Characteristics of hydrogen production from bean curd manufacturing waste by anaerobic microflora

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Abstract The hydrogen production from bean curd manufacturing waste by anaerobic microflora was investigated using batch experiments at 35 ^{o}C. The anaerobic microflora was obtained from fermented soybean-meals and maintained using a sucrose-limited medium in continuous culture. A solution of an organic substrate without solid component such as rough fiber in bean curd manufacturing waste was used for the experiments. After the inoculation, hydrogen production immediately occurred and almost ceased at 12 h. The final concentration of hydrogen in gas produced was 63% H_2. During hydrogen production, carbohydrate was rapidly degraded while protein degradation was hardly observed, suggesting that carbohydrate was the main source of the hydrogen production. The hydrogen yield was 2.54 mol of H_2 mol^{-1} of hexose utilized if hydrogen gas was produced from only carbohydrate degradation. At a carbohydrate concentration greater than 3,720 mg l^{-1}, the rate of hydrogen production rate significantly decreased. The rate of alcohol production was remarkably increased with increasing carbohydrate while the rate of volatile fatty acid production was hardly changed. The results indicated that the metabolic pathway and the amount of hydrogen production would be significantly influenced by the carbohydrate concentration.

Keywords Bean curd manufacturing waste; hydrogen; molecular weight; soluble carbohydrate; soluble protein; volatile fatty acids

Introduction

It is well-known that hydrogen is a clean and renewable energy resource not directly contributing to global climate changes. During the energy crisis of the 1970s, hydrogen was considered to be the fuel of the future (Gregory, 1973). With the 1990s concerns about the greenhouse effect, hydrogen is recognized again as an important fuel (Benemann, 1996).

Hydrogen can be generated in a number of ways, for example, through fossil fuel processing or by electrolysis using solar power. However, these processes are energy-intensive and therefore expensive. The biological production of hydrogen is potentially more attractive, especially if wastewater or other biomass could be used as the raw material. The ability to produce hydrogen has been recognized in a large number of microbial species. Microbial hydrogen production is classified into two categories. One is the hydrogen production by photosynthetic bacteria, and the other is that by fermentative hydrogen-producing bacteria. Fermentative hydrogen-producing bacteria have an advantage over photosynthetic bacteria in that hydrogen can be continuously produced in a reactor without light. Recently, hydrogen production from organic waste was investigated from an engineering point of view. Some previous studies indicated practical hydrogen production from organic solid waste. Oi *et al.* (1982) investigated the hydrogen fermentation of rice straw and kitchen leftovers. The microbial fermentation of sugars polysaccharides was investigated further as a potentially practical source of hydrogen (Roychowdhury *et al.*, 1988). *Bacillus licheniformis* produced hydrogen from damaged wheat grains (Kalia *et al.*, 1994). Ueno *et al.* (1996) investigated hydrogen production using wastewater from a sugar factory. Sparling *et al.* (1997) reported hydrogen production from a model lignocellulosic waste

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using *Clostridium thermarum*. However, little is known about the characteristics of organic substance decomposition during hydrogen production.

Bean curd (Tofu) is one of the traditional foods made from soybeans in Japan. Most of the bean curd manufacturing waste, which is produced at about 7 x 10^5 ton per year, is incinerated as an industrial waste (Yoshii *et al.*, 1996). The purpose of this study is to investigate both the hydrogen production from bean curd manufacturing waste and the characteristics of substrate degradation using batch experiments.

Materials and Methods

Hydrogen-producing anaerobic microflora. The hydrogen-producing anaerobic microflora was obtained from fermented soybean-meal in a silo (ESPRIT Co., Ltd., 1989). Inoculum was maintained on a sucrose-limited medium in continuous culture at a temperature of 35±1 °C and an HRT of 10 hours. The medium contained the following ingredients in 1 litre of tap water: sucrose, 18 g; NH_4HCO_3, 3800 mg; K_2HPO_4, 130 mg; $MgCl_2 \cdot 6H_2O$, 100 mg; $FeSO_4 \cdot 7H_2O$, 282 mg; $Na_2CO_3 \cdot 10H_2O$, 2500 mg; $CoCl_2 \cdot 6H_2O$, 2.5 mg; $MnCl_2 \cdot 4H_2O$, 2.5 mg; KI, 2.5 mg; $Na_2CmoO_4 \cdot 2H_2O$, 0.5 mg; $H_{3B}O_4$, 0.5 mg; $NiCl_2 \cdot 6H_2O$, 0.5 mg; $ZnCl_2$, 0.5 mg. Hydrogen and carbon dioxide in the headspace of the reactor were 43 and 57%, respectively. Methane was not detected during the incubation. The pH was 5.0(0.2. The biomass concentration was 1630 mg VSS 1^{-1}.

