Characteristics of fermentative hydrogen production by the chemoheterotrophic bacterium, *Citrobacter* sp. Y19

Eun-hee Seol, You-Kwan Oh, Sang kil Lee, and Sunghoon Park*
Department of Chemical Engineering, Pusan National University, Pusan 609-735, Korea
TEL (051) 510 - 3049, FAX (051) 515 - 2716

ABSTRACT

Fermentative hydrogen production by *Citrobacter* sp. Y19 was investigated in batch culture. Optimal hydrogen production activity was observed at pH 6 – 7 and temperature of 36 °C, and hydrogen yield and maximal hydrogen production rate were 1.12 mmol/mmol glucose and 32.3 mmol/g cell·h, respectively. With glucose as a substrate, the bacterium produced ethanol, acetate, and carbon dioxide as major glucose fermentation by-products. Y19 could utilize various sugars such as galactose, fructose, lactose, sucrose, and starch for cell growth and hydrogen production.

INTORUCTION

Hydrogen is highly efficient energy that is easy to transport and store. It is considered the cleanest energy because the combustion product is only water, and is getting more attentions with time.[1] Many strains have been investigated for the biological production of H_2,[2,3,4] but generally grow slowly, are sensitive to oxygen inhibition, or require light for growth. Recently, a new chemoheterotrophic bacterium *Citrobacter* sp. Y19 was isolated and characterized.[5,6] In this study, fermentative H_2 production from various sugars using Y19 was investigated in batch culture. Carbon mass balance and H_2 conversion yield were also presented. These results will be of use to better understand the potential of Y19 for the mass H_2 production.

MATERIALS AND METHODS

Miroorganism and Culture Conditions

A mineral salt medium supplemented with 3g of yeast extract/l MSYE and various sugars was employed.[5] Batch cultivations were performed at 36 °C (unless stated other wise) in a gyratory incubator at a shaking speed of 250 rpm. A serum bottle of 165 ml (working volume, 50 ml) was used. After inoculation, the bottle was flushed with argon gas for 5 min for anaerobic condition and sealed with a 12 mm-thick butyl rubber and aluminum cap.

Measurement of specific H_2 production activity

The specific H_2 production activity (mmol/g cell·h) was determined under various temperature and pH. The cells grown in MSYE supplemented with 140 mM phosphate and 10 g glucose/l

were harvested at 6h by centrifugation, washed once with 140 mM phosphate buffer solution, and resuspended in MSYE containing 10 g glucose/l and various buffer (see below). Then, 2.0 ml of the cell suspension was placed in an 8.0 ml serum vial with butyl rubber septum and aluminum cap. All the procedures except for centrifugation were conducted in an anaerobic chamber. The H_2 production was monitored for 1 h, while shaking the vial at 100 strokes/min in a water bath. For determining the pH dependence of the H_2 production activity, different buffer solutions were used as follows (all in 140 mM): acetate buffer (pH 4.0 and 5.0), phosphate buffer (pH 6.0 and 7.0), and Trizma base buffer (pH 8.0 and 9.0).

Analyses

Cell concentration was determined by measuring absorbance at 600 nm.[6] Glucose was analyzed by enzymatic method. The gas concentration (H_2 and CO_2) was determined by GC equipped with a thermal conductive detector.[6] Organic acids and ethanol in culture broth were also analyzed by GC, but equipped with a flame ionization detector.[7]

RESULTS AND DISCUSSION

Figs. 1 and 2 show the effect of temperature and pH on the specific H_2 production activity. In order to get a maximum H_2 production activity without the influence from the accumulation of organic acids or gas production such as H_2 and CO_2, initial rate during 1 h was measured. Fig. 1 shows that, with increasing temperature from 25 to 36 °C, H_2 production activity (q) increased from 6.95 ± 0.10 to 12.88 ± 0.77 mmol/g cell·h. At the higher temperature of 40 °C, the activity decreased considerably. The activation energy from Arrhenius plot (Insert in Fig. 1) was estimated as 10.24 kcal/mol, a little lower value than the one reported for *Enterobacter cloacae* IIT-BT 08 as 12.84 kcal/mol.[8] Fig. 2 shows that the specific activity is the highest at pH 6 –7 as of 10.57 – 11.67 mmol H_2/g cell·h. Especially, it should be noticed that no H_2 production activity is observed at pH 4.0. . This is in a well agreement with the previous results[6] and clearly shows that the maintenance of proper pH is the most critical for the successful H_2 production with the strain Y19.

Fig. 3 shows the time course profiles and kinetics of batch fermentation under the partially optimized.[6] MSYE supplemented with 180 mM phosphate and 8.5 g glucose/l was used as culture medium. In addition to cell mass and H_2, various fermentation products such as CO_2, ethanol and organic acids were monitored to analyze the quantitative fate of the carbon consumed. Exponential cell growth was observed from the beginning and the maximum rate was estimated to be 0.694 ± 0.014 h^{-1}. The pH gradually decreased but remained above 5.5 throughout the experiment. Chromatographic analyses exhibited ethanol and acetic acid are the

major by-products of H_2 fermentation. Their concentrations gradually increased in a similar trend with the cell growth and H_2 production. The production of higher organic acids such as propionic, n-butyric, and n-valeric acids was very low (data not shown). The H_2 conversion yield was 1.12 mmol/mol glucose. The maximum rates of H_2 production and glucose consumption could be estimated from the initial rate as 32.3 ± 0.1 mmol/g cell ·h and 21.7 ± 3.3 mmol/g cell·h, respectively.

