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Effects of Hydraulic Retention Time on Hydrogen Production

Young Joon Lee†
National Institute of Environmental Research
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수리학적체류시간이 수소생성에 미치는 영향

이 영 준†
국립환경연구원

요 약

연속반응조에서의 수소생산에 대한 수리학적체류시간(희석율, D)의 영향을 1% sucrose를 함유한 37±1℃ 조건에서 조사하였다. 실험결과 수리학적체류시간(희석율)의 각각의 조건에 따라 생성된 가스중 수소성분은 50~ 71%의 범위로 발생되었다. H_2/CO_2 비율은 희석율이 증가할 때 H_2/CO_2 비율도 증가하였다. 최대수소생성 수율은 희석율 0.14 1/h까지는 증가하다가 이후에는 감소하였고, 수소생성 수율은 0.81 *l*/g sucrose이었다. Acetate 생성 수율은 butyrate 생성 수율 보다 희석율 조건변화에 민감하게 변화하였다. propionate 및 solvents는 희석율 변화에 영향을 받지 않았다. biomass 수율은 희석율이 0.2 1/h까지는 증가하였으나, 그 이상의 조건에서는 감소하였다.

주요어: hydrogen production, HRT, solvents, sucrose, volatile fatty acids

I. Introduction

The limited supply and the probability of future increases in petroleum prices has initiated interest in chemical and fuel production from biomass fermentations. The future of hydrogen as a clean and sustainable energy sources is receiving wide attention in both political and technical circles.

Especially, biological hydrogen production from renewable biomass and waste water represent an important development in the area of bioenergy production. Practically, some hydrogen producing bacteria are able to grow on cheap plant substances containing carbohydrates. *Clostridium perfringens* strain C was proposed for the production of hydrogen from carbohydrates.[1] Recently some investigators have been using natural anaerobic microorganisms, taken from sludge compost, to generate hydrogen from sugary wastewater in a chemostat culture.[2] On the other hand, an interesting feature that the saccharolytic hydrogen producing bacteria share with many other fermentative organisms is the ability to form a number of products (ethanol, acetoin, butanol, propanol, acetic and butyric acids, and gaseous hydrogen and carbon dioxide), depending on the microorganisms and environmental conditions used. The variety of chemicals such as hydrogen, acids and neutral solvents, produced in clostridial fermentations is one of the factors that has brought them to prominence as bacteria with biotechnological potential. The influence of environmental factors such as pH and hydraulic retention time (HRT), have been claimed to enhance continuous hydrogen/solvents production.[2-4] Optimization of culture conditions are only partly understood.

The purpose of this study is to determine the optimum HRT(dilution rate, *D*) value on hydrogen production and to determine the VFAs and solvents distribution in hydrogen fermentation using sucrose as a model of substrate.

†Corresponding author : National Institute of Environmental Research
Tel : 011-348-1893, Fax : 82-62-970-3988
E-mail : 8djoon@hanmail.net

II. Materials and Methods

1. Microorganism and media

The organism, which was isolated from soybean-

meal obtained from a silo which exploded owing to the accumulation of biological produced hydrogen, was used for batch experiments. This microorganism was cultivated at a temperature of 37± 1°C, pH 5.0 and HRT of 10 hours by being fed with 20 g/*l* sucrose. In this condition, hydrogen content in biogas and volatile suspended solids (VSS) in effluent was 35-40%, 1.3 g/*l* respectively. Methane was not found in biogas. The culture medium contained the following components per liter of distilled water: NH_4HCO_3, 2 g; KH_2PO_4, 1 g; $MgSO_4 \cdot H_2O$, 100 mg; NaCl, 10 mg; $Na_2MoO_4 \cdot H_2O$, 10 mg; $CaCl_2 \cdot H_2O$, 10 mg; $MnSO_4 \cdot H_2O$, 15 mg; sucrose, 10 g.

2. Experimental system

A schematic diagram of the experimental set up for the hydrogen fermentation of sucrose is shown in Fig. 1. The reactor was a 15-cm internal diameter, 23-cm-long plexiglass tube with a working volume of 3.0 liters. The temperature was controlled at 37±1°C using an air-bath incubator. A complete-mix condition was achieved by a powerful magnetic mixer at an agitation speed of approximately 300 revolutions/min (rpm) for minimizing attachment of microorganisms on the fermentor vessel components. The pH was controlled by a pH controller. Both potassium hydroxide (5N KOH) and hydrochloric acid (2N HCl) were combined with influent flow before entering the reactor to adjust its pH value. The former were provided by micro tube pumps and the latter was passed through a ceramic pump to adjust the hydraulic retention time (HRT) of the reactor. The amount of biogas produced was recorded daily using a wet gas meter. A biogas sampling port was installed between the meter and the reactor to allow direct biogas sampling with a syringe.

