Continuous hydrogen production from organic waste

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Abstract The antibiotic effects of lactic acid bacteria, *Lactobacillus paracasei*, on hydrogen production were investigated using glucose as the substrate for the batch experiments. The effects of lactic acid bacteria on hydrogen fermentation depended on pH and the inhibition of hydrogen-producing bacteria was prevented by keeping the pH over 5.0. Then, a continuous hydrogen production experiment was conducted by using bean curd manufacturing waste as an actual organic waste at pH 5.5 at 35 °C. The increase of the substrate concentration and the addition of nitrogen gave precedence to acetic and butyric acids production in the metabolic pathway and suppressed propionic acid production. As the result, continuous hydrogen production from municipal organic waste was enabled.

Keywords Hydrogen production; continuous; lactic acid bacteria; antibiotic effects; nitrogen

Introduction

Hydrogen gas is recognized as a promising energy resource in the future. Microbial hydrogen fermentation would be an attractive process for hydrogen recovery. Noike and Mizuno (2000) investigated microbial hydrogen production from actual organic wastes such as bean curd manufacturing waste, rice bran and wheat bran using batch experiments, and a good amount of hydrogen could be recovered from each waste in short time. However, it was frequently observed that hydrogen production was stopped by feeding the actual organic waste as the substrate continuously to the reactor and the medium of the batch reactor could not serve as the inoculum for the next experiment. In the past, most of the studies on hydrogen fermentation from actual organic waste have been conducted using batch experiments. Continuous hydrogen fermentation from actual organic waste has not succeeded yet. In order to realize this process, it is absolutely necessary to make clear the cause of stoppage of hydrogen production in the continuously fed reactor and the reason why such a phenomenon occurs must be clarified.

The first objective of this study is to investigate the influence of pH on lactic acid bacteria in continuous hydrogen production. Noike *et al.* (2002) discovered the inhibition of hydrogen fermentation of organic wastes at low pH value of 4.5 by lactic acid bacteria in the batch experiment. The antibiotic effects of lactic acid bacteria on hydrogen fermentation over a wide pH range were investigated using the supernatant of the culture medium by the batch and continuous experiments in this study. The second objective is to clarify the cause of stoppage of hydrogen production as mentioned before and to investigate the operational conditions for continuous hydrogen production from organic waste. Bean curd manufacturing wastes were employed as a representative organic waste.

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Control of pH to prevent the antibiotic effects of lactic acid bacteria

Materials and method

Material. *Clostridium acetobutylicum* IAM19012 were employed as the hydrogen producing bacteria. They were kept in a refrigerator at below 4 °C after cultivation in a glucose medium (Table 1) and pH was controlled at 4.5, 5.0 and 5.5. *Lactobacillus paracasei* were employed as the lactic acid bacteria. They were cultivated in the MRS alternated medium (Table 2).

Method. The influence of the coexistence of hydrogen production bacteria and lactic acid bacteria was investigated. The supernatant of the culture medium (described as the supernatant in the following) filtered with a 0.2 micrometre sterilized filter was used to investigate the influence of the metabolic products. Experiment conditions were set up at pH 4.5, and 5.0 and 5.5, as shown in Table 3. The following four series of experiments were conducted as shown in Table 3. Pure culture (without lactic acid bacteria and supernatant) was inoculated by only hydrogen producing bacteria as the control. Lactic acid bacteria with the supernatant were inoculated by hydrogen production bacteria. Lactic acid bacteria were inoculated and the supernatant was not introduced to hydrogen producing bacteria to observe the influence of coexistence. Lactic acid bacteria were not inoculated and only the supernatant were introduced to observe the influence of the supernatant. A chemostat-type 500 ml reactor and 250 ml substrate was used. The gaseous phase of the reactor was replaced by N_2 and the control of pH was carried out with a pH controller.

Table 1 Glucose medium

	Concentration (mg/L)
Glucose	5,000
Yeast extract	500
NH_4Cl	2,600
K_2HPO_4	250
$MgCl_2{\cdot}6H_2O$	125
$FeSO_4{\cdot}7H_2O$	5.0
$CoCl_2{\cdot}6H_2O$	2.5
$MnCl_2{\cdot}4H_2O$	2.5
KI	2.5
$Na_2MoO_4{\cdot}2H_2O$	0.5
H_3BO_4	0.5
$NiCl_2{\cdot}6H_2O$	0.5
$ZnCl_2$	0.5

Table 2 MRS alternated medium

	Concentration (mg/L)
Pepton	10
Yeast extract	10
Glucose	20
K_2HPO_4	5
$NH_4OCOCH_2(OH)(COOH)CH_2COONH_4$	2
CH_3COONa	5
$MgSO_4{\cdot}7H_2O$	0.58
$MnSO_4{\cdot}4H_2O$	0.28