Preparation of organic substrate solution. The characteristics of bean curd manufacturing waste are shown in Table 1. Bean curd manufacturing waste was crushed soybeans which were squeezed in the manufacturing of bean curd. Bean curd manufacturing waste contains solid component such as rough fiber which cannot be easily decomposed by the anaerobic microflora during a short incubation period. Therefore rough fiber in bean curd manufacturing waste was removed by filtration. A 210 g VS of bean curd manufacturing waste was introduced into 4-litre distilled water. The mixture was completely stirred and filtered using a cloth filter. The filtered solution was used as the organic substrate for batch experiments. Characteristics of the filtered solution are shown in Table 2.

Batch experimental setup. The batch experiment investigating the characteristics of substrate degradation was performed in a 1.2-litre serum vial with a 1-litre culture volume. The experimental apparatus is shown in Figure 1. The culture was continuously stirred with a magnetic stirrer. In order to measure the volume of biogas produced, the serum vial was connected to a biogas collection cylinder placed in an acidic saturated salt solution of NaCl with 2% sulfuric acid. The headspace of the serum via was flushed with O_2-free N_2 gas. Traces of oxygen were removed from the N_2 by passing it through a vertical Pyrex column packed with copper turnings and heated to approximately 400 °C. The organic substrate was supplemented with 142 mg 1^{-1} of $FeCl_2 \cdot 4H_2O$ and 1 ml of 0.2% resazurin. A 250 ml of

Table 1 Characteristics of bean curd manufacture

Water content (wt %)	77
Total solids (wt %)	23
Volatile solids (wt %)	21
Ash (wt %)	2.0
Carbohydrate* (wt %)	5.8

*Bean curd manufacturing waste was treated with alkali at 120 °C for 20 min, and then the total carbohydrate content was determined by the phenol-sulfuric acid method (Oi et al., 1982).

Table 2 Characteristics of organic substrate

Total carbohydrate (mg 1-1)	3750
Soluble carbohydrate (mg 1-1)	2660
Total protein (mg 1-1)	5010
Soluble protein (mg 1-1)	3280
VSS (mg 1-1)	15100
SS (mg 1-1)	16600

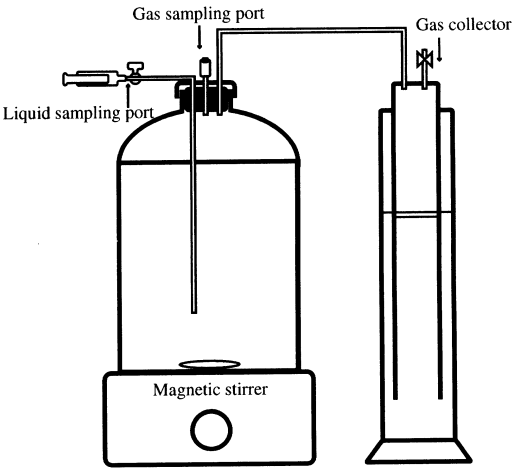

Figure 1 Experimental apparatus

the anaerobic microflora was anaerobically inoculated into 750 ml or organic substrate. The initial pH was adjusted to 6.0 with 1N HCl and 1N NaOH. The serum vial was incubated at a temperature of 35±1 °C and used without any pH control. On the other hand, a serum vial (120-ml) was employed for the batch experiments investigating the effect of substrate concentration on the rate of hydrogen production. 40 ml of the anaerobic microflora was inoculated into 40 ml of organic substrate. The headspace of the batch reactor was flushed with O_2-free N_2 gas. The serum vial was sealed with a butyl rubber bung and an aluminum crimp. For shaken cultures, a reciprocating water bath shaker was used at a speed of 80 strokes per min and a temperature of 35(1 °C. The initial pH was adjusted to 6.0 with 1N HCl and 1N NaOH. The serum vial was used without any pH control.