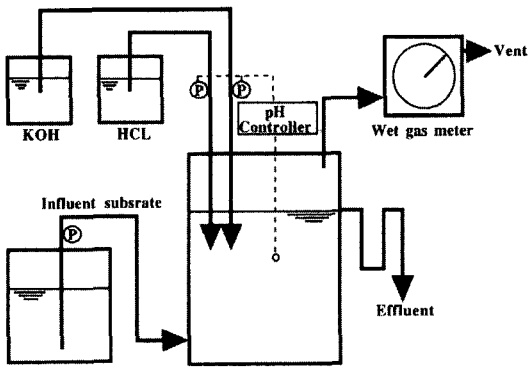

Fig. 1. A schematic view of chemostat reac-tor system.

3. Analytical methods

Gas samples were taken from the fermenter using a pressure-lock gas tight syringe. The sample was analyzed for its hydrogen content in a Shimadzu 8A gas chromatograph equipped with a thermal conductivity detector (TCD). The separation was effected in column packed with Porapak Q 50/80 mesh (GL Sciences). Nitrogen was used as the carrier gas at a flow rate of 30 m*l*/min. The column, injection port, and detector block were maintained at 100, 70 and 100°C, respectively. The detector current was set to 80 mA. Methane and carbon dioxide were determined by gas chromatography (Shimadzu 8A), using TCD detector and column packed with Porapak T (50/80 mesh). The operational temperatures of the injection port, the oven and the detector block were the same as those for the hydrogen analysis. The concentrations of the major acids produced by the fermentation were determined using FID detector and column packed with Unisole F-200 (30/60 mesh) (GL Sciences). The operational temperatures for the injection port, the oven and the FID were 170, 145 and 170°C, respectively. The components were eluted from the column in the following order: acetic acid, propionic acid, i-butyric acid, butyric acid, i-valeric acid and valeric acid. The solvents, including ethanol, propanol and butanol, were analyzed using FID detector and column packed with Gaskuropack 54 (60/80 mesh). The operational temperatures for the injection port, the oven and the FID were 200, 185 and 200°C, respectively. Helium was used as the carrier gas for the determinations of VFAs and solvents at a flow rate of 30 m*l*/min. Calibrations based on peak height were made using a standard solution containing all the components listed above. Samples and standards were injected into the column using a Hamilton model 701 Microliter syringe. The pH of samples were determined by a TOA pH meter. The concentrations of volatile suspended solids (VSS) were determined by the procedures described in Standard Methods.[5] Carbohydrate analysis was deter-

mined by the phenol-sulfate method,[6] and was measured spectrophotometrically at 490 nm (Hitachi, model 100-20). Glucose concentration in the samples was then determined by comparing the optical density reading with the standard glucose solutions' calibration curves.

4. Experimental procedure

In the experiment on the influence of HRT (dilution rate, *D*), the feed substrate concentration was maintained at pH 6.5 and 10 g/*l* sucrose throughout, and was investigated at different HRTs (3, 5, 7, 10 and 12 hours).

III. Results and Discussion

The gas phase consisted of hydrogen and carbon dioxide; no methane could be detected depending on the dilution rates. The solute products consisted of VFA (acetic acid, propionic acid, and butyric acid) and solvent (ethanol, propanol and butanol). There was significant difference in the yield of the culture at different dilution rates.

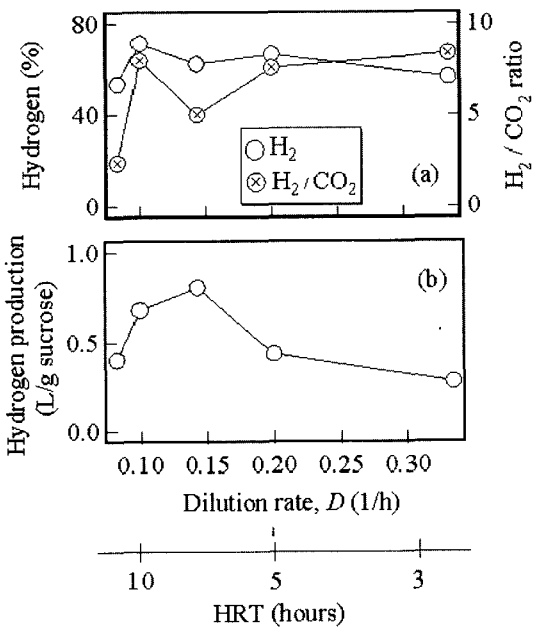

Fig. 2. Influence of the HRT on hydrogen production. (a) hydrogen contents and H_2/CO_2 ratio for the produced biogas, (b) hydrogen production yield.

