P429

# Effect of environmental and nutritional conditions on $H_2$ production from glucose by the chemoheterotropic facultative bacterium, *Citrobacter* sp. Y19

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## ABSTRACT

*Citrobacter* sp. Y19 was studied for $H_2$ production from glucose in batch culture. Important conditions studied include phosphate concentration, temperature, glucose concentration, and gas-phase replacement. Optimal $H_2$ production was observed at 140 – 180 mM of phosphate and 36°C. 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 observed to be 113.2 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] Extensive studies on biological $H_2$ production have been carried out and many microorganisms, including photosynthetic organisms or strict anaerobes, have been isolated.[2,3,4] However, most microorganisms generally grow slowly, are sensitive to oxygen inhibition, or require light for growth.

We have isolated a novel chemoheterotrophic bacterium, *Citrobacter* sp. Y19 from an anaerobic sludge digester. Y19 was a facultative anaerobe, grew fast, and effectively catalyzed the gas-shift reaction that produced $H_2$ from CO and $H_2O$.[5]

In the present study, we report the production of $H_2$ from glucose instead of CO and $H_2O$. The effect of environmental and nutritional conditions on $H_2$ production was studied. These results will be of use for evaluating the potential of Y19 for the $H_2$ production from organic carbon sources.

## MATERIALS AND METHODS

### Miroorganism and Culture Conditions

*Citrobacter* sp. Y19 was cultivated in the modified PFN (+) medium which contained bacto-tryptone and yeast extract.[5] $H_2$ 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 $H_2$ 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 profile of the batch culture of Y19 in the modified PFN (+) medium. Both cell growth rate and $H_2$ production rate was initially high but lowered after 4 h, mainly due the decrease in pH. Furthermore, the low pH of 4.0 at 24 h completely stopped the production of $H_2$. The pH drop is attributed to the accumulation of short-chain organic acids and seems to be the most important factor affecting $H_2$ production of Y19.

Fig. 2 shows the effect of phosphate concentration on cell growth, final pH, and $H_2$ production. At a low phosphate concentration of 10 mM, the final pH was very low as 3.9 due to the drastic change in pH during the cultivation. The lowered pH during batch culture resulted in the severe inhibition in cell growth and $H_2$ production. As the phosphate concentration increased above 50 mM, the pH change was much stabilized and, $H_2$ production and cell growth was improved to a great extent. The optimal concentration was determined to be 140 mM, and this concentration was employed in the following experiments.

Fig. 3 shows the effect of culture temperature. With increasing temperature in the range of 25 – 36°C, $H_2$ production gradually increased. Final pH and cell growth were not affected by the temperature change except for the case of 36°C where cell growth was reduced by about 25%.

Fig. 4 shows the effect of glucose concentration. When glucose concentration increased from 0.10 to 2.0% (w/v), both final cell concentration and $H_2$ production gradually increased from 0.34 mg/ml and 15.3 ml $H_2$ to 1.75 mg/ml and 91.2 ml $H_2$. The final pH, on the contrary, gradually decreased in this range from 7.5 to 4.7. At the high glucose concentration of 5% (w/v), no more improvement in cell growth and $H_2$ production was observed. 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 4.7 for both the glucose concentrations of 2% (w/v) and 5% (w/v), these results seem to be mainly attributed to the inhibition by the lowered pH.

Fig. 5 shows the effect of gas-phase replacement. The $H_2$ production rate was initially high but lowered after 8 h, 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 113.2 ml $H_2$/h-l, which was about 1.4-fold higher than that without purging. This indicates that the high partial pressure of $H_2$ suppresses its $H_2$ production activity of Y19 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 *Citrobacter sp.* Y19 in batch culture. The maximum productivity of $H_2$ at 24 h under the optimal conditions (phosphate of 140 – 180 mM, 36℃, glucose of 2% (w/v), and intermittent gas-phase replacement) was 113.2 ml $H_2$/h-l. More experiments to evaluate Y19 for large-scale $H_2$ production from organic carbon sources are under progress.

