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Effects of pH and Carbon Sources on Biohydrogen Production by Co-Culture of *Clostridium butyricum* and *Rhodobacter sphaeroides*

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To improve the hydrogen yield from biological fermentation of organic wastewater, a co-culture system of dark- and photo-fermentation bacteria was investigated. In a pure-culture system of the dark-fermentation bacterium *Clostridium butyricum*, a pH of 6.25 was found to be optimal, resulting in a hydrogen production rate of 18.7 ml-H_2/l/h. On the other hand, the photosynthetic bacterium *Rhodobacter sphaeroides* could produce the most hydrogen at 1.81 mol-H_2/mol-glucose at pH 7.0. The maximum specific growth rate of *R. sphaeroides* was determined to be 2.93 h^{-1} when acetic acid was used as the carbon source, a result that was significantly higher than that obtained using either glucose or a mixture of volatile fatty acids (VFAs). Acetic acid best supported *R. sphaeroides* cell growth but not hydrogen production. In the co-culture system with glucose, hydrogen could be steadily produced without any lag phase. There were distinguishable inflection points in a plot of accumulated hydrogen over time, resulting from the dynamic production or consumption of VFAs by the interaction between the dark- and photo-fermentation bacteria. Lastly, the hydrogen production rate of a repeated fed-batch run was 15.9 ml-H_2/l/h, which was achievable in a sustainable manner.

Keywords: Biohydrogen, co-culture, dark fermentation, photo fermentation, *Clostridium butyricum*, *Rhodobacter sphaeroides*

Renewable energy is essential from the point of view of socioeconomic and environmental issues. Because of its many advantages, including a lack of carbon dioxide emissions and high energy density, hydrogen has been found to be more environmentally friendly as a potential energy source compared with petroleum and other fossil fuels [1]. Among various techniques for hydrogen production, biological fermentation is a feasible method since it can be carried out even under ambient temperature and pressure using various organic compounds or wastes as an energy source [7].

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There are two types of fermentation processes capable of producing hydrogen [12, 15]. One is a dark-fermentation process, in which anaerobic bacteria such as *Clostridium* spp. produce hydrogen and various volatile fatty acids (VFAs) through acidogenic hydrogenesis [5, 18, 20]. The other is a photo-fermentation process, using photosynthetic bacteria such as *Rhodobacter* or *Rhodopseudomonas* spp., which enable the production of hydrogen from various VFAs at the expense of light energy [4, 10, 17]. The structure of the dark-fermentation system is very similar to a commercial anaerobic digestion process for methane production, so it may be easy to attain commercial access by retrofitting conventional plants [7]. However, the oxygen sensitivity and low hydrogen yield of dark-fermentation bacteria are known to be major drawbacks that have not yet been resolved [13]. Theoretically, *Clostridium* spp. produce four moles of hydrogen from one mole of glucose with residual organic acids, which should be treated further to meet affordable effluent quality. Compared with dark-fermentation bacteria, photo-fermentation bacteria give a higher hydrogen yield and can even utilize VFAs as the sole carbon source [10]. The limitation of not only substrate utilization but also low hydrogen production efficiency of a single stage of the dark-fermentation system would be eliminated. Therefore, some researchers have suggested a co-culture system of dark- and photo-fermentation bacteria [16, 21]. A higher hydrogen yield can be obtained when dark- and photo-fermentative systems are combined. Further consumption of organic acids by photo-fermentation bacteria prevents pH drops in the fermenter owing to the accumulation of fatty acids by dark-fermentation bacteria. In addition, the fermented liquid from the co-culture system satisfies the demand of intensified COD removal that is not met by the single dark fermentation. Despite previous research on the co-culture system, little information about pH and substrates is available in the literature.

Therefore, we examined hydrogen production by pure or co-cultures of dark- and photo-fermentation bacteria after investigating the effects of pH and carbon sources on

the hydrogen production of each system in batch tests. An evaluation of a repeated fed-batch run of the co-culture system based on continuous hydrogen production and fermentation outlet composition was performed.

