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Effects of pH on Continuous Hydrogen Fermentation

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연속반응실험에서 수소생성에 대한 pH 영향

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ABSTRACT

The influences of pH on hydrogen production were also investigated over the pH range from 4.1 to 8.0 at HRT 10 hours. The hydrogen content for the produced gas was changed from 41 to 71% with corresponding pHs throughout this experiment. The produced hydrogen/carbon dioxide ratio was not vary significantly up to 6.0, then steepenly increased with increases in the pH. The maximal hydrogen yield was found to be 3.16 *l*/g sucrose at pH 5.0. Acetate production yield increased with increased pH, but butyrate production yield decreased with increased pH. Biomass yield increased with increased pH.

Keywords: hydrogen production, pH, solvents, sucrose, volatile fatty acids

요 약

연속반응조에서의 수소생산에 대한 pH의 영향을 HRT 10시간으로 유지하고, pH 4.1부터 8.0까지의 범위에서 조사하였다. 실험조건에서의 생성된 수소가스 성분은 41~71% 범위로 발생되었다. H_2/CO_2 비율은 pH 6.0 이상에서는 크게 변화가 없었으나, 대체적으로 pH가 증가함에 따라 H_2/CO_2 비율도 증가하였다. 최대 수소생성수율은 pH 5.0에서 3.16 *l*/g sucrose이었다. Acetate 생성은 pH 증가에 따라 증가하였으나, butyrate 생성은 pH 증가에 따라 감소하였다. 미생물량은 pH 증가에 따라 증가하였다.

I. Introduction

Hydrogen is a carbonfree fuel which oxidizes to water as combustion product. And so hydrogen is considered as one of the candidates to reduce global pollution such as green house effect. Four basic processes are available for the production of hydrogen gas from nonfossil primary energy sources: water electrolysis, thermochemical, radiolytic and biological processes.[1] Electrolytic hydrogen is presently produced competitively for industrial use, but only in areas where cheap electricity is readily available.[2] Therefore, 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.[3] Recently some investigators have been using natural anaerobic microorganisms, taken from sludge compost, to generate hydrogen from sugary wastewater in a chemostat culture.[4]

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 environ-

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mental 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. However, what controls the switch to hydrogen production has not been determined in detail because the regulation of hydrogen/solvent production is complex and dependent on more than one factor.[4,5]

The purpose of this study is to determine the optimum pH value on hydrogen production and to determine the VFAs and solvents distribution in hydrogen fermentation using sucrose as a model of substrate.

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 and procedure

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

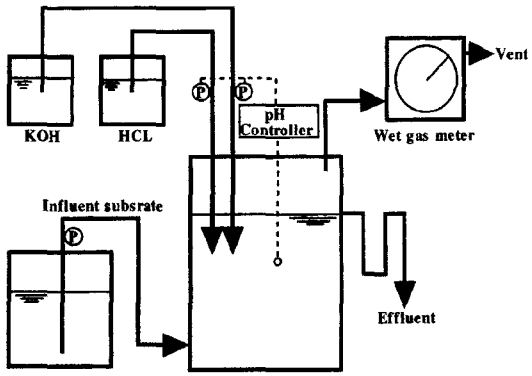

Fig. 1. A schematic view of the chemostat reactor system.

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. In the experiment on the influence of pH, the feed substrate concentration was also maintained at HRT 10 and 10 g/*l* sucrose throughout, and was investigated at different pHs (4.1, 4.5, 5.0, 6.0, 6.5, 7.0 and 8.0).

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 ml/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 ml/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.[7] Carbohydrate analysis was determined by the phenol-sulfate method,[8] 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.

III. Results and Discussion

The gas phase consisted of hydrogen and carbon dioxide; no methane could be detected. The solute products consisted of VFA (acetic acid, propionic acid and butyric acid) and solvent (ethanol, propanol and butanol) depending on the pH values. The following pH values were studied: 4.1, 4.5, 5.0, 6.0, 6.5, 7.0 and 8.0. All these values were imposed at dilution rates of 0.1 1/h.