Various sugars were tested as substrates for H_2 production by Y19 (Fig. 4). Concentration of each sugar was fixed at 8.5 g/l and phosphate concentration was at 140 mM. Simple sugars such as galactose and fructose showed a similar trend with glucose in H_2 production and cell growth. The final H_2 production and the H_2 yield, however, were slightly higher in the formers. When disaccharides or starch were used as carbon source, the final H_2 production was lower than that of simple sugars.

In summary, we studied the fermentative H_2 production from various sugars with *Citrobacter.* sp. Y19 in batch culture. Optimal hydrogen production activity was observed at pH 6 – 7 and temperature of 36 °C. The H_2 yield and maximal H_2 production rate were 1.12 mmol/mmol glucose and 32.3 mmol/g cell·h, respectively. The H_2 production from various sugars was also observed. These results suggest that Y19 is a promising strain which can be used for large-scale H2 production.

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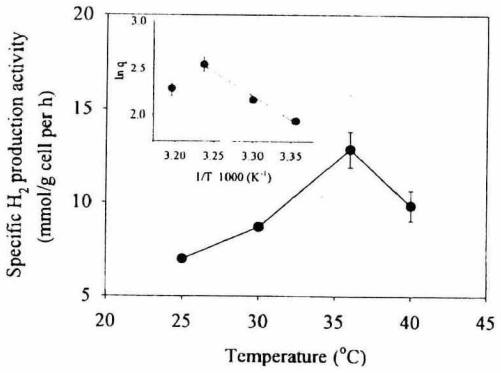

Fig. 1. Effect of temperature on specific H_2 production activity. Insert shows the Arrhenius plot (ln q vs. 1/T) to determine the activation energy

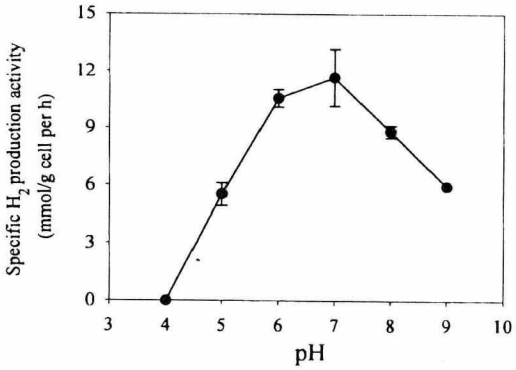

Fig. 2. Effect of pH on specific H_2 production activity

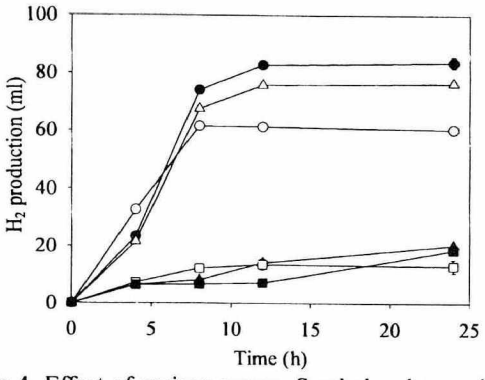

Fig.4. Effect of various sugars. Symbols: glucose (○), galactose (●), fructose (△), lactose (▲), sucrose (□), and starch (■).

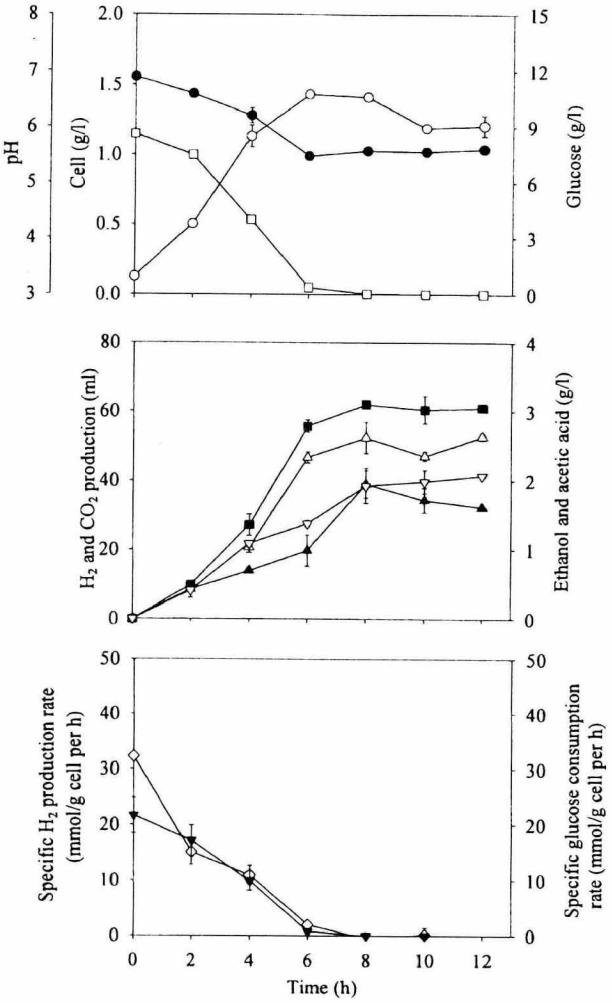

Fig. 3. Fermentation profiles of glucose. Symbols: cell concentration (○), pH (●), glucose (□), H2 (■), CO2 (△), ethanol (▲), acetic acid (▽), specific H2 production rate (▼), and specific glucose consumption rate (◇).