Materials and Methods

Microorganisms and Media

A dark-fermentation bacterium, *Clostridium butyricum*, and a photo-fermentation bacterium, *Rhodobacter sphaeroides*, were used as the hydrogen-producing microorganisms in this study. These bacteria were purchased from the Korean Culture Center of Microorganisms (KCCM). *C. butyricum* was cultivated at 30°C for 7 h in PYG medium (pH 6.5), which was composed of K_2HPO_4 (0.9 g/l), KH_2PO_4 (0.9 g/l), NaCl (0.9 g/l), $(NH_4)SO_4$ (0.9 g/l), $MgSO_4$ (0.09 g/l), $CaCl_2$ (0.09 g/l), peptone (10 g/l), yeast extract (5 g/l), cysteine·HCl (0.5 g/l), $Na_2CO_3 \cdot 10H_2O$ (4.0 g/l), aminobenzoic acid (100 µl/l), and glucose (10 g/l). This medium was sterilized by autoclaving at 121°C for 15 min before being inoculated with *C. butyricum*. The bioreactor was purged with argon for 10 min to keep the culture system anaerobic.

R. sphaeroides was cultivated in sterilized Sistrom's minimal medium at 30°C for 72 h under tungsten lamps at 5,000 lux [21]. Sistrom's minimal medium was composed of K_2HPO_4 (34.8 g/l) or KH_2PO_4 (27.2 g/l), $(NH_4)_2SO_4$ (5.0 g/l) or NH_4Cl (1.95 g/l), succinic acid (40.0 g/l), L-glutamic acid (1.0 g/l), L-aspartic acid (0.4 g/l), NaCl (5.0 g/l), nitrilotriacetic acid (2.0 g/l), $MgSO_4 \cdot 7H_2O$ (3.0 g/l) or $MgCl_2 \cdot 6H_2O$ (2.44 g/l), $CaCl_2 \cdot 2H_2O$ (0.334 g/l), $FeSO_4 \cdot 7H_2O$ (0.020 g/l), $(NH_4)_6Mo_7O_{24}$ (0.2 ml/l of a 1% solution), trace element solution (1 ml/l), and vitamin solution (1 ml/l). The trace element solution was prepared by adding 1.765 g EDTA, 10.95 g $ZnSO_4 \cdot 7H_2O$, 5.0 g $FeSO_4 \cdot 7H_2O$, 1.54 g $MnSO_4 \cdot H_2O$, 0.392 g $CuSO_4 \cdot 5H_2O$, 0.248 g $Co(NO_3)_2 \cdot 6H_2O$, and 0.114 g H_3BO_3 to 100 ml of distilled water. For the vitamin solution, 1.0 g nicotinic acid, 0.5 g thiamine·HCl, and 0.010 g biotin were added to 1 l of distilled-deionized water and the pH was adjusted to 7.0. The media and solutions were stored at 4°C until use.

Experimental Procedure

A dark-fermentation batch experiment was carried out in an acrylic reactor with a 1 l working volume. Precultivated *C. butyricum* was harvested after centrifugation at 4,000 rpm for 20 min and then resuspended in modified PYG medium (0.1 g dry cell/l). The modified medium was composed of 1.5 g/l KH_2PO_4, 4.2 g/l $Na_2HPO_4 \cdot 12H_2O$, 0.18 g/l $MgCl_2 \cdot 6H_2O$, 0.1 g/l $FeSO_4 \cdot 7H_2O$, 5 g/l peptone, 2 g/l yeast extract, and 10 g/l glucose. The pH of the medium in the bioreactor was 5.5 or 6.25. Evolved hydrogen was collected using Tedlar gas bags, and the volume of gas was measured with an inverted cylinder containing a 10% NaOH solution.

Another reactor with a 1 l working volume was used for a photo-fermentation batch experiment. Precultured *R. sphaeroides* was inoculated into modified Sistrom's minimal medium, where multi-organic acids of acetate, propionate, butyrate, and lactate replacing succinic acid as the carbon source were input. Glutamic acid at 10 mM was also added instead of $(NH_4)_2SO_4$ as the nitrogen source. Different runs at pH 6.25 and 7.0 were carried out to determine the practical operation conditions for a single culture of *R. sphaeroides*. Then, acetate or glucose was used as the sole carbon source for photo fermentation at pH 6.25 in order to investigate the effects of various substrates on hydrogen production. The photo-fermentation experiment was carried out under an illumination of 5,000 lux and 30°C after the replacement of the gas phase in the reactor with argon [21].

Co-culture of *C. butyricum* and *R. sphaeroides* was accomplished using modified PYG medium containing 10 g/l glucose as the sole carbon source in a 1 l acrylic reactor. A repeated fed-batch run of the co-culture system (pH 6.25) was conducted at 30°C and a light intensity of 5,000 lux.

Analytical Methods

The hydrogen in the evolved biogas was analyzed using gas chromatography (Agilent 7890, USA) equipped with a capillary column (Agilent 19095P-MS6; 300°C, 30 m, 535 µm, 25 µm) and a thermal conductivity detector. The operation temperature of the oven, injector, and detector was 50, 200, and 250°C, respectively. Helium was used as the carrier gas at a flow rate of 11.15 ml/min.