Table 3 Experiment series of batch study

Experiment series	Without lactic acid bacteria and supernatant	With lactic acid bacteria and supernatant	With lactic acid bacteria and without supernatant	Without lactic acid bacteria and with supernatant
pH		4.5, 5.0, 5.5		
Culture solution of *C. acetobutylicum* (ml)		10		
Weight of *C. acetobutylicum* (mg)		5.5		
Culture solution of *L. paracasei* (ml)	0	10	0	0
Supernatant of *L. paracasei* (ml)	0	0	0	10
Weight of *L. paracasei* (mg)	0	15	15	0

Results and discussion

The amount of hydrogen production in each condition is shown in Figure 1. When pH was 4.5, the reduction in the rate of hydrogen production occurred in the reactor to which *L. paracasei* and supernatant were added. When pH was raised to 5.0, no reduction was observed. When pH was 5.5, the amount of hydrogen production was almost the same under all conditions. Comparing the experimental results of the control with those with or without *L. paracasei*, and with those with or without the supernatant, the rate of hydrogen production decreased as pH dropped. The reason why the prevention of hydrogen production by the addition of supernatant occurred at the low pH of 4.5 is considered to be due to the antibiotic effect of lactic acid bacteria on hydrogen producing bacteria. However, the remarkable reduction in hydrogen production was not observed when pH was over 5.0. When pH is controlled to be very low, the prevention of hydrogen production occurred in the reactors to which lactic acid bacteria or the supernatant was added. On the other hand, when pH is controlled to be higher, those phenomena did not occur, which indicates that the influence of lactic acid bacteria can be prevented by the pH control. The concentrations of organic acid in each condition are shown in Figure 2. A large amount of lactic acid was produced at pH 4.5 and hydrogen production was greatly inhibited. As the pH rose, the production of lactic acid decreased and the production of butyric acid increased. According to the above-mentioned experimental results, it is indicated that the influence of lactic acid bacteria on hydrogen fermentation depends on pH and the inhibition of hydrogen producing bacteria is prevented by keeping the pH at over 5.0.