Quantification of substrate and fermentation products. Total solids (TS) and volatile solids (VS) were estimated at 104 °C for 24h and at 600 °C for 1h, respectively. The biomass was analyzed according to standard methods (APHA, 1992). The amount of the biogas production from a 124-ml serum vial was measured by the method of Owen (Owen *et al.*, 1979). Hydrogen (H_2) in biogas was determined with a gas chromatograph (Shimadzu GC-8A) equipped with a thermal conductivity detector and a 1.5-m stainless column filled with activated carbon. Nitrogen was used as the carrier gas. The temperatures of the injection port and column were 140 and 70 °C, respectively. Insoluble substances were separated by centrifugation at 14,000 rpm for 15 min at 4 °C. The supernatant was filtered using a 0.45 (m filter (Milipore Co., Ltd.) and acidified with 1N HCl for analysis of volatile fatty acids and alcohols. Volatile fatty acid was measured with a gas chromatograph (Shimadzu GC-8A) equipped with a flame ionization detector and a 1.5-m (5-mm inside diameter) glass column filled with Greensorb. The temperatures of the injection port and column were 180 and 160 °C, respectively. Helium carrier gas was passed through the column. Alcohol was determined with a gas chromatograph (Shimadzu GC-8A) equipped with a flame ionization detector and a 2.0-m (5-mm inside diameter) glass column filled with Gaskuropack 54 (60/80). The temperatures of the injection port and column were 210 and 180 °C, respectively. Helium carrier gas was passed through the column. Carbohydrate concentration was colorimetrically measured by the phenol-sulfuric acid method described by Dubois *et al.* (1956). Glucose was used as the standard for calibration. The protein concentration was colorimetrically measured by the method of Lowry with bovine serum albumin as the standard (Lowry *et al.*, 1951). High molecular soluble carbohydrate was separated and determined by gel chromatograph with Toyopearl HW 65F. (Toso Co., Ltd.).

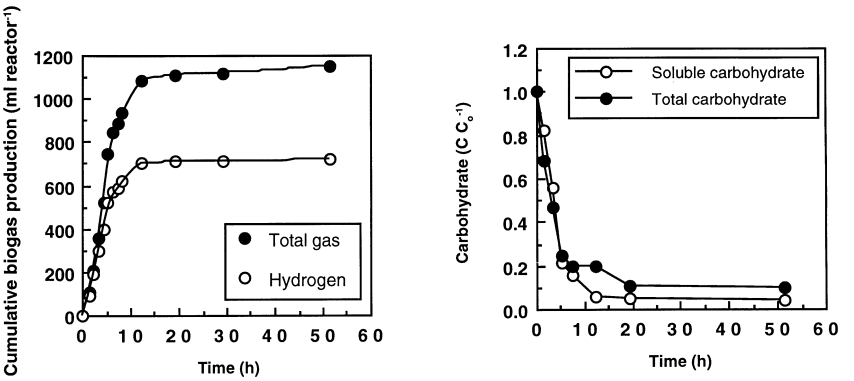

Figure 2 The time courses of hydrogen production and carbohydrate degradation. C_0; initial concentration of carbohydrate

Results and Discussion

Hydrogen production and degradation of organic substrate. Figure 2 shows the time courses of hydrogen production and the carbohydrate degradation of the substrate. After the inoculation, hydrogen production occurred without a lag time, and then almost ceased at 12 h incubation. The gas production was simultaneously completed. The final hydrogen content in the headspace increased to 63%. Methane was never detected during the incubation. The hydrogen production was observed following the degradation of carbohydrate. Carbohydrate was almost consumed at 10 h after inoculation. The anaerobic microflora also degraded insoluble carbohydrate. On the contrary, as shown in Figure 3, soluble protein was slightly degraded during the first 10 h of incubation. Hydrogen production appeared to be linked to carbohydrate consumption. Although the anaerobic microflora employed in this study was obtained from fermented soybean-meal, they could not degrade soluble protein originated from soybean. The results indicate that carbohydrate is the main source of hydrogen production and soluble protein cannot contribute to the hydrogen production.

Figure 4 shows the distribution of carbohydrate and the time-course degradation of soluble carbohydrate. Only one peak was detected between 55 and 70 ml of elution. The molecular weight of the soluble carbohydrate was estimated to be about 46,000. The soluble carbohydrate of 46,000 MW was rapidly degraded during the first 10 h of incubation. Although the anaerobic microflora were incubated using a sucrose-limited medium, they could easily degrade high molecular carbohydrate, suggesting that the anaerobic microflora could be available for hydrogen recovery from industrial wastewater containing carbohydrate.

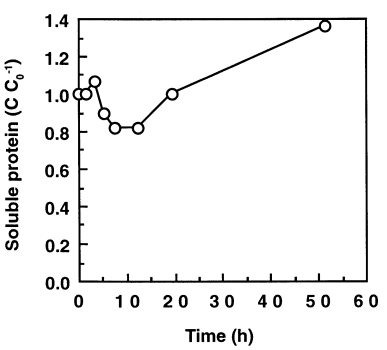

Figure 3 The time course degradation of soluble protein. C_0; initial concentration of soluble protein

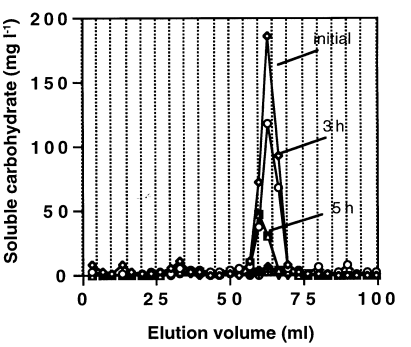

Figure 4 The distribution and the time-course degradation of soluble carbohydrate