VFAs in the fermentation effluents were also measured on a gas chromatograph equipped with a capillary column (Restek 922303; 260°C, 30 m, 530 µm, 0.25 µm) and a flame ionization detector. Helium gas was flowed as the carrier gas at a splitting ratio of 10:1. The temperature of the oven was increased to 145°C at a rate of 20°C/min and thereafter maintained at 95°C for 2 min, and then increased again to 200°C at a rate of 50°C/min. Injector and detector termperatures were set at 200 and 240°C, respectively.

The glucose concentration was detected by the 3,5-dinitrosalicylic acid method according to previous literature [2]. The cell concentration in the medium was determined as suspended solid (SS) according to standard methods [3].

Results and Discussion

Effects of pH on Dark- and Photo-Fermentation Processes

Fig. 1 shows the concentrations of SS and glucose as well as the accumulated amount of hydrogen evolved at different pH values in a *C. butyricum* pure-culture system. As

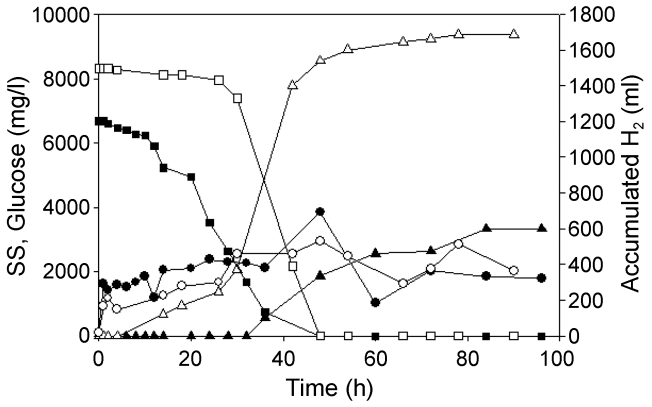

Fig. 1. Time-dependent concentrations of SS (●, ○) and glucose (■, □) as well as accumulated amount of hydrogen evolved (▲, △) in a *C. butyricum* pure-culture system at pH 5.5 (closed symbols) and pH 6.25 (open symbols).

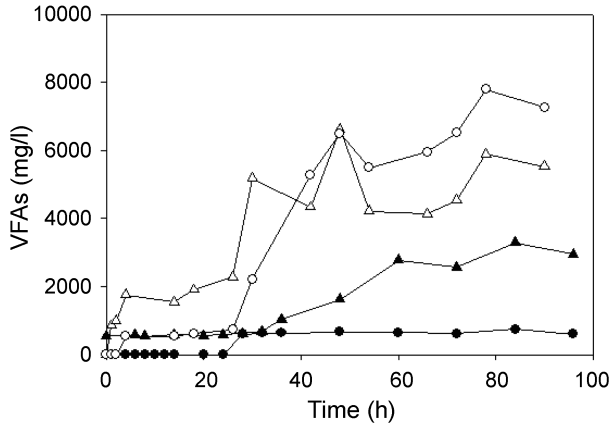

Fig. 2. Time-dependent concentrations of butyric acid (●, ○) and acetic acid (▲, △) in a *C. butyricum* pure-culture system at pH 5.5 (closed symbols) and pH 6.25 (open symbols).

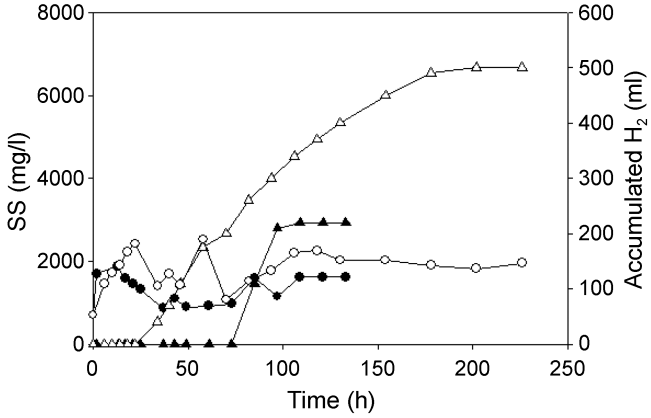

Fig. 3. Time-dependent concentration of SS (●, ○) as well as accumulated amount of hydrogen evolved (▲, △) in an *R. sphaeroides* pure-culture system at pH 6.25 (closed symbols) and pH 7.0 (open symbols).

