Hydrogen fermentation of organic municipal wastes

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Abstract Hydrogen gas is recognized as a promising energy resource in the future. Microbial hydrogen fermentation would be an attractive process for hydrogen recovery. In particular, hydrogen production using fermentative bacteria has some advantages such as a high rate of hydrogen production without light. In this study, the hydrogen production from organic wastes was investigated using batch experiments. Bean curd manufacturing waste, rice bran and wheat bran were used as the organic wastes. The effects of solid concentration on the hydrogen production potential and the characteristics of substrate decomposition were investigated. The percentages of hydrogen in the produced gas were between 54–78%, 43–68% and 42–72% for bean curd manufacturing waste, rice bran and wheat bran, respectively. The hydrogen production potentials of bean curd manufacturing waste, rice bran and wheat bran were 14–21, 31–61 and 10–43 ml.g VS^{-1}, respectively. The hydrogen yields from carbohydrate degradation were 2.54, 1.29 and 1.73 mol of H_2 mol^{-1} of hexose for bean curd manufacturing waste, rice bran and wheat bran, respectively. The carbohydrate was rapidly consumed just after inoculation. On the other hand, soluble protein was hardly degraded for each substrate, indicating that carbohydrate was the main source of the hydrogen production.
Keywords Bean curd manufacturing waste; hydrogen fermentation; organic wastes; rice bran; soluble carbohydrate; wheat bran

Introduction

Hydrogen could be the ideal fuel in the global environmental era. During the oil crisis in the 1970s, hydrogen was recognized as a renewable energy resource. Despite its many advantages, the movement shifting from fossil fuels to hydrogen has been rapidly stalled because the world oil market was stabilized again. However, in the 1990s, interests in the hydrogen system have been increased significantly, because it is clear that hydrogen does not cause global warming (Gregory, 1973; Benemann, 1996).

The air pollutants such as sulfur dioxide, hydrocarbons, particulates and photochemical oxidants cannot he produced in a hydrogen flame, and its combustion by-product is only water. However, the application of hydrogen gas has been delayed for good reasons (e.g., in the fiery crash of the Hindenburg, a hydrogen filled zeppelin in 1937). Although hydrogen can be stored as a form of compressed gas or as a cryogenic liquid, it has been recognized as a dangerously explosive gas. Fortunately, metal hydrides, materials absorbing pressurized hydrogen gas, can solve the problem of hydrogen storage. Hydrogen combined as a metal hydride is safer than in the gaseous form because the metal hydride is non-flammable and non-explosive. Thus, doubts about the safety of hydrogen would be solved by the development of metal hydride technology. The industrial uses of hydrogen were investigated by Ramachadran and Mennon (1998). In particular, the recent development of hydrogen fuel cells is promising for the use of hydrogen gas. For example, some car manufacturers will introduce new vehicles with such improved fuel cells in the 21st century.

At the present time, hydrogen can be recovered by water electrolysis, coal gasification and biomass conversion. The biological hydrogen production from organic substances was also reviewed by Nandi and Sengupta (1998). In this paper, the current status of the hydrogen fermentation researches was outlined and then hydrogen production from organic wastes was investigated using batch experiments.

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Microbial hydrogen production

Microbial hydrogen production is potentially attractive because wastewater or other biomass materials could be used as the raw material. It has been recognized in a large number of microbial species (Gray and Gest, 1965). Microbial hydrogen production is classified into two categories. One is hydrogen production by photosynthetic bacteria, and the other is that by fermentative bacteria. Hydrogen production can be considered a device for disposing of electrons released during metabolic oxidation. Fermentative bacteria have advantages over photosynthetic bacteria and they can continuously produce hydrogen in a reactor without light. As the growth rate is generally high, hydrogen production by fermentative bacteria can establish a high-rate hydrogen production. Some researchers noted the Clostridial-type microorganism as a hopeful hydrogen producing bacterium, which could rapidly convert organic substances into metabolites such as hydrogen, carbon dioxide, acids and solvents. Their ability to produce hydrogen was recognized to be quite high, suggesting that it could he applied to industrial hydrogen production. It has been well investigated that the metabolic pathway of the Clostridial types is significantly influenced by various factors such as pH, nutrients, carbon source concentration, stirring rate, dissolved H_2 and dissolved CO_2 (Chung, 1976; Monot *et al.*, 1983; Bahl and Gottschalk, 1984; Andel *et al.*, 1985; Yerushalmi and Volesky, 1985; Yerushalmi *et al.*, 1985; Dabrock *et al.*, 1992; Tanisho *et al.*, 1998). To enhance the hydrogen yield, those factors should he optimized. In general, organic wastes are recognized to be nutrient-rich and the physical factors can be overcome by improving the reactor.