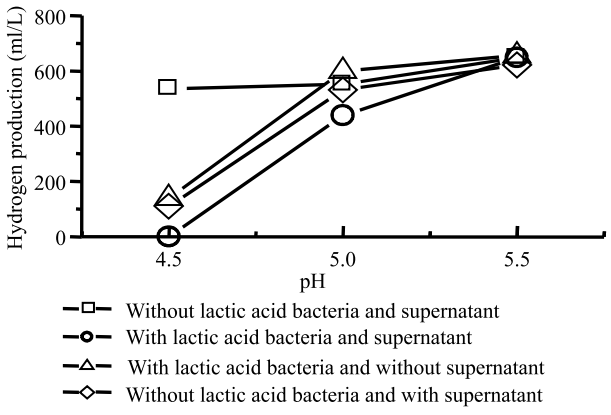

Figure 1 Influence of pH on the amount of hydrogen production

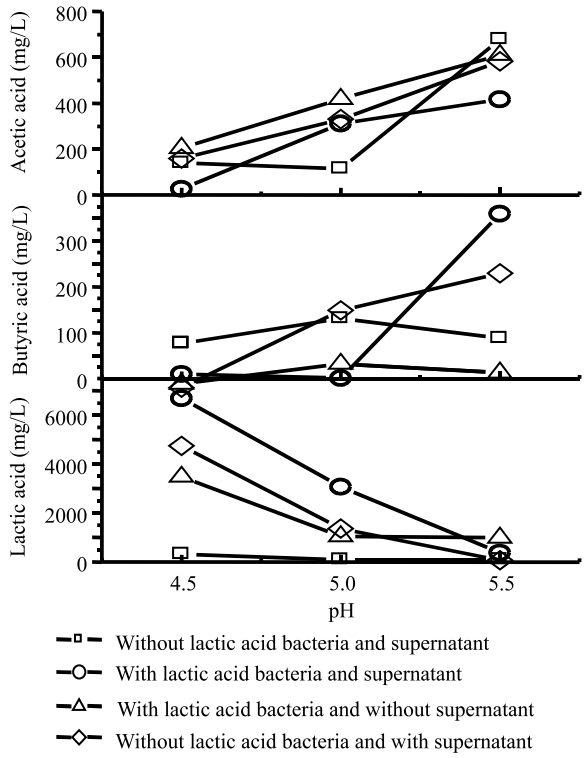

Figure 2 Influence of pH on the amount of organic acid production

Continuous hydrogen production from actual organic waste

Material and methods

Seed bacterial strain and substrate. The bacterial strains used in the continuous hydrogen production from organic waste were the following three kinds of facultative anaerobic hydrogen producing bacteria: *Enterobacter aerogenes* IAM12348T, *Enterobacter cloacae* IAM12349T and *Enterobacter sakazakii* IAM12660T. The mixture of the above-mentioned bacteria was inoculated into the reactor after the subculture using PYG media. Bean curd manufacturing waste (okara in Japanese) was used as a substrate in this study. Approximately 0.75 million tons of okara is produced per annum in Japan. As okara is perishable and has a high water content, only a small amount of okara is available for food and animal fodder and most of it has been disposed of by incineration. In Japan, okara is designated as industrial waste by the Waste Disposal and Public Cleansing Law. In this experiment, okara diluted by tap water was heat-treated at 70 °C for 30 min and most of the total solids in it was filtered, before it was used as the substrate. The okara substrates which contain almost the same concentrations of soluble carbohydrate and protein of 3000 mg/L, 4000 mg/L and 5000 mg/L, were defined as the substrates of 3000 ppm, 4000 ppm and 5000 ppm, respectively. The effective volumes of those reactors were 1350 mL, 350 mL and 400 mL, respectively. The substrate of 3000 ppm to which NH_4Cl of 1250 mg-N/l was added was defined as the substrate of 3000 ppm + NH_4Cl. The above-mentioned four kinds of okara substrates were used for the four series of continuous experiments. Table 4 shows the composition of the substrate for each experiment.

Table 4 Composition of the substrate in each experiment

Each experiment	3000 ppm	4000 ppm	5000 ppm	3000 ppm + NH_4Cl
Dilution times of okara with water	5	3.75	3	5
TS (g/kg)	12.5	12.7	12.5	12.5
VS (g/kg)	11.0	11.4	11.3	11.0
Total carbohydrate (mg/l)	4230	5410	7110	4100
Soluble carbohydrate (mg/l)	2980	3890	4970	2900
Total protein (mg/l)	5500	8170	11140	5700
Soluble carbohydrate (mg/l)	3090	4120	5260	3010
Acetic acid (mg/l)	160	210	270	120
Butyric acid (mg/l)	80	100	120	50
DOC (mg-C/l)	2190	3180	3710	2200
Ammonium nitrogen (mg-N/l)	155	240	295	1250

Experimental conditions and procedure. The bioreactor was operated at HRT of 6 hrs at 35 °C. The pH was adjusted to 5.5 ± 0.1 with 3N of NaOH. The substrate was continuously fed to the reactor and the same volume of the culture medium was continuously drawn from the reactor. Figure 3 shows the schematic diagram of the experimental apparatus. At the beginning of the experiment, the inoculum of one-sixth of the effective volume was added to the reactor and other five-sixths of the volume was filled with the substrate. The atmosphere of the reactor was exchanged by nitrogen gas.

Results and discussion

Figure 4 shows the time course of the hydrogen production rate in each experiment. In the reactor to which the substrate of 3000 ppm was fed, hydrogen was absorbed in the medium and did not recover from production. In the reactor to which the substrate of 4000 ppm was fed, hydrogen production attained the average value of 12 mL/L/h at 225 hours after the experiment began. In the reactor to which the substrate of 5000 ppm was fed, hydrogen production attained the average value of 50 mL/L/h at 280 hours after the experiment began. In the reactor to which the substrate of 3000 ppm + NH_4Cl, hydrogen production reached the average value of 35 mL/L/h at 225 hours after the experiment began. Hydrogen was stably and continuously produced throughout each experimental period in the above-mentioned three reactors to which the substrates of 4000 ppm, 5000 ppm and 3000 ppm + NH_4Cl were fed respectively. Table 5 shows the average values of hydrogen production rates and hydrogen production yields after the hydrogen production from each reactor reached the steady state. Those values increased as the substrate concentration increased and nitrogen source was added to the substrate.

Figure 5 shows DOC material balances in the reactors to which the substrates of 4000 ppm, 5000 ppm and 3000 ppm + NH_4Cl were fed. In all the reactors, soluble carbohydrate was completely decomposed to produce hydrogen and organic acids. In the

