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Biological production of H_2 from glucose by the chemoheterotropic facultative bacterium, *Rhodopseudomonas palustris* P4

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ABSTRACT

Rhodopseudomonas palustris P4 was studied for H_2 production from glucose in batch culture. Important conditions studied include phosphate concentration, initial pH, temperature, glucose concentration, and gas-phase replacement. Optimal H_2 production was observed at 60 – 300 mM of phosphate and 7.8 – 8.6 of initial pH. The effect of culture temperature was negligible. When glucose concentration increased from 0.1 to 5% (w/v), H_2 production increased up to 2% and remained constant thereafter. Intermittent purging of the reaction bottle with Ar gas stimulated the H_2 production by alleviating the inhibition by H_2. The maximum productivity was 111.1 ml H_2/h-l.

INTORDUCTION

Hydrogen is a useful energy source with favorable characteristics such as high efficiency, easy transportation and storage. It is also a clean energy, leaving H_2O as the only combustion product, and expected to draw more attentions in the future.[1] Many strains have been investigated for the biological production of H_2.[2,3,4] However, most microorganisms generally grow slowly, were sensitive to oxygen inhibition, or require light for growth.

Recently, a novel chemoheterotrophic bacterium, *Rhodopseudomonas palustris* P4 was isolated and characterized.[5] In this study, batch culture conditions were optimized for the H_2 production of P4. These results will be of use for evaluating the potential of P4 in utilization of organic carbon for H_2 production.

MATERIALS AND METHODS

Miroorganism and Culture Conditions

Rhodopseudomonas palustris P4 was cultivated in the modified PFN (+) containing bacto-tryptone and yeast extract. H_2 production was carried out at 30°C in a 165 ml serum bottle (working volume, 50 ml) sealed with a butyl rubber septum and aluminum cap (Wheaton, USA). After inoculation with exponentially growing cells at 10% (v/v), the bottles were flushed with Ar gas for 4 min and incubated for 24 h in a rotary shaking incubator at 250 rpm. H_2 production was monitored by GC.

Analyses

Cell density was determined by measuring absorbance at 600 nm[5]. The hydrogen content was determined by a gas chromatograph equipped with a thermal conductive detector and a 6 ft x 1/8-in stainless-steel column packed with Molecular sieve 5A (80/100 mesh, Alltech, USA). The temperatures of oven, injector and detector were 80, 90, and 120°C, respectively. Ar was used a carrier gas at the flow rate of 30 ml/min.

RESULTS AND DISCUSSION

Fig. 1 shows the time course profiles of the typical batch culture of P4. Both cell growth rate and H_2 production rate was initially high but lowered after 4 h, mainly due the decrease in pH. Further decrease of pH during fermentation inhibited significantly both cell growth and H_2 production. This indicates that the pH change is an important factor affecting H_2 production of P4.

Fig. 2 shows the effect of phosphate concentration on cell growth, final pH, and H_2 production. At a low phosphate concentration of 10 – 30 mM, the final pH decreased significantly from the initial pH of 7.6 to 3.9 – 4.2 and, subsequently, cell growth and H_2 production were inhibited. As the phosphate concentration increased in the range of 50 - 300 mM, the final pHs were maintained above 5.5 and, H_2 production and cell growth was improved. The optimal concentration was determined to be 180 mM, and this concentration was employed in the following experiments.

Fig. 3 shows the effect of initial pH. When initial pH increased from 6.6 to 8.7, final pH gradually increased from 6.4 to 7.2. With increasing initial pH, cell concentration and H_2 production also slightly increased and optimal pH was observed in the range of 7.8 – 8.6. The effect of incubation temperature was studied in the range of 25 to 36°C. Cell concentration, final pH, and H_2 production were almost constant regardless of temperature (data not shown).

Fig. 4 shows the effect of glucose concentration. When glucose concentration increased from 0.10 to 2.00% (w/v), both final cell concentration and H_2 production gradually increased from 0.33 mg/ml and 17.0 ml H_2 to 1.80 mg/ml and 94.1 ml H_2. The accumulation of H_2 produced in the serum bottle might inhibit the further production of H_2, but considering the final pH was same as 5.2 for both the glucose concentrations of 2% (w/v) and 5% (w/v), these results seem to be attributed to the inhibition by the lowered pH. The final pH, on the contrary, gradually decreased in this range from 7.4 to 5.2. At the high glucose concentration of 5% (w/v), no more

improvement in cell growth and H_2 production was observed.