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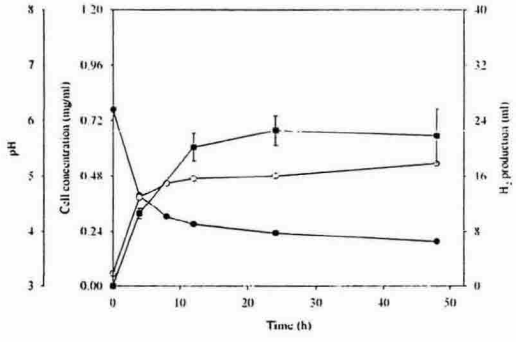


Fig. 1. Time course profiles of cell concentration (○), pH (●), and $H_2$ production (■). Experimental conditions: phosphate of 10 mM, 30°C, glucose of 0.5% (w/v), and initial pH of 6.2.

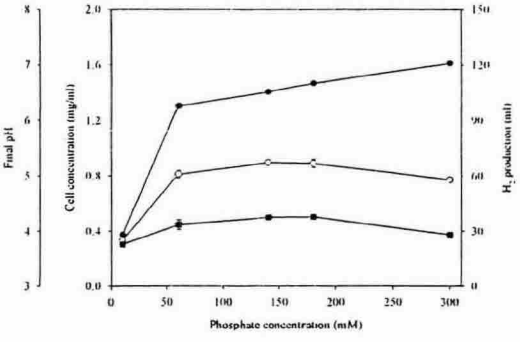


Fig. 2. Effect of phosphate concentration on cell concentration (○), final pH (●), and $H_2$ production (■). Experimental conditions: 30°C and glucose of 0.5% (w/v), and initial pH of 7.5.

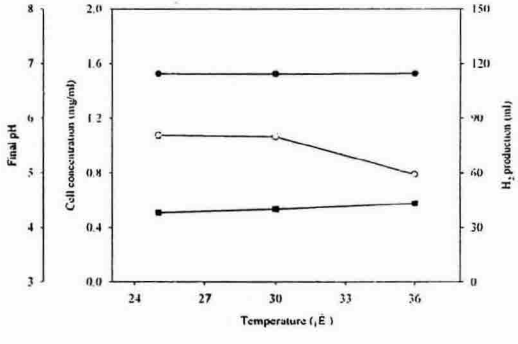


Fig. 3. Effect of temperature on cell concentration (○), final pH (●), and $H_2$ production (■). Experimental conditions: phosphate of 180 mM, 30°C, initial pH of 7.8, and glucose of 0.5% (w/v).

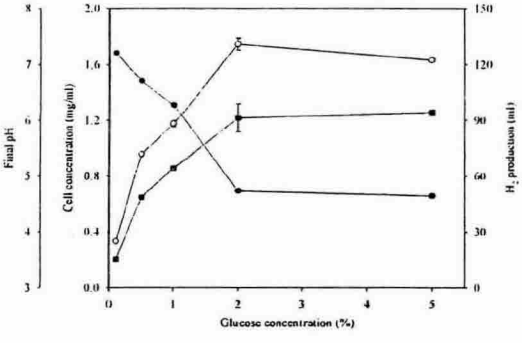


Fig. 4. Effect of glucose concentration on cell concentration (○), final pH (●), and $H_2$ production (■). Experimental conditions: phosphate of 180 mM, initial pH of 7.8, and 36°C.

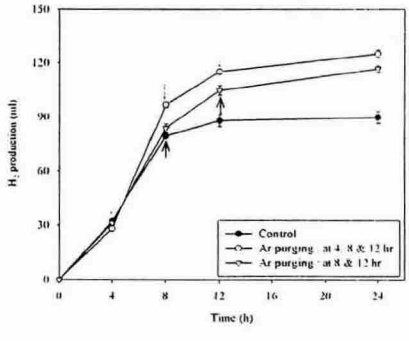


Fig. 5. Effect of purging of the reaction bottle with Ar gas on $H_2$ production. Arrows indicate the purging times. Experimental conditions: phosphate of 180 mM, initial pH of 7.8, 36°C, and glucose of 2% (w/v).