expected, the pH strongly influenced hydrogen production as well as glucose consumption by the dark-fermentation bacterium. At pH 6.25, 1,680 ml of hydrogen could be produced, whereas 600 ml of hydrogen was evolved at pH 5.5. In addition, a long lag phase for hydrogen production was observed at pH 5.5. Therefore, pH 6.25 was preferable for hydrogen production by *C. butyricum*. At this pH, the highest hydrogen production rate and yield were determined to be 18.7 ml-H_2/l/h and 1.22 mol-H_2/mol-glucose, respectively. Alalayah *et al*. [2] also reported that the maximum hydrogen production rate by *Clostridium* sp. was attained at an initial pH of 6.0 (±0.2). This result is meaningful in view of the possibility that *C. butyricum* can be co-cultivated in a pH range of 6.5 to 7.0, which is preferred by photo-fermentation bacteria for the production of hydrogen [21].

During acidogenic hydrogenesis of sugars, various kinds of metabolites are known to be generated along with evolved hydrogen [11]. In this study, the main metabolites of the dark-fermentation process were found to be acetic and butyric acids (Fig. 2), but other VFAs such as lactic and propionic acids were not detected by our analytical method. It is interesting to note that butyric acid was present at a very low concentration, below 700 mg/l, at pH 5.5, but was detected at a very high concentration, above 7,000 mg/l, at pH 6.25. This result could be explained on the basis of a metabolic pathway of dark fermentation with sugars. Butyric acid has been reported to be one of the end-products of dark fermentation by *Clostridium* spp. [9, 11]. At a low pH of 5.5, the fermentative activity of *C. butyricum* must have been inhibited metabolically, resulting in the low rate of conversion of glucose into acetic or butyric acids in this study (Fig. 1 and 2).

In the case of the photo-fermentation process, VFAs have been reported to be efficient carbon sources for hydrogen production [9, 19]. Thus, a VFA mixture composed of acetic acid (2 g/l), butyric acid (1 g/l), lactic acid (2 g/l), and propionic acid (1 g/l) was used as the carbon source for photo-fermentative hydrogen production by *R. sphaeroides* in this study. Fig. 3 and 4 show the time-dependent

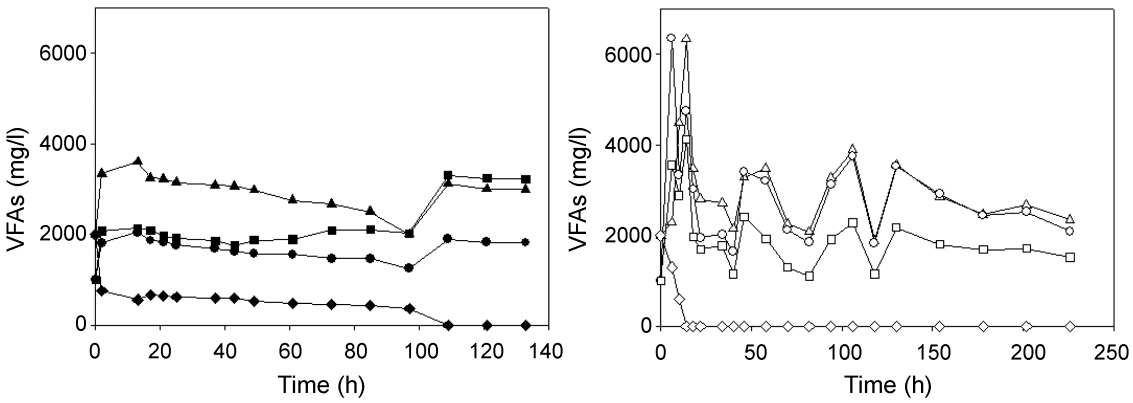

Fig. 4. Time-dependent concentrations of propionic acid (●, ○), butyric acid (■, □), acetic acid (▲, △), and lactic acid (◆, ◇) in an *R. sphaeroides* pure-culture system at pH 6.25 (closed symbols) and pH 7.0 (open symbols).