1. Hydrogen

The hydrogen production of this fermentor under steady state condition are shown in Fig. 2. The hydrogen content for the produced gas was changed from 41 to 71% with corresponding pHs throughout this experiment. The produced hydrogen/carbon dioxide ratio was not vary significantly up to 6.0,

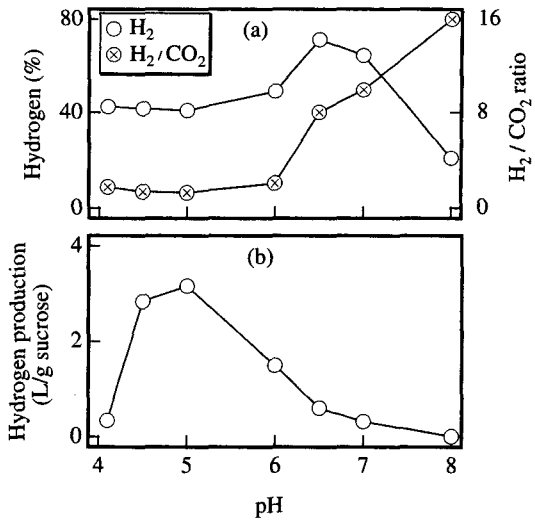

Fig. 2. Influence of the pH values on hydrogen production. (a) hydrogen contents and H_2/CO_2 ratio for the produced biogas; (b) hydrogen production yield.

then steepenly increased with increases in the pH (Fig. 2a). However, the hydrogen production yield increased up to pH 5.0, then decreased with increases in the pH. The maximal hydrogen yield was found to be 3.16 *l*/g sucrose at pH 5.0 (Fig. 2b). At a pH level of 4.1, 88% of the influent substrate was utilized as opposed to its almost total utilization at the higher pH levels. This finding is consistent with the well-known influence of pH on most microbial activities.

2. Volatile fatty acids, solvents and biomass

Fig. 3 shows the VFAs and solvents produced at different pH values. The solute products consisted of VFAs (acetate, propionate and valerate) and solvents (ethanol, propanol and butanol). According to Crabbenendam,[9] these products can be considered normal products of carbohydrate fermentation. For the pH range considered in this study acetate and butyrate were the main products. In all experiments, isobutyrate and valerate amounts were defected, if at all, in trace amounts. The relative amounts of the two major products were found to be strongly dependent on pH. As shown in Fig. 3(a), butyrate predominated below a pH value of 6.0 and acetate predominated above a pH value of 6.5, the region between 6.0 and 6.5 being the

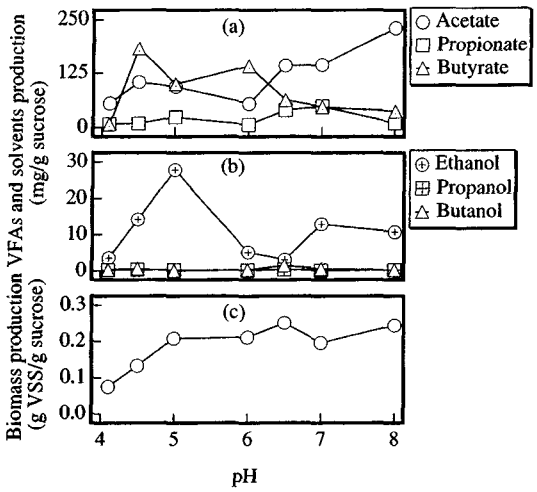

Fig. 3. Influence of the pH values on metabolite production yield in hydrogen. (a) volatile fatty acids; (b) solvents; (c) biomass.