On the contrary, fermentative bacteria produce a relatively small amount of hydrogen, typically only 10–20% stoichiometrically, suggesting that the hydrogen production is not suitable on a commercial basis at the present time. Economic feasibility will be established when hydrogen yield reaches 60–80% (Benemann, 1996).

The mixed culture of hydrogen-producing bacteria is more important for industrial application because it may be difficult to use a pure culture for hydrogen production from organic wastes, and the pure culture is easily contaminated by various hydrogen utilizers such as methane producing bacteria and sulfate-reducing bacteria. Recently, practical hydrogen production from organic wastes was investigated from an engineering point of view. Some previous studies indicated practical hydrogen production from organic solid wastes. Oi *et al.* (1982) investigated hydrogen fermentation of rice straw and kitchen leftovers. The microbial fermentation of sugars and polysaccharides was further investigated as a potentially practical source of hydrogen (Roychaudhury *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 using *Clostridium thermarum*. Mizuno *et al.* (1997) reported hydrogen production using hydrogen-producing mixed culture from bean curd manufacturing waste.

Hydrogen production from organic municipal wastes

Organic solid waste

Bean curd manufacturing waste, rice bran and wheat bran were used as the organic wastes. Bean curd (Tofu) is one of the traditional foods made from soybeans in Japan. About 7×10^5 ton of the bean curd manufacturing waste is produced in Japan in a year and is incinerated as an industrial waste (Yoshii *et al.*, 1996). Rice bran and wheat bran are agricultural wastes. The characteristics of bean curd manufacturing waste, rice bran and wheat bran are shown in Table 1. Total solids and volatile solids were estimated at 105ºC for 24 h and at 600ºC for 1 h, respectively. In order to determine the carbohydrate percentage, rice bran and wheat bran were treated with NaOH, and then the total carbohydrate content was determined by the phenol-sulfuric acid method (Oi *et al.*, 1982).

Hydrogen-producing anaerobic microflora

The hydrogen-producing anaerobic microflora was obtained from fermented soybean-meal in a silo (ESPRIT Co. Ltd., 1989). An inoculum was maintained on a sucrose-limited medium in a 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; $Na_2MoO_4 \cdot 2H_2O$, 0.5 mg; H_3BO_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 anaerobic microflora used in this study was obtained from fermented soybean-meal and maintained with a sucrose-limited medium, which was sterilized. The hydrogen production by the anaerobic microflora has been quite stable during an eight-year incubation period in our laboratory.

Hydrogen production potential

Serum vials (120-ml) were used for the batch experiment to investigate the influence of the solids (total solids) concentration on the hydrogen production potential. A 10-ml aliquot of the anaerobic microflora from the reactor was anaerobically introduced into a 120-ml

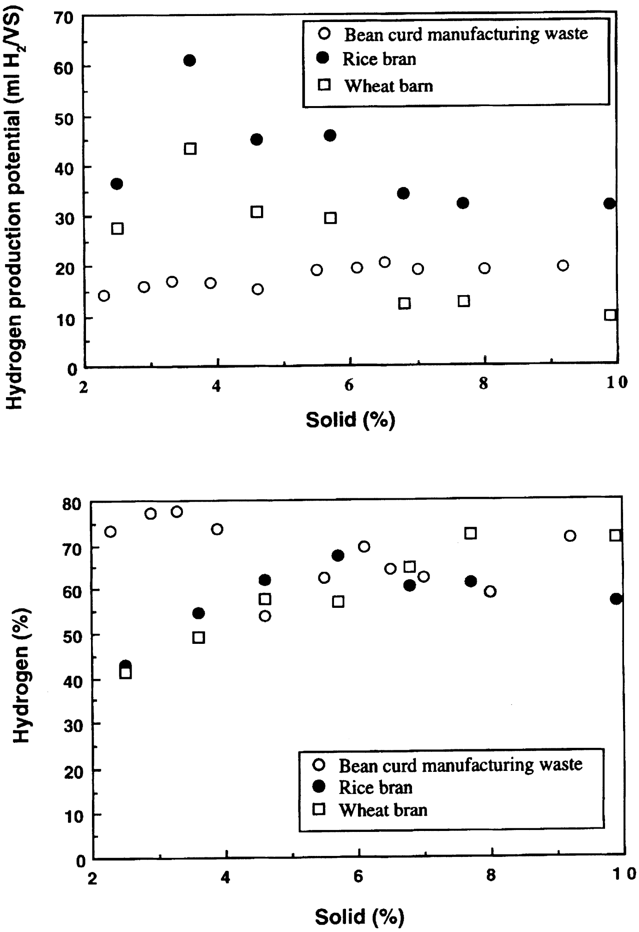

Figure 1 The influence of solid concentration on hydrogen production potential and hydrogen percentage of the produced gas