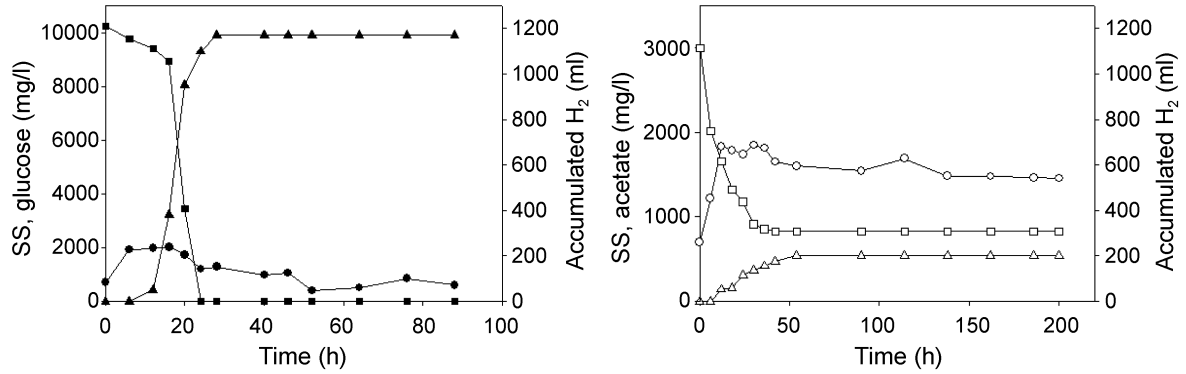

Fig. 5. Time-dependent concentrations of SS (●, ○), acetic acid (□), and glucose (■) as well as accumulated amount of hydrogen evolved (▲, △) in an *R. sphaeroides* pure-culture system at pH 6.25.

concentrations of SS and VFAs as well as the accumulated amount of hydrogen evolved in the *R. sphaeroides* pure-culture system at different pHs. More hydrogen could be produced at pH 7.0 and there was no lag phase (Fig. 3). The hydrogen production rate and yield at pH 6.25 were 0.80 and 1.81 mol-H_2/mol-glucose, respectively. The values at pH 7.0 were determined to be 1.65 and 2.21 ml-H_2/l/h, respectively. This result was coincident with those of other researchers. Yokoi *et al*. [21] reported that the photo-fermentative production of hydrogen from acetic acid was optimal in a pH range of 6.5 to 7.0. Thus, it could be concluded that neutral pH is preferable for hydrogen production by a pure culture of the photo-fermentation bacterium *R. sphaeroides*. However, an acidic pH range of 5 to 6 has been known to be preferable for the dark-fermentation process based on both hydrogen production and hindrance of methanogenic activity [6–8, 14]. Hence, it was found that a pH of 6.0 (±0.25) was reasonable in considering the preferences of both dark- and photo-fermentation bacteria.

As shown in Fig. 4, the concentration of lactic acid decreased significantly, while that of the other VFAs (especially acetic acid) increased dramatically in inverse proportion. This result implies that lactic acid was easily degraded by *R. sphaeroides* and converted into the smaller molecular acids. At pH 7.0, it was observed that the other VFAs were gradually consumed after the depletion of lactic acid. It has been reported that *R. sphaeroides* consumed acetic acid first, followed by propionic acid and then butyric acid in a VFA mixture [19].

Effect of Carbon Source on Photo-Fermentation Process

Besides VFAs, photo-fermentation bacteria are known to utilize glucose as a carbon source as well [9]. Thus, the glucose-utilizing activity of *R. sphaeroides* was examined and compared with its acetate-utilizing activity. As can be seen in Fig. 5, *R. sphaeroides* grew fast with little consumption of glucose for 16 h and then produced hydrogen up to about 1,200 ml. Acetic acid at 43 mM was produced by the photo fermentation of glucose in this study. This value was nearly half the theoretical value, but it was more than double that reported by Fang *et al*. [9]. The reason our result differed greatly from the latter seems to be that the pH was constantly controlled at 6.25 in our study but shifted from 7.5 to 6.5 in their study.

When acetic acid was used as the sole carbon source for photo fermentation, *R. sphaeroides* successfully used it for growth as well as hydrogen production (Fig. 5). However, about 800 mg/l of acetic acid remained in the medium. In addition, the accumulated amount of hydrogen evolved from 2,200 mg/l of acetic acid consumed was only 200 ml. Thus, it might be concluded that *R. sphaeroides* used acetic acid more efficiently for growth than for hydrogen production. A similar result has been reported in the literature [9].

Table 1 shows some factors to be considered when choosing a carbon source for hydrogen production by *R.*

Table 1. Cell growth and hydrogen production coefficients of *R. sphaeroides* according to the type of carbon source.

