Continuous Hydrogen Production from Starch by a Soil Bacterium Belonging to the Enterobacteriaceae

Osamu Hirayama*, Yasuhiko Kumada** and Yoshiyuki Naruse***

* Department of Food Science and Nutrition, Faculty of Agriculture, Kinki University, Nara 631-8505, Japan.
** Okunots-seihun Inc., 3-7-24, Minamihorie, Nani-ku, Osaka 550, Japan.

Synopsis

A soil bacterium, strain 05B, belonging to the family Enterobacteriaceae, produced hydrogen from heated and non-heated starch with both growing cells and resting cells. Continuous production of hydrogen by both kinds of cells was carried out using a cartridge filter under high cell density conditions. Efficient and stable production of hydrogen by the growing cells was achieved for about 12 days, followed by a gradual decrease in the yield. In contrast, hydrogen production by the resting cells showed a similar initial rate of hydrogen production, followed by a slightly rapid decrease with operation time. The decrease in the hydrogen production rate, however, was easily recovered with the addition of new resting cells. Therefore, when considering the cost of operation and the contamination in the effluent, the resting cell mode appears to be more practical for the hydrogen production accompanied by wastewater treatment.

Introduction

The biological conversion of biomass or organic wastewater to hydrogen is an energy saving process with attracting advantages. In previous paper, we have isolated a bacterial strain, SO5B, which was capable of producing hydrogen gas from starch. The growing cells of the strain produced hydrogen from both heated and non-heated starch, and the hydrogen-producing ability was appreciably tolerant to oxygen. Thus, in the present study, continuous production of hydrogen from starch by the strain has been investigated, to develop a new technique for microbial treatment of industrial wastewater and hydrogen production. For continuous production of hydrogen by microorganisms, their immobilized cells have been frequently employed so far with advantages. In the production of hydrogen from starch by strain SO5B, however, gel trapped cells would be inappropriate, because the high molecular substrates are difficult to penetrate into gel and the organic acids produced by the cells are slow to release from gel, resulting in decrease in the ability of the cells. Therefore, in this study, a new approach for continuous production of hydrogen was performed using a cartridge filter to keep bacterial cells in high concentrations and to remove reaction products.

Materials and Methods

Organism and growth conditions. A new strain SO5B isolated from soil, belonging to...
Enterobacteriaceae) was used. The growth medium (PY medium) used contained the following in 1000 ml of distilled water: peptone 10 g, yeast extract 5 g, L-cysteine-HCl 0.5 g, CaCl₂ 8 mg, MgSO₄ 8 mg, KH₂PO₄ 40 mg, NaHCO₃ 0.4 g, and NaCl 80 mg, supplemented with 10 g of starch as a carbon source. Fifteen ml of the growth medium containing bacterial cells (inoculation, 5% volume) was placed in a 32-ml test tube, and the tube was sealed with a silicone W-cap, flushed with nitrogen gas, and incubated at 36°C with shaking. Hydrogen gas evolved was measured by GLC.²

Hydrogen production in a batch culture by growing or resting cells. Twenty ml of the growth medium were placed in a 50-ml flat-bottom flask with a glass syringe connected to it through a silicone tube. The mixture was inoculated (5% volume) with freshly grown SO5B cell culture, and flushed with nitrogen gas, and incubated with stirring by magnetic stirrer. Hydrogen gas evolved was measured by the glass syringe. For hydrogen production by the resting cells, SO5B cells harvested from the freshly grown cultures by centrifugation, were added to 20 ml of 50 mM phosphate buffer (pH 7.0) in the same 50-ml flask. Incubation and measurement of hydrogen gas evolved were carried out in the same manner as described above.

Apparatus for continuous hydrogen production and operation. To keep bacterial cells in a high concentration, a cartridge filter (TCR-020: ø 45 mm x h 100 mm, pore size 0.2 μm; ADVANTEC, Japan) was used. As shown in Fig. 3, the cartridge filter (A) was set in a reaction vessel (B) (inner volume, 1700 ml). Cell suspension in medium (1000 ml) was placed in the outer space of the cartridge filter. The mixture was stirred by magnetic stirrer (C), and pH was controlled with an automatic pH controller (D) by adding 4 N NaOH solution (E) with a micro-tubing pump (F). In-flow of the substrate solution into the reaction vessel from a substrate solution tank (G) and out-flow from the vessel to a treated water tank (H) were performed synchronously by a micro-tubing pump (I). Hydrogen gas produced was collected in a gas-liquid exchange tank (J) containing 1 N NaOH solution, and the volume of hydrogen

Fig. 3. Apparatus for continuous hydrogen production. A, cartridge filter; B, reaction vessel; C, magnetic stirrer; D, pH controller; E, 4N KOH solution in graduated cylinder; F, pump; G, substrate solution tank; H, treated water tank; I, pump; J, gas-liquid exchange tank containing 1N KOH solution; K, 1N KOH solution in graduated cylinder.
produced was estimated by measuring the volume of released NaOH solution (K). The apparatus was set in a thermostat controlled at 36°C.

**Analytical method.** Organic acids were determined by HPLC on TSKgel SCX column (Tohsoh Corp., Japan). Glucose and starch were assayed by the phenol-sulfuric acid reaction. Cell concentration was determined by measuring optical density at 600 nm.

