirring. The symbols are the same as those in Fig. 2.

value, that is, 6 mol of hydrogen were produced per mol of lactate.

Zürer and Bachofen⁷⁾ reported that on continuous culture of *Rhodospirillum rubrum* under laboratory conditions 1,250 to 1,550 ml of hydrogen was produced per day per liter culture medium at the rate of 20 ml/hr/g dry cells (the culture contained 3.0 to 3.5 mg dry cells/ml), with a yield of 65%. We obtained more hydrogen per liter and a much higher specific activity. This may have been due to the use of a more active strain and of stronger illumination in the present study.

In order to examine the effect of the illumination interval on hydrogen production, we conducted cultures of strain B6 under periodic illumination for 12 hr intervals with and without stirring. In the dark period, feeding of the culture medium was stopped for both culture conditions.

Using medium B, the steady state of both cultures was obtained with a dilution rate of 0.015 hr^{-1} , and at $A_{660 \text{ nm}}$ = around 1.3 in the stirred culture and at $A_{660 \text{ nm}}$ = around 1.15 in the culture without stirring. As shown in Fig. 3, about 840 and 700 ml of hydrogen per liter per day was produced with conversion efficiencies of 73.9 and 61.6% in the cultures with and without stirring, respectively. There was a short lag time before hydrogen production in both cultures at the beginning of illumination.

Since hydrogen production continued at almost the same rate under repeated periodic illumination, even

without stirring, the results indicate that this bacterium is suitable for daytime continuous outdoor culture for hydrogen production.

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