transition range. Zoetemeyer *et al.*[10] reported the transition range was 6.4-7.6 for glucose acidogenesis. Kisaalita *et al.*[11] reported the transition range was 5.0-5.5 for lactose acidogenesis. The reason for the change in major products is not known at present. However, it can be speculated that it is due to either a change in the metabolism of the population or a change in the composition of the population or a combination of both these changes. Based on the product concentrations, it would be expected that a butyric acid bacteria predominated over the pH range considered since their normal products are butyrate and acetate.[12] Pure cultures of strict anaerobic bacteria isolated from primary sewage sludge that produced hydrogen also produced butyric acid, those that did not produce hydrogen did not produce butyric acid.[1] The ethanol production yield changed to the corresponding pH values but the butanol and propanol were relatively stable over a range of pH (Fig. 3b). Fig. 3(c) shows biomass yield as functions of pH. Generally, low microbial biomass yield at low pH is due to lower adenosine triphosphate (ATP) production per mole of substrate utilized.[13] It has also been pointed out that microorganisms display an optimum pH for growth around a pH of 7.0, with the majority favoring the pH range of 5.0-8.0.[14]

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 41 to 71% with corresponding pHs throughout this experiment. The produced hydrogen/carbon dioxide ratio was not vary significantly up to 6.0, then steepenly increased with increases in the pH. However, the hydrogen production yield increased up to pH 5.0, then decreased with increases in the pH. The maximal hydrogen yield was found to be 3.16 *l*/g sucrose at pH 5.0.

(2) Acetate production increased with increased pH, but butyrate production decreased with increased pH. The ethanol production yield changed to the corresponding pH values but the butanol and propanol were relatively stable over a range of pH. Biomass yield increased with increases in the pH.

Acknowledgement

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References

1. Zajic, J. E., Kosaric, N. and Brosseau, J. D. : Microbial production of hydrogen. *Adv. Biochem. Eng.*, **7**, 57-109, 1978.
2. Rajeshwar, K., Ibanez, J. G. and Swain, G. M. : Electrochemistry and the environment. *J. Appl. Electrochem.*, **24**, 1077-1091, 1994.
3. Kondratieva, E. N. and Gogotov, I. : Production of molecular hydrogen in microorganisms. *Adv. Biochem. Eng.*, **28**, 139-191, 1983.
4. Ueno, Y., Otsuka, S. and Morimoto, M. : Hydrogen production from industrialwastewater by anaerobic microflora in chemostat culture. *J. Ferment. Technol.*, **82**(2), 194-197, 1996.
5. Bowles, L. K. and Ellefson, W. : Effects of butanol on *Clostridium acetobutylicum*. *Appl. Environ. Microbiol.*, **50**, 1165-1170, 1985.
6. Dabrock, B., Bahl, H. and Gottschalk, G. : Parameters affecting solvent production by *Clostridium pasteurianum*. *Appl. Environ. Microbiol.*, **58**(4), 1233-1239, 1992.
7. APHA : *Standard methods for the examination of water and wastewater*, 19th ed. American Public

Health Association, Washington, DC, USA, 2-57, 1995.
8. Dubios, M., Gilles, K. L., Hamilton, J. K., Rebers, P. A. and Smith, F. : Colorimetric method for determination of sugars and related substances. *Anal. Chem.*, **28**, 350-356, 1956.
9. Crabbendam, P. M., Neijssel, O. M. and Tempest, D. W. : Metabolic and energetic aspects of the growth of *Clostridium butyricum* on glucose in chemostat culture. *Arch. Microbiol.*, **142**, 375-382, 1985.
10. Zoetemeyer, R. J., van den Heuvel, J. C. and Cohen, A. : pH influence on acidogenic dissimilation of glucose in an anaerobic digestor. *Wat. Res.*, **16**, 303-311, 1982.
11. Kissalita, W. S., Pinder, K. L. and Lo, K. V. : Acidogenic fermentation of lactose, *Biotechnol. Bioeng.*, **30**, 88-95, 1987.
12. Doelle, H. W. : *Bacterial metabolism*, (2nd ed). Academic, New York, 1979.
13. Atkinson, A. and Mavituna, F. : *Biochemical engineering and biotechnology handbook*, Nature, New York, 1983.
14. Bryant, M. P. : Microbial methane production-theoretical aspects. *J. Anim. Sci.*, **48**, 193-201, 1979.