Table 1 Characteristics of organic waste

	Bean curd manufacturing waste	Rice bran	Wheat bran
Water content (wt%)	77	11	12
Total solid (wt%)	23	89	88
Volatile solid (wt%)	21	79	82
Ash (wt%)	2	10	6
Carbohydrate (wt%)	5.8	18	23

Table 2 Characteristics of substrate for batch experiment

	Bean curd manufacturing waste	Rice bran	Wheat bran
Total carbohydrate (mg · l^{-1})	3750	6300	7920
Soluble carbohydrate (mg · l^{-1})	2660	5110	5150
Total protein (mg · l^{-1})	5010	3740	1060
Soluble protein (mg · l^{-1})	3280	2680	943
Carbohydrate/protein (total)	0.75	1.68	7.47
Carbohydrate/protein (soluble)	0.81	1.91	5.46

serum vial containing 2.2 g VS of organic wastes and a 5 ml nutrient solution described by Owen *et al.* (1979). The solid concentration was controlled by adding distilled water. The headspace of the serum vial was flushed with O_2-free N_2 gas. For shaken cultures, a reciprocating water bath shaker was used at a speed of 80 strokes per min and at a temperature of 35±1ºC. The serum vials were used without any pH control. The amount of gas production from serum vial was measured using a glass syringe by the method of Owen *et al.* (1979). Hydrogen percentage versus time was monitored by gas chromatography.

Figure 1 shows the influence of the solid concentration on the hydrogen production potential and the hydrogen percentage of the produced gas. After the inoculation, hydrogen production occurred at each solids concentration. The hydrogen production potentials of rice bran and wheat bran obviously decreased as the solids concentration increased. Even at a solid concentration on over 9.0 %, hydrogen production was observed in each substrate. The percentages of hydrogen in the produced gas were between 54–78%, 43–68% and 42–72% for bean curd manufacturing waste, rice bran and wheat bran, respectively. The hydrogen production potentials of bean curd manufacturing waste, rice bran and wheat bran were 14-21, 31-61 and 10–43 ml· g VS^{-1}, respectively. At a high solids concentration, the hydrogen production potential of wheat bran was lower than that of rice bran. On the other hand, no significant changes in the hydrogen production potential of bean curd manufacturing waste were observed. Methane was never detected during the incubation.

The solids concentration is an important factor for hydrogen production from an engineering point of view because hydrogen production under a high solids condition has some advantages. In this study, hydrogen production was successfully carried out at a high solids concentration of over 9.0%. These results indicate that hydrogen recovery can be carried out using the anaerobic microflora from unsterilized organic wastes. However, hydrogen production potentials of rice bran and wheat bran decreased as the solids concentration increased.

Characteristics of degradation of organic substances

Organic wastes contain solid substances such as rough fiber which cannot be easily decomposed by the anaerobic microflora during a short incubation period. In the experiment to investigate the characteristics of organic substance degradation, the solid substance in the

Table 3 Hydrogen production from organic wastes by mixed culture

Organic wastes	Hydrogen yield (mol H_2/mol hexose)	Reference
Sugar factory wastewater	1.91–2.59	Ueno *et al.* (1996)
Wheat bran	1.12	Kalia *et al.* (1994)
Bean curd manufacturing waste	2.54	This study
Rice bran	1.29	This study
Wheat bran	1.73	This study