Carbon source (pH 6.25)	Half saturation rate coefficient, Ks (mg/l)	Maximum specific growth rate μmax (h^{-1})	H_2 production rate (NmL-H_2/l/h)	H_2 production yield (NmL-H_2/g COD)
VFAs[a]	0.16	0.25	1.48	92.6
Glucose (10 g/l)	0.22	0.37	12.0	98.5
Acetic acid (3 g/l)	1.54	2.93	0.9	56.1

[a]Mixture composed of 2 g/l acetic acid, 1 g/l butyric acid, 2 g/l lactic acid, and 1 g/l propionic acid.

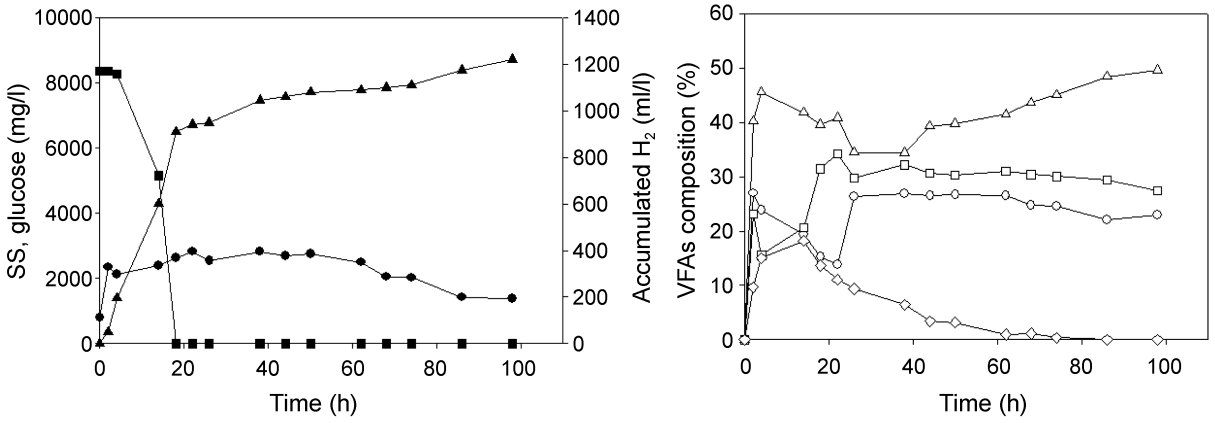

Fig. 6. Time-dependent concentrations of SS (●) and glucose (■), and composition of acetic acid (△), butyric acid (□), lactic acid (◇), and propionic acid (○), as well as accumulated amount of hydrogen evolved (▲) in a *C. butyricum* and *R. sphaeroides* co-culture system at pH 6.25.

sphaeroides. The maximum specific growth rates of this photo-fermentation bacterium were determined to be 0.25, 0.37, and 2.93 h^{-1} when using 6 g/l VFAs, 10 g/l glucose, and 3 g/l acetic acid, respectively, indicating that acetic acid best supported *R. sphaeroides* cell growth. When considering hydrogen production, however, acetic acid as a single substrate was not sufficient since it resulted in the lowest hydrogen yield in this study. *R. sphaeroides* was able to produce the most hydrogen from glucose at the highest production rate of 12.0 ml-H_2/l/h and 98.5 ml-H_2/g COD. When the mixture of VFAs was used as the carbon source, *R. sphaeroides* quickly grew *via* the utilization of acetic acid and produced hydrogen using the various VFAs as electron donors, especially lactic acid.

Hydrogen Production in the *C. butyricum* and *R. sphaeroides* Co-Culture System

Co-culture of *C. butyricum* and *R. sphaeroides* was performed with 10 g/l glucose as the sole carbon source. As shown in Fig. 6, hydrogen could be rapidly produced in inverse proportion to the depletion of glucose in the co-culture system. There was a distinguishable inflection point in the accumulated amount of hydrogen evolved. The hydrogen production rate was very high up to 19 h, after which it decreased significantly. This result was due not only to the different utilization time of glucose but also the dynamic production and consumption of VFAs by both the dark- and photo-fermentation bacteria.

Fig. 6 also shows dynamic changes in VFA concentration in the co-culture system. Lactic acid was produced in the initial stages but disappeared at the end. This result clearly indicates that lactic acid was most preferable for hydrogen production by the photo-fermentation bacterium *R. sphaeroides*. The concentrations of other VFAs, such as acetic, butyric, and propionic acids, increased steadily over the entire period of the fermenter run. In particular, acetic acid was largely generated even after glucose was used up. In addition, the concentrations of propionic and lactic acids were higher than those in the pure-culture system. These results imply that the co-fermentation system derived the

