Biological hydrogen potential of materials characteristic of the organic fraction of municipal solid wastes

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Abstract The purpose of this study is to investigate the biological hydrogen production potential of individual organic fraction of municipal solid wastes (OFMSW) by batch experiments. Seven varieties of typical organic solid wastes including rice, cabbage, carrot, egg, lean meat, fat and chicken skin were selected to estimate the hydrogen production potential. Among the OFMSW, carbohydrate produced the most hydrogen through biological hydrogen fermentation compared with proteins or lipids. Subsequently, the biological hydrogen production potentials of some individual carbohydrate were measured: cabbage, 26.3–61.7 mL/g-VS; carrot, 44.9–70.7 mL/g-VS; and rice, 19.3–96.0 mL/g-VS. The hydrogen percentages of the total biogas produced from cabbage, carrot and rice were 33.9–55.1%, 27.7–46.8% and 44.0–45.6%, respectively.

Keywords Anaerobic microflora; biological hydrogen production potential; moisture content; organic fraction of municipal solid wastes

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Introduction

After the oil crisis of the 1970s, there has been an increase in effort to develop the biological production of fuels from renewable biomass. In connection to global environmental considerations such as the greenhouse effect, the hydrogen produced by microbiological fermentation represents a partial answer to the depletion of hydrocarbon fuel reserves and to the accumulation of carbon dioxide.

Among renewable biomasses, OFMSW are potentially useful since they can be converted into an alternative energy source. However, the characteristics of hydrogen fermentation from various substances in a wide range of moisture content are not clear at present. Also, the design of systems for generating hydrogen from OFMSW would be facilitated by better understanding the influence of OFMSW composition on the potential and rate of hydrogen production.

The purpose of this study is to investigate the biological hydrogen production potential of individual OFMSW by batch experiments. For this purpose, seven varieties of typical organic solid wastes including rice, potato, cabbage, carrot, egg, lean meat, fat and chicken skin were selected and their hydrogen production potential estimated.

Materials and methods

Experimental apparatus

Organic fraction of municipal solid wastes (OFMSW) have complex compositions including various organic and inorganic compounds. In order to investigate the influence of organic composition on the hydrogen production characteristics of seven different materials, (cabbage, carrot, rice, egg, lean meat, fat and chicken skin) were used as the main ingredients. Before feeding to the batch experiments, rice, egg and lean meat were boiled and broken into pieces by a blender; cabbage, carrot, fat and chicken skin were broken into pieces by a blender without boiling. The experiments were performed under the mesophilic condition of 37ºC using a glass vial of 120 mL volume. A rotary cell culture at a rotation

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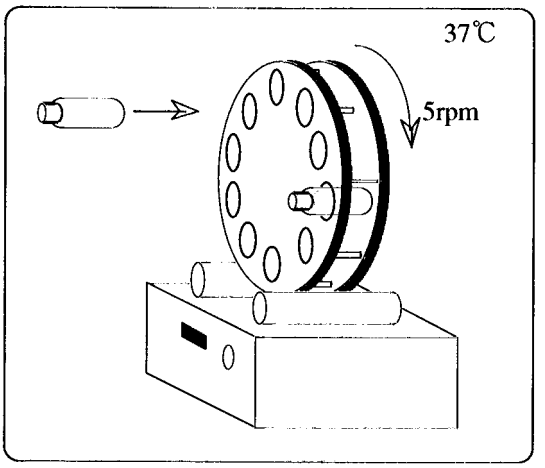

Figure 1 Experimental apparatus

Table 1 Experimental condition

	Substrate						
	cabbage	carrot	rice	egg	lean meat	chicken skin	fat
setting TS	2	2	4	4	4	4	4
concentration	3	3	8	8	8	8	8
(%)	4	4	12	10	12	12	12
	5	5	15	12	15	15	15