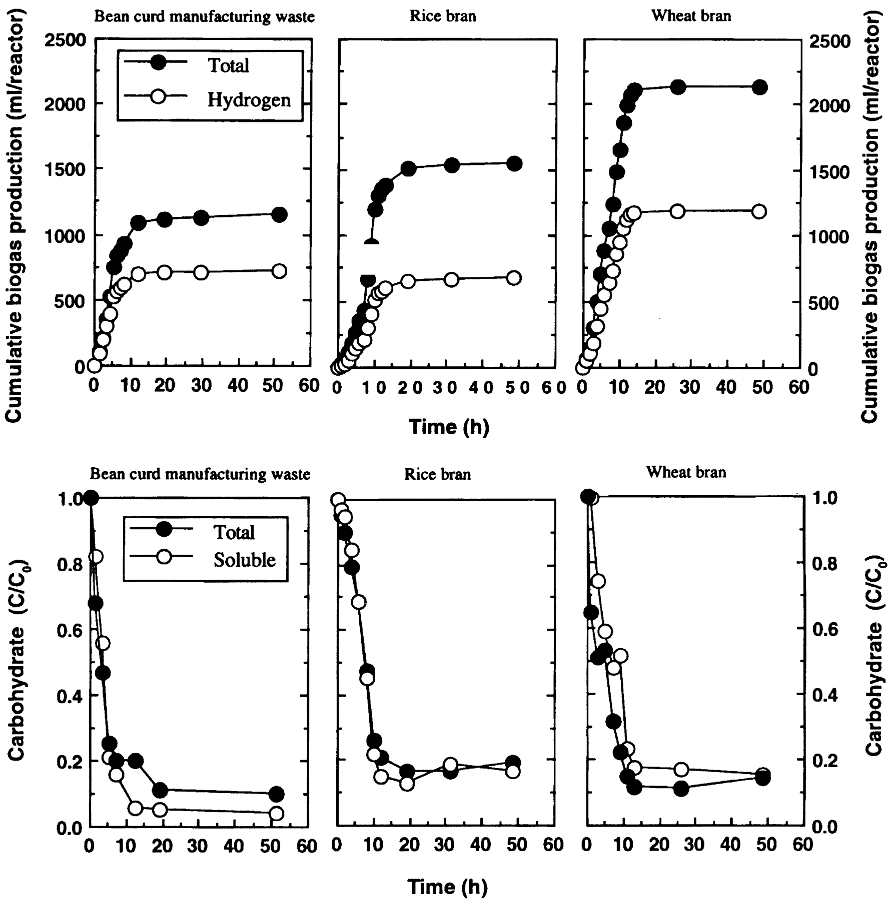

Figure 2 Hydrogen production and decomposition of carbohydrate C, initial concentration of carbohydrate

organic waste was removed by filtration. A 210 g VS of organic waste was soaked in 4 litres of distilled water. The mixture was stirred and then filtered using a cloth filter. The filtered solutions were used as the organic substrate for the batch experiments. The characteristics of the filtered solutions are shown in Table 2.

The batch experiment to investigate the characteristics of the organic substrate degradation was performed in a 1.2 litre serum vial with a 1 litre culture volume. The culture was continuously stirred with a magnetic stirrer. In order to measure the volume of produced gas, the serum vial was connected to a gas collection cylinder placed in an acidic saturated salt solution of NaCl with 2% sulfuric acid. A 250 ml aliquot of the anaerobic microflora from the reactor was anaerobically introduced into a 750 ml 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 was used without any pH control.

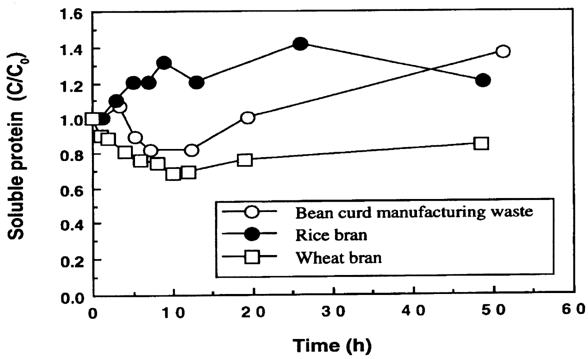

Figure 3 Decomposition of soluble protein. C_0, initial concentration of soluble protein