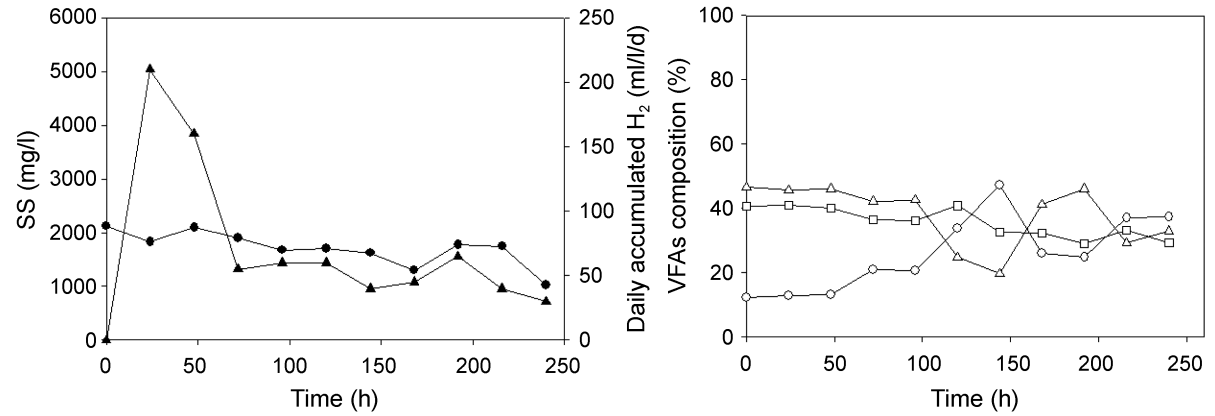

Fig. 7. Time-dependent concentration of SS (●), and composition of acetic acid (△), butyric acid (□), and propionic acid (○), as well as daily accumulated amount of hydrogen evolved (▲) by the repeated fed-batch operation of the co-culture system at pH 6.25.

production of other intermediates from glucose and resulted in changes in the concentrations of VFAs. The hydrogen production rate by the co-culture system was determined to be 12.4 ml-H_2/l/h.

The *C. butyricum* and *R. sphaeroides* co-culture system was also operated in a repeated fed-batch run, where 200 ml of the culture broth was replaced with the same volume of fresh medium every day. Fig. 7 shows the time-dependent concentration of SS, composition of VFAs, and accumulated amount of hydrogen evolved. Hydrogen production was stabilized in 3 days and its daily production rate was determined to be 77 ml/day. The hydrogen production rate of the repeated fed-batch run was 15.9 ml-H_2/l/h, a result which implies that sustainable hydrogen production could be achieved in the co-culture system. Residual glucose and lactic acid were not detected over the entire period of the test, indicating that the easy-degradable carbon sources were completely consumed in the co-culture system.

In conclusion, the optimum pH was 6.25 in the *C. butyricum* pure-culture system, whereas neutral pH was preferable for the production of hydrogen by a pure culture of the photo-fermentation bacterium *R. sphaeroides*. The results presented on this research show that a pH of 6.25 in the co-culture system is reasonable, considering not only enhanced hydrogen production but the mitigation of methanogenic hindrance.

Acetic acid best supported *R. sphaeroides* cell growth with the maximum specific growth rate. However, acetic acid as a single substrate was not sufficient, due to its resulting in the lowest hydrogen yield compared with that obtained with either glucose or a mixture of VFAs (acetic, butyric, lactic, and propionic acids). *R. sphaeroides* quickly grew with acetic acid and produced a considerable amount of hydrogen (92.6 NmL-H_2/g COD) using various VFAs as electron donors when the mixture of VFAs was used as the carbon source.

In the *C. butyricum* and *R. sphaeroides* co-culture system, hydrogen could be steadily evolved without any lag phase with a marked inflection point on the time-dependent graph of the accumulated hydrogen evolved. This was the result of the dynamic production of VFAs by the dark-fermentation bacterium and the simultaneous consumption of VFAs by the photo-fermentation bacterium. Further study about dynamic changes between substrate and intermediate products during fermentation is needed. Lastly, the repeated fed-batch run of the co-culture system clearly showed the possibility of sustainable hydrogen production.