speed of 5 rpm was used for mixing the content of the bottles (Figure 1). The moisture contents of the whole bottle were adjusted ranging from 85 to 98%, and are shown as total solid concentration of the whole bottle in Table 1. These moisture contents of the whole bottle were controlled by adding 40 g of seed sludge and 40 g of appropriate mixture of substrate and distilled water. Pretreated anaerobic digested sludge was used as seed sludge in those batch experiments. Medium of 0.5 g also was added. The medium reported by Bahl *et al.* (1986) was used for this batch experiments with a minor modification. One litre of each medium contains 2 g of NH_4HCO_3, 1 g of KH_2PO_4, 0.1g of $MgSO_4 ? 7H_2O$, 10 mg of NaCl, 10 mg of $Na_2MoO_4 ?2H_2O$, 10 mg of $CaCl_2 ?2H_2O$, 13 mg of $MnSO_4 ?7H_2O$, 2.78 mg of $FeSO_4 ? 7H_2O$, 0.1 mg of biotin, 2 mg of thiamine hydrochloride, and 2 mg of *p*-aminobenzoic acid. All operations proceeded under an atmosphere of 80% N_2 and 20% CO_2 to assure an anaerobic condition. The pH of the vial content was initially adjusted to approximately 7.0 with 1.0N HCl because the bioactivity of hydrogen-producing microorganisms is re-established rapidly by neutralizing the culture (Roychowdhury *et al.*, 1988). The pH of each content was allowed to change during the batch fermentation periods. The batch fermentation experiments had been done until hydrogen production from each bottle reached zero.

Seed microorganisms

The pretreated anaerobic digested sludge was introduced for converting OFMSW components to hydrogen. This sludge was taken from a ten-litre laboratory-scale anaerobic digester and was boiled 15 minutes to inhibit the bioactivity of hydrogen consumers and to

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develop its H_2-producing capabilities. The digester was operated at a temperature of 37±1ºC and a hydraulic retention time (HRT) of 20 days for over three years by feeding it with high-solids organic wastes. The solids concentration in it was maintained at 2–3 wt.%. Previous study (Lay *et al.*, 1996) demonstrated that the sludge contained in the digester had a high methanogenic activity on the treatment of OFMSW.

Analytical method

Biogas production was measured by the displacement of the water lubricated plungers of glass syringes. The percentage of hydrogen in biogas was determined using a gas chromatograph (Shimadzu GC-8A) equipped with a thermal conductivity detector (TCD) and a 2 m stainless column packed with Porapak Q (50/80 mesh). The operational temperatures at the injection port, the column oven and the detector were 100, 70 and 100ºC, respectively. Nitrogen was used as the carrier gas at a flow rate of 30 mL/min. The methane and carbon dioxide was analyzed using a second GC-TCD of the same model with a 2 m stainless column packed with Porapak T (50/80 mesh). The operational temperatures of the injection port, the column oven and the detector were the same with those of the hydrogen analysis. The concentrations of the volatile fatty acids (VFAs) were determined using a third GC of the same model with a flame ionization detector (FID) and a 2 m glass column packed with Unisole F-200 (30/60 mesh). The operational temperatures at the injection port, the column oven and the detector were 180, 165 and 180ºC, respectively. The ethanol, propanol, butanol and acetone were analyzed using a GC-FID of the same model with a 2 m glass column packed with Gaskuropack 54 (60/80 mesh). The operational temperatures at the injection port, the column oven and the detector were 200, 185 and 200ºC, respectively. Helium was used as the carrier gas for the determinations of methane, VFAs and solvents at a flow rate of 30 mL/min. Ammonium (NH_4^+) concentration was determined using an ionic chromatograph (Shimadzu) with a Shimadzu Shim Pack IC-C3 column at 40ºC. The IC was equipped with a conductivity detector (COD 6A). The mobile phase consisted of 2.5 mmol oxalic acid dihydrate at a flow rate of 1.2 mL/min. The pH was measured by a pH meter equipped with a GST-5425C probe. The concentrations of total solids (TS) and volatile solids (VS) were determined according to the procedures described in Standard Methods (APHA, 1995). Carbohydrates concentration was measured by the phenol-sulfuric acid method using glucose as the standard.