1. Hydrogen

The hydrogen production of this fermentor are shown in Fig. 2. The hydrogen content for the produced gas was changed from 50 to 71% with corresponding dilution rates throughout this experiment. The produced hydrogen/carbon dioxide ratio was increased with increases in the dilution rate (Fig. 2a). However, the hydrogen production yield increased up to dilution rate 0.14 1/h, then decreased with increases in the dilution rate. The maximal hydrogen yield was found to be 0.81 *l*/g sucrose at dilution rate 0.14 1/h (Fig. 2b). In terms of sucrose consumption, the sucrose was completely consumed at all of the dilution rates examined. When the dilution rate was increased to more than 0.2 1/h, a small amount of sucrose remained in the effluent. van Andel *et al.*[7] reported that the NADH-ferredoxin oxidoreductase system is accelerated at high dilution rate. According to Jungermann *et al.*[8] this enzyme system is activated by an increased level of acetyl-CoA in cell-free extracts of *C. pasteurianum*. Decker *et al.*[9] reported for *C. kluyveri* that an increased flux through a bacterial system resulted in an increased level of acetyl-CoA. If this argument holds for hydrogen-producing microorganisms used in this study as well, this is a possible explanation for the higher hydrogen production rate measured with increasing dilution rates.

2. Volatile Fatty Acids, Solvents and Biomass

In Fig. 3, the VFAs and solvents production yields are shown as function of the dilution rate. The major accumulated components were acetate and butyrate. Fig. 3(a) showed that acetate production yields were more related to the dilution rate than butyrate production yields. Hydrogen gas production among strict anaerobic bacteria, therefore, appears to be associated with butyric acid production. As the dilution rate was increased during growth of *C. butyricum* on glycerol, acetyl-CoA accumulated and the carbon flux switched from butyrate to acetate formation; it appeared likely that this was due to limiting activity of the enzyme thiolase which did not change. The concentration of NADH also increased under these conditions. Acetate might be an indirect acceptor of hydrogen not releasable by hydrogenase in the fermentations of carbohydrates. Thus the observed

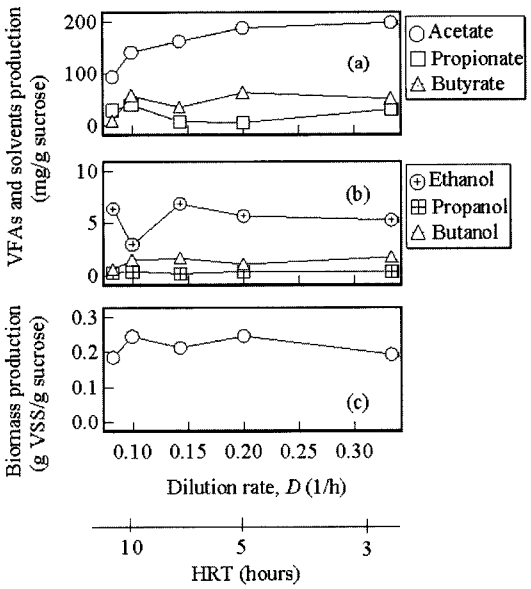

Fig. 3. Influence of the HRT on metabolite production yield in hydrogen. (a) volatile fatty acids, (b) solvents, (c) biomass.

hydrogen gas production was higher than on glucose, indicating that at least part of the NADH + H^+ regenerated through the ferredoxin-hydrogenase system, an important fraction still had to be oxidized through the butyrate pathway through additional acetate. Propionate production had no significant difference on the yield of the culture at different dilution rates. On the other hand, solvent production was very low in comparison to the VFAs production (Fig. 3b). This means that high solvent production is obtained at sufficiently low dilution rate (0.03 1/h).[10] The biomass (VSS) measured were depicted in Fig. 3(c). The VSS yield increased up to dilution rate 0.2 1/h, then decreased with increases in the dilution rate. It is interesting to note that the observed drop in biomass concentration with dilution rates coincides with the main organic acids distribution shift that was considered to be a consequence of microbial population shift.[11] The VSS concentration was 1.65-2.15 g/*l*.

IV. Conclusions

The principle conclusions can be drawn from the results are as follows:

1. The hydrogen content for the produced gas was changed from 50 to 71% with corresponding dilution rates throughout this experiment. The produced hydrogen/carbon dioxide ratio was increased with increases in the dilution rate. However, the hydrogen production yield increased up to dilution rate 0.14 1/h, then decreased with increases in the dilution rate. The maximal hydrogen yield was found to be 0.81 *l*/g sucrose at dilution rate 0.14 1/h.
2. The acetate production yields were more related to the dilution rate than butyrate production yields. Propionate had no significant difference in the yield of the culture at different dilution rates. Solvents had no significant difference with dilution rate. The biomass yield increased up to dilution rate 0.2 1/h, then decreased with increases in the dilution rate.

Acknowledgement

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