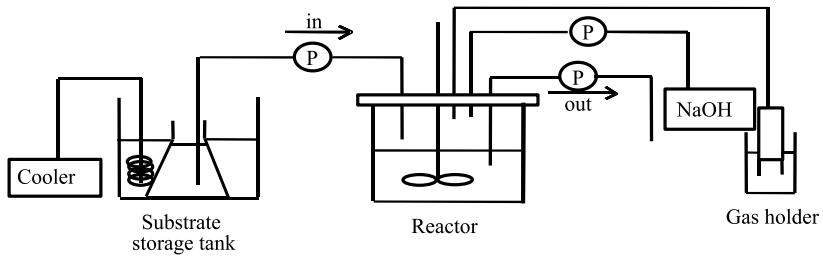

Figure 3 Experimental apparatus for continuous hydrogen production from okara

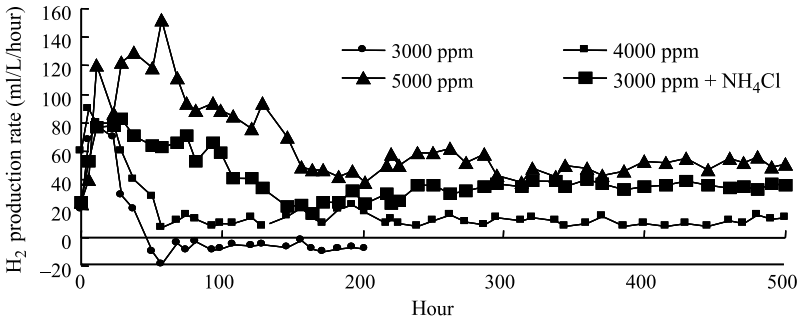

Figure 4 Time course of H_2 production rate in each experiment

reactor to which the substrate of 4000 ppm was fed, the proportion of propionic acid was larger than that in the reactors to which the substrate of 5000 ppm and 3000 ppm + NH_4Cl were fed. On the other hand, in the reactor to which the substrate of 5000 ppm and 3000 ppm + NH_4Cl were fed, the proportions of acetic and butyric acids were larger than those in the reactor to which the substrate of 4000 ppm was fed. The hydrogen production rate and yield increased as the carbohydrate concentration in the substrate increased. As mentioned before, in the reactor to which the substrate of 4000 ppm was fed, the proportion of propionic acid was larger than in the other reactors, while the proportions of acetic and butyric acids were less than those in the other reactors. According to the metabolic pathway of glucose, when propionic acid is produced from glucose, hydrogen is not produced, on the other hand, hydrogen is produced when acetic and butyric acids are produced. It is considered that when the carbohydrate concentration in the substrate is low, acetic and butyric acid metabolisms do not occur, but propionic acid metabolism mainly occurs. That is the reason why hydrogen was not produced in the reactor to which the substrate of 3000 ppm was fed.

In the reactor to which the substrate of 3000 ppm was fed, hydrogen was absorbed in the medium and did not recover production, but hydrogen was continuously and stably produced in the reactor to which the substrate of 3000 ppm + NH_4Cl was fed. Comparing the experimental results in the reactor fed with the substrate of 4000 ppm with those of the 3000 ppm + NH_4Cl, it is known that acetic and butyric acid metabolisms mainly occurred by the addition of NH_4Cl to the substrate. It can be considered that in the metabolism occuring in anaerobic reactor, there exist the following two kinds of anaerobic bacteria: acetic and butyric acids producing bacteria and acetic and propionic acids producing bacteria. Zoetemeyer *et al.* (1982) reported that the main metabolism of the substrate was acetic and propionic acids producing metabolisms in the continuous anaerobic digestion experiment in which the substrate feeding rate was low. It is considered that the reactor has insufficient nutrition when the substrate feeding rate is low, and under the such conditions, acetic and propionic acids producing bacteria grow more rapidly than acetic and butyric acids producing bacteria. The reason why a small amount of hydrogen was produced in the reactor to which the substrate of 4000 ppm was fed, is considered to be due to a lack of nitrogen.

Table 5 Average values of H_2 production rate and production yield

Each experiment	3,000 ppm	4,000 ppm	5,000 ppm	3,000 ppm + NH_4Cl
H_2 production rate (mL/L/hour)	–	12	50	35
H_2 production yield (mol H_2/mol Hexose)	–	0.16	0.52	0.62

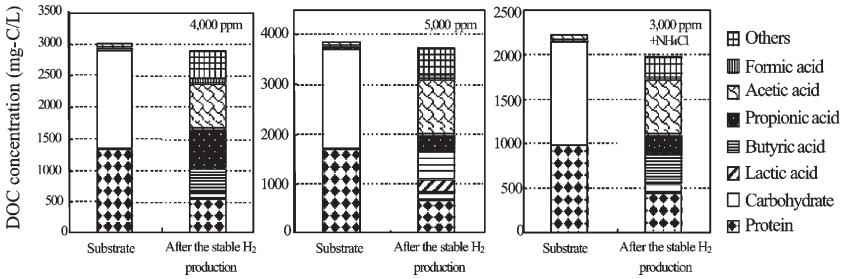

Figure 5 DOC material balances in each reactor

Conclusions

The principal conclusions derived from this investigation are as follows. The effect of lactic acid bacteria on hydrogen fermentation depends on pH and the antibiotic effect on hydrogen-producing bacteria is prevented by keeping the pH at over 5.0. The nitrogen deficiency causes the stopping of continuous hydrogen production. The increase of the substrate and the addition of nitrogen enabled the continuous hydrogen production from actual municipal organic waste and suppressed propionic acid production. As a result, acetic and butyric acids are predominantly produced and hydrogen production yield is upgraded.

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