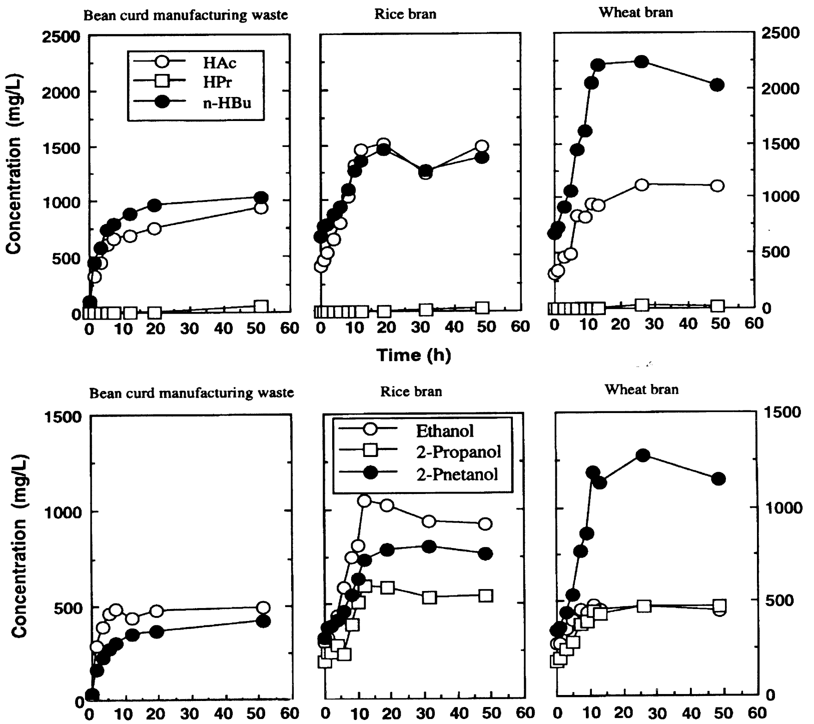

Figure 4 Metabolites production from decomposition of organic substances

Figure 2 shows the hydrogen production and carbohydrate degradation of the substrate. Hydrogen production began immediately after the inoculation. The produced gas in the headspace of the serum vial was found to consist of hydrogen and carbon dioxide. Methane was never detected during the incubation of each substrate. During the first 15 h of incubation hydrogen production rapidly increased and then reached a plateau for each substrate, whereas gas production was simultaneously completed. The final hydrogen content in the headspace increased to 63% for bean curd manufacturing waste, 44% for rice bran and 54% for wheat bran. The soluble carbohydrate removals of bean curd manufacturing waste, rice bran and wheat bran were 96, 83 and 85% respectively. Carbohydrate was totally consumed during the first 10 h of incubation, and then hydrogen production simultaneously ceased. The anaerobic microflora could also degrade insoluble carbohydrates. It was obvious that hydrogen production was apparently linked to the carbohydrate consumption.

Figure 3 shows the soluble protein degradation of each substrate. Unlike the degradation of carbohydrate, soluble protein of the wheat bran was hardly degraded during the incubation.

Figure 4 summarizes the results of the end products after 50 h of incubation. During the hydrogen production, complex organic substances were hydrolyzed into volatile fatty acids, such as acetate, propionate, and butyrate, and neutral products, such as ethanol, propanol and pentanol. Among the VFAs, acetate and *n*-butyrate were detected as the major soluble metabolites, but trace levels of propionate and *i*-butyrate were also detected.

Hydrogen yield

Hydrogen production by the mixed culture has been reported in previous studies. Table 3 summarizes the hydrogen yield from various wastes. The hydrogen yields of bean curd manufacturing waste, rice bran and wheat bran were 2.54, 1.29 and 1.73 mol H_2 mol^{-1} of hexose consumed, respectively. It should be noted hat the hydrogen yields were calculated based only on the consumed carbohydrate. Ueno *et al. (*1995) investigated the hydrogen production from cellulose using natural anaerobic microflora in a sludge compost and established a high hydrogen yield of 2.4 mol of H_2 mol^{-1} of hexose. Moreover, Ueno *et al. (*1996) successfully carried out continuous hydrogen production from sugar factory waste-water by anaerobic microflora. They reported a high hydrogen yield of 2.6 mol of H_2 mol^{-1} of hexose.

Conclusion

The results presented here showed that hydrogen production from organic wastes was possible. The hydrogen yields from carbohydrate degradation were 2.54, 1.29 and 1 .73 mol of H_2 mol^{-1} of hexose for bean curd manufacturing waste, rice bran and wheat bran, respectively. The carbohydrate was rapidly consumed just after inoculation while soluble protein was hardly degraded for each substrate, indicating that carbohydrate was the main source of hydrogen production. The organic wastes used in this study contain solid substances such as rough fiber. The solid substances would require a long incubation period for complete decomposition. Actually, the degradation of solid substances was not observed during the batch incubation in this study, and it is suggested that soluble and insoluble carbohydrates are the important substrates for hydrogen production. Therefore, the effluent after hydrogen production using the anaerobic microflora will contain solid substances and protein. Further investigation will be needed to upgrade the degradation of solid substances and protein.

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