Acknowledgment

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References

1. Afgan, N. H. and M. G. Carvalho. 2004. Sustainability assessment of hydrogen energy systems. *Int. J. Hydrogen Energy* **29:** 1327–1342.
2. Alalayah, W. M., M. S. Kalil, A. A. H. Kadhum, J. M. Jahim, and N. M. Alauj. 2008. Hydrogen production using *Clostridium saccharoperbutylacetonicum* N1-4 (ATCC 13564). *Int. J. Hydrogen Energy* **33:** 7392–7396.
3. Clesceri, L. S., A. E. Greenberg, and A. D. Eaton (Eds.) 1998. *Standard Methods for the Examination of Water and Wastewater*, 20th Ed. American Public Health Association, Washington, DC.
4. Barbosa, M. J., J. M. S. Rocha, J. Tramper, and R. H. Wifjjels. 2001. Acetate as a carbon source for hydrogen production by photosynthetic bacteria. *J. Biotechnol.* **85:** 25–33.
5. Brosseau, J. D. and J. E. Zajic. 1982. Hydrogen-gas production with *Citrobacter intermedius* and *Clostridium pasteurianum. J. Chem. Technol. Biotechnol.* **32:** 496–502.
6. Chen, C. C., C. Y. Lin, and J. S. Chang. 2001. Kinetics of hydrogen production with continuous anaerobic cultures utilizing sucrose as the limiting substrate. *Appl. Microbiol. Biotechnol.* **57:** 56–64.
7. Demirel, B., P. Scherer, O. Yenigün, and T. T. Onay. 2010. Production of methane and hydrogen from biomass through conventional and high-rate anaerobic digestion processes. *Crit. Rev. Environ. Sci. Technol.* **40:** 116–146.
8. Fang, H. H. P. and H. Liu. 2002. Effect of pH on hydrogen production from glucose by a mixed culture. *Bioresour. Technol*. **82:** 87–93.
9. Fang, H. H. P., H. Zhu, and T. Zhang. 2006. Phototrophic hydrogen production from glucose by pure and co-cultures of *Clostridium butyricum* and *Rhodobacter sphaeroides. Int. J. Hydrogen Energy* **31:** 2223–2230.
10. Fascetti, E., E. D'Addario, O. Todini, and A. Robertiello. 1998. Photosynthetic hydrogen evolution with volatile organic acids derived from the fermentation of source selected municipal solid wastes. *Int. J. Hydrogen Energy* **23:** 753–760.
11. Jo, J. H., D. S. Lee, D. Park, and J. M. Park. 2008. Biological hydrogen production by immobilized cells of *Clostridium tyrobutyricum* JM1 isolated from a food waste treatment process. *Bioresour. Technol.* **99:** 6666–6672.
12. Kapdan, I. K. and F. Kargi. 2006. Bio-hydrogen production from waste materials. *Enzyme Microb. Technol.* **38:** 569–582.
13. Kraemer, J. T. and D. M. Bagley. 2007. Improving the yield from fermentative hydrogen production. *Biotechnol. Lett.* **29:** 685–695.
14. Kim, J. O., Y. H. Kim, J. Y. Ryu, B. K. Song, I. H. Kim, and S. H. Yeom. 2005. Immobilization methods for continuous hydrogen gas production biofilm formation versus granulation. *Process Biochem.* **40:** 1331–1337.
15. Manish, S. and R. Banerjee. 2008. Comparison of biohydrogen production processes. *Int. J. Hydrogen Energy* **33:** 279–286.
16. Odom, J. M. and J. D. Wall. 1983. Photoproduction of H_2 from cellulose by an anaerobic bacterial coculture. *Appl. Environ. Microbiol.* **45:** 1300–1305.
17. Shi, X. Y. and H. Q. Yu. 2006. Continuous production of hydrogen from mixed volatile fatty acids with *Rhodopseudomonas capsulata. Int. J. Hydrogen Energy* **31:** 1641–1647.

18. Taguchi, F., N. Mizukami, T. Saito-Taki, and K. Hasegawa. 1995. Hydrogen production from continuous fermentation of xylose during growth of *Clostridium* sp. strain No. 2. *Can. J. Microbiol.* **41:** 536–540.
19. Uyar, B., I. Eroglu, M. Yücel, and U. Gündüz. 2009. Photofermentative hydrogen production from volatile fatty acids present in dark fermentation effluents. *Int. J. Hydrogen Energy* **34:** 4517–4523.
20. Van Andel, J. G., G. R. Zoutberg, P. M. Crabbendam, and A. M. Breure. 1985. Glucose fermentation by *Clostridium butyricum* grown under a self-generated gas atmosphere in chemostat culture. *Appl. Microbiol. Biotechnol.* **23:** 21–26.
21. Yokoi, H., S. Mori, J. Hirose, S. Hayashi, and Y. Takasaki. 1998. H_2 production from starch by a mixed culture of *Clostridium butyricum* and *Rhodobacter* sp. M-19. *Biotechnol. Lett.* **20:** 895–899.