**Results**

**Hydrogen production from starch by growing cells and resting cells of strain S05E.** In the previous paper, we found that the growing cells of strain S05E was able to produce hydrogen from both heated and non-heated starch efficiently. It was also found that the resting cells produced hydrogen from heated starch as the growing cells did. So, here, we first examined the possibility of hydrogen production from non-heated starch by the resting cells. Fig. 1 shows the results compared with those by the growing cells. The concentration of the resting cells added was approximately similar to that of the growing cells at the stationary phase. The hydrogen production by the resting cells increased a little earlier than that of the growing cells, showing that the maximum peak of the former production rate was at 6h after incubation and the latter was at 9h. The former yield of hydrogen production was about 80% of the latter. Apparently, this slow start of the production in the growing cells was due to small cell numbers at the early incubation period. Fig. 2 shows the hydrogen production by the resting cells from non-heated soluble and natural starch. The rate of conversion to hydrogen from potato starch and soluble-starch were almost similar, while wheat starch and corn starch showed a slower rate.

**Fig. 1.** Hydrogen production from non-heated starch by growing and resting cells. The cells were grown in 500 ml of growth medium containing 1% soluble starch under anaerobic conditions with stirring. The resting cells (600 mg dry weight) were incubated in 500 ml of 50 mM phosphate buffer (pH 6.5) containing 1% soluble starch under the same conditions as those of growing cells. Symbols: ○, • growing cells; △, ▲ resting cells.

**Fig. 2.** Hydrogen production from non-heated starch by resting cells. The incubation conditions were the same as those in Fig. 1.

**Continuous hydrogen production from starch.**

For continuous production of hydrogen, an apparatus shown in Fig. 3 was used and operated as described in MATERIAL AND METHODS. The rate of hydrogen production was dependent on the flow rate or hydraulic retention time (HRT) of substrate solution in the reaction vessel.
Thus, the relationship between the yield of hydrogen production and the flow rate of substrate solution was examined. The resting cells (3 g in dry weight) suspended in 1000 ml of substrate solution (1% soluble-starch in 50 mM phosphate buffer, pH 7.0) were placed in the reaction vessel and bubbled with nitrogen gas for 5 min. The mixture was continuously stirred at 36°C and pH was kept to pH 7.0 by a pH controller. The substrate solution was supplied, increasing the flow rate from 8.3 to 41.6 ml/h (HRT from 5 to 1 day). The results were summarized in Fig. 4. Hydrogen production increased with decrease in HRT, and the substrate consumption rate was maintained to more than 90% until HRT of 2 days. These data suggest that the best flow rate was around 2 days of HRT.

Fig. 5 shows a typical hydrogen production from heated soluble-starch by growing cells. Preculture (at early stationary phase) of 1000 ml was placed in the reaction vessel, and depleted oxygen. After batch incubation for 12 h, continuous incubation was followed by supplying the substrate solution at the same flow rate as in the growing cells. Hydrogen-producing rate rapidly increased and reached the maximum after 2-days incubation. After that the rate decreased slowly through 7-days incubation, but when new
resting cells (1g in dry weight) were added, the rate was recovered to the initial level. The hydrogen production pattern was closely corresponded to the change in cell concentration. The substrate consumption and the production of organic acids also proceeded in a similar pattern.

Fig. 6. Continuous hydrogen production by resting cells.

Discussion

The present data showed that a new approach using a cartridge filter was useful for continuous microbial production of hydrogen from starch. The preliminary study showed that a cartridge filter, ADVANTEC TCR-020 (pore size, 0.2 μm), was able to keep almost all cells in the reaction vessel against flow of the medium (a flow rate of 20 ml/h) for long time, and that the clogging up of the filter did not occurred even at a high flow rate (5 ml/min). On the other hand, the reaction products, organic acids, easily passed through the filter. High molecular starch of the substrate also passed through the filter, but under the conditions in the experiment the loss of starch by leaking was a little, because its biological decomposition proceeded rapidly before released through the filter.

In the present study, continuous production of hydrogen was carried out by two modes of the growing cells and the resting cells. In both modes the substrate solution was continuously supplied without removing dissolved oxygen, because the anaerobic level necessary to SO5B cells seemed to be maintained under the conditions. Both modes showed efficient and stable production of hydrogen from starch. These results should be attributed to the favorable properties of the cartridge filter, in which the bacterial cells were maintained in a high concentration, and the acids produced were released efficiently.

Compared with the growing cell mode, the resting cell mode showed a apparently higher decrease in the hydrogen-producing rate with operation time. Thus, the former mode seems to be somewhat superior to the latter mode in the efficiency and stability of hydrogen production. However, the high decrease in the hydrogen-producing rate was easily recovered by adding new resting cells or new cell culture. And, when the cost of growth medium reagents and the contamination in the effluent in the growing cell mode were considered, the resting cell mode would be more practical especially for treatment of organic wastewater aiming both its purification and energy production.

Acknowledgement

We thank Prof. F. Taguchi, Department of Microbiology, Kitasato University School of Hygienic Science, for his generous gift of an anaerobic strain. This work was partly supported by the Environmental Science Program of Environmental Science Institute of Kinki University.
References


Enterobacteriaceae科嫌気性土壌細菌による
デンプンからの連続的水素生成

平山 修*，熊田泰彦 **，成瀬喜之***

* 近畿大学農学部食品栄養学科
** 奥本製粉株式会社
*** 六甲バター株式会社

要 約

Enterobacteriaceae科嫌気性土壌細菌、SO5B 株、の水素発生能を調べ、デンプンからの連続的水素生成を検討した。生育菌体と同様休止菌体でも、天然生デンプンから水素をよく生成することが分かった。カートリッジフィルターをセットした水素生産装置を用い、菌増殖条件で運転する方法（生育菌体法）と休止菌体を用いて運転する方法（休止菌体法）の2つのモードで連続的水素生成を行った。両者は効率的な水素生成を示したが、後者では水素生産速度の低下が速く進んだ。しかし、この低下は新しい休止菌体を追加することにより容易に回復させることができた。したがって、廃水処理とエネルギー生産の両方を指向する場合、生育菌体法よりも休止菌体法の方が実用的であると考えられる。