Results

Degradation characteristics of carbohydrates

Figure 2 shows the degradation characteristics of carrot with TS5% as an example for carbohydrate degradation. After 5.5 hours incubation, it is evident that carrot was hydrolyzed

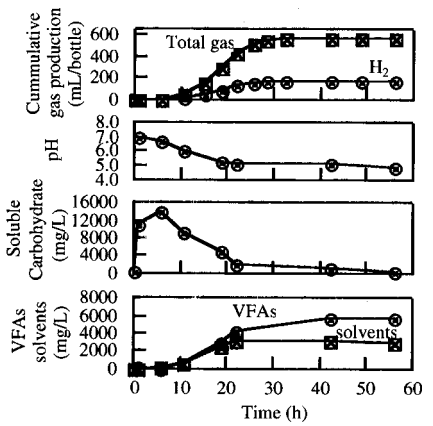

Figure 2 Degradation characteristics of carrot with TS5%

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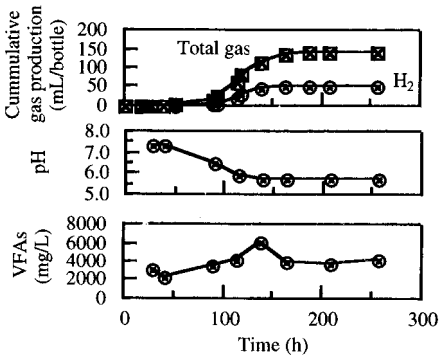

Figure 3 Degradation characteristics of fat with TS8%

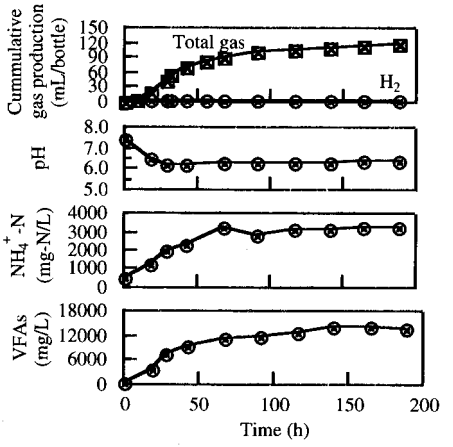

Figure 4 Degradation characteristics of lean meat with TS4%

and soluble carbohydrate concentration increased. At that time soluble carbohydrate concentration was 13.9 g/L. Subsequently hydrogen, VFAs and solvents were produced with the degradation of soluble carbohydrate. After 50 hours incubation, hydrogen production was stopped, cumulative hydrogen production, acetate, butyrate, ethanol were 72.6 mL/g-VS, 2,160 mg/L, 3,500 mg/L and 2,980 mg/L, respectively. 4.0% of carrot was converted to hydrogen gas based on COD. On the experiment of carrot with TS5%, ethanol production was the largest of all substrates. The pH was dropped to 4.96 with acids production.

Degradation characteristics of lipids

Figure 3 shows the degradation characteristics of fat with TS8% as an example for lipid degradation. The lag time on hydrogen production using fat as the substrate was much longer than that using carbohydrates and proteins as the substrates. A hydrogen production potential of 9.75 mL/g-VS indicates that 0.2% of fat was converted to hydrogen gas based on COD. Acetate, propionate, butyrate and iso-butyrate were produced as for VFAs. After 160 hours incubation, acetate, propionate, butyrate and iso-butyrate production were 894 mg/L, 945 mg/L, 1,720 mg/L and 751 mg/L, respectively. On the other hand, solvent was not produced at all. The pH eventually dropped to 5.79.

Degradation characteristics of proteins

Figure 4 shows the degradation characteristics of lean meat with TS4% as an example of protein degradation. A hydrogen production potential of 2.47 mL/g-VS indicates that 0.1% of lean meat was converted to hydrogen gas based on COD. Ammonia nitrogen and VFA concentrations were gradually increased during the experimental period. After 188 hours incubation, the ammonia nitrogen concentration was 3,220 mg/L. Acetate, propionate, butyrate, iso-butyrate and iso-valerate concentrations were 7,330 mg/L, 873 mg/L, 2,920 mg/L, 917 mg/L and 1,710 mg/L, respectively. On the other hand, small amounts of ethanol and acetone were produced. Although the pH dropped to 6.22 after 42 hours, the pH eventually rose to 6.42 after 188 hours due to ammonia production.