Table 3 Fermentation products from the degradation of organic substrate

		Volatile fatty acid (mg l^{-1})				Alcohol (mg l^{-1})		
	pH	HAc	HPr	i-HBu	n-HBu	Ethanol	2-Propanol	2-Pentanol
Initial	6.0	327	ND	ND	443	288	167	291
Final	4.7	931	56	ND	1026	490	418	705

The metabolites from substrate degradation were shown in Table 3. Acetate, butyrate, ethanol, 2-propanol and 2-pentanol were the main by-products from the substrate degradation. On the other hand, propionate and *i*-butyrate were in low concentration. The hydrogen yield was 2.54 mol of H_2 mol^{-1} of hexose utilized, which was calculated by the consumption of total carbohydrate. In previous studies, various hydrogen yields were reported. Ueno *et al.* (1995) established a high hydrogen yield of 2.4 mol of H_2 mol^{-1} of hexose on cellulose degradation by microflora in a sludge compost. Kumar *et al.* (1995) reported a hydrogen yield of 0.67–1.56 mol of H_2 mol^{-1} of glucose utilized using immobilized microorganisms. Ueno *et al.* (1996) successfully carried out continuous production of hydrogen from sugar factory wastewater. They reported a high yield of hydrogen of 2.6 mol of H_2 mol^{-1} of hexose. The degradation of solid component not considered in this study is the subject of further investigation.

Effect of substrate concentration on the rate of metabolite production. The effect of substrate concentration on the rate of hydrogen production is shown in Figure 5. The rate of hydrogen production increased with increasing carbohydrate concentration. Up to 3,720 mg l^{-1} of carbohydrate, the rate of hydrogen production was significantly decreased. Figure 6 shows the effect of substrate concentration on the rates of VFA and alcohol production. The rate of alcohol production gradually increased with increasing carbohydrate concentration. It was obvious that the rate of alcohol production was higher than that of the VFA production at a higher concentration of soluble carbohydrate. It was suggested that the metabolic pathway was significantly influenced by the concentration of soluble carbohydrate. Monot *et al.* (1983) reported that the initial glucose concentration in the medium also has a strong influence on the stoichiometry and kinetics of acetone-butanol fermentation. At low glucose concentration only acids were produced. At high initial glucose concentration, butanol and acetone were the main metabolites produced. The similar phenomenon was observed in this study, suggesting that hydrogen recovery is significantly influenced by carbohydrate concentration.

Conclusions

1. The hydrogen production was observed following the degradation of carbohydrate while soluble protein was slightly degraded during hydrogen production, suggesting that carbohy-

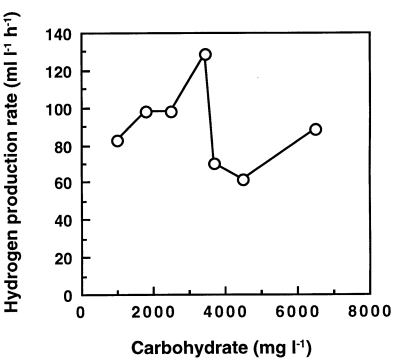

Figure 5 The effect of carbohydrate concentration on the rate of hydrogen production

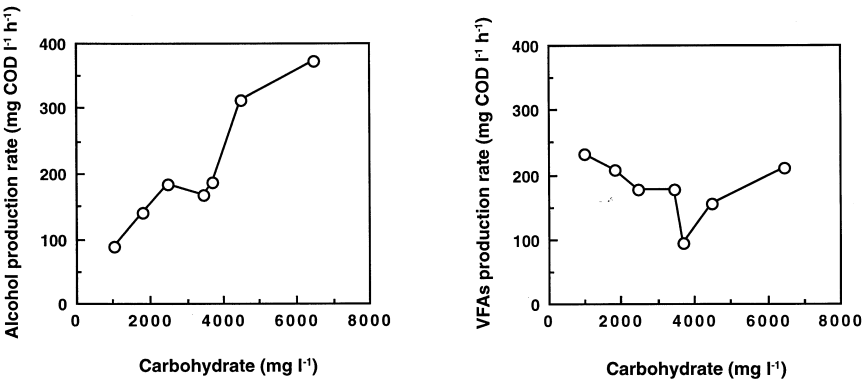

Figure 6 The effect of carbohydrate concentration on the rate of metabolite production

drate was the main source of hydrogen production. The final hydrogen content in the headspace increased to 63%. The hydrogen yield was 2.54 mol of H_2 mol^{-1} of hexose utilized.

2. The rate of hydrogen production was greatly influenced by the concentration of carbohydrate. The rate of alcohol production was higher than that of VFA production at a higher concentration of soluble carbohydrate. The metabolic pathway and the amount of hydrogen production would be influenced by the carbohydrate concentration.

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