Fig. 5 shows the effect of gas-phase replacement. The H_2 production rate was initially high but lowered after 12 hr, mainly due to the decrease in pH (data not shown). When bottles were purged by Ar gas, H_2 production was stimulated in comparison to that of the not-purged. Also, as increasing the number of purging, the H_2 production was more stimulated. The maximum H_2 production with Ar gas purging was 111.1 ml H_2/h-l, which was about 1.5-fold higher than that without purging. This indicates that the high partial pressure of H_2 suppresses its H_2 production activity of P4 to a considerable extent. The H_2 inhibition has been reported in the literature many times.[6)]

In summary, we studied the H_2 production from glucose by *R. palustris* P4 in batch culture. The maximum productivity of H_2 at 24 h under the optimal conditions (phosphate of 60 – 300 mM, initial pH of 7.8 – 8.6, 30°C, glucose of 2% (w/v), and intermittent gas-phase replacement) was 111.1 ml H_2/h-l. More experiments to evaluate P4 for the large-scale H_2 production from organic carbon sources are under progress.

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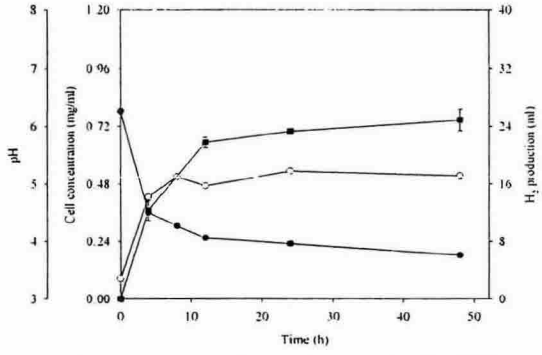

Fig. 1. Time course profiles of cell concentration (○), pH (●), and H_2 production (■). Experimental conditions: phosphate of 10 mM, 30°C, glucose of 0.5% (w/v), and initial pH of 6.2.

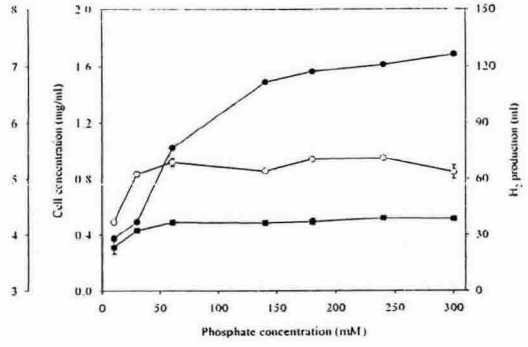

Fig. 2. Effect of phosphate concentration on cell concentration (○), final pH (●), and H_2 production (■). Experimental conditions: 30°C and glucose of 0.5% (w/v), and initial pH of 7.5.

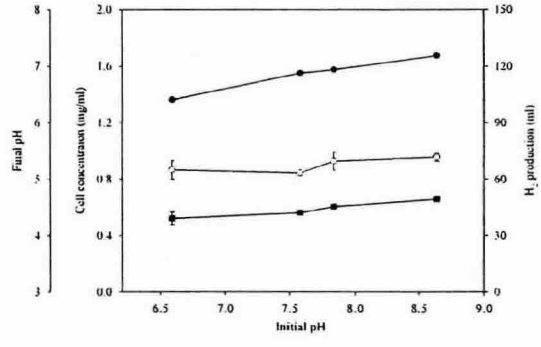

Fig. 3. Effect initial pH on cell concentration (○), final pH (●), and H_2 production (■). Experimental conditions: phosphate of 180 mM, 30°C, and glucose of 0.5% (w/v).

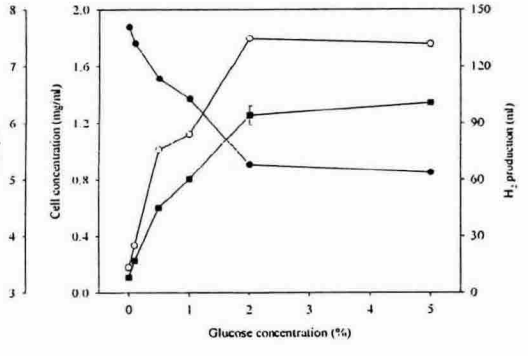

Fig. 4. Effect of glucose concentration on cell concentration (○), final pH (●), and H_2 production (■). Experimental conditions: phosphate of 180 mM, initial pH of 7.8, and 30°C.

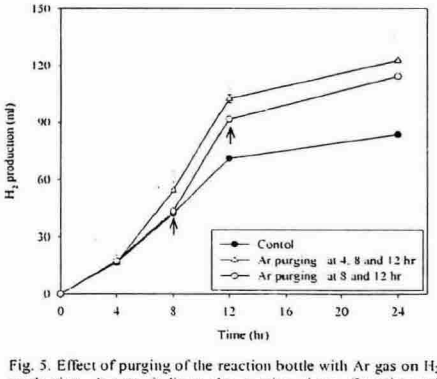

Fig. 5. Effect of purging of the reaction bottle with Ar gas on H_2 production. Arrows indicate the purging times. Experimental conditions: phosphate of 180 mM, initial pH of 7.8, 30°C, and glucose of 2% (w/v).