Hydrogen production potential

Figure 5 shows the influence of moisture contents on biological hydrogen production potential of various substrates. Hydrogen production potentials were as follows: cabbage,

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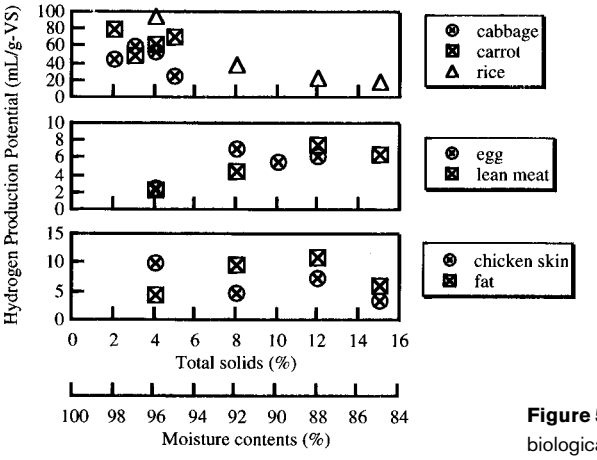

Figure 5 Influence of moisture contents on biological hydrogen production potential

26.3–61.7 mL/g-VS; carrot, 44.8–70.7mL/g-VS; rice, 19.3–96.0 mL/g-VS; egg, 2.6–7.1 mL/g-VS; lean meat, 2.5–7.7 mL/g-VS; chicken skin, 3.6–10.2 mL/g-VS; and fat, 4.4–11.1 mL/g-VS. It is clear that carbohydrate has much higher biological hydrogen production potential than lipids and proteins. Figure 4 shows that hydrogen production of carbohydrate may be preferable at moisture contents of 95–98% (2–5% TS). Hydrogen production potentials of various substrates were summarized in Table 1. Glucose has much higher hydrogen production potential than other substrates such as cellulose and OFMSW (this study), because hydrogen production from them requires hydrolysis reaction which requires long time.

Discussion

Carbohydrates

Hydrogen gas produced in the reactors to which cabbage, carrot and rice were individually fed. Acetate and butyrate were predominant VFAs in the soluble fraction of hydrogen fermentation products. It was reported that *Clostridium* was predominant space in the hydrogen fermentation reactor which was operated with digested sludge when the hydrogen production from the reactor was maintained (Cohen, 1979; Kanbe, 1992a, b). In this study, the compositions of the metabolites from the degradation of cabbage, carrot and rice were similar to those metabolites by *Clostridium* species. It was considered that *Clostridium* were predominant species in this study. It is well known that metabolic pathway of *Clostridium* species is affected by environmental factors (e.g. pH, acids concentration, hydrogen partial pressure etc.). An undissociated butyric acid plays an important role during the metabolic pathway selection for *Clostridium acetobutylicum* (Fond, 1985; Monot, 1983, 1984; Bahl, 1982). Substrate concentration is also a factor for the selection of metabolic pathway because it is a precursor of organic acids which are by-products of hydrogen fermentation. *Clostridium acetobutylicum* produces organic acids with low substrate concentration, on the other hand produces solvent with high substrate concentration (Long, 1984; Monot, 1983; Bahl, 1982). *Clostridium fallax* produces hydrogen, acetate and butyrate with low substrate concentration, on the other hand, if substrate concentration is over the threshold, *Clostridium fallax* changes metabolic pathway and produces lactate as a predominant product (Ueki, 1991). It is also known that metabolism pathways of *Clostridium acetobutylicum* (Yerushalmi, 1985), *Clostridium butyricum* (Andel, 1985) and *Clostridium cellobioparum* (Chung, 1976) were changed by hydrogen partial pressure. The

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Table 2 Comparison with previous studies

	Specific hydrogen production (mL/mg-COD)	Cultivation method	Seed	Reference
Glucose	148.6–210.4	continuous	activated sludge	Cohen (1979)
	195.8	continuous	secondary sludge	Zoetemeyer (1982)
	189.63	continuous	*Clostridium butyricum* LMG 77-11	Andel (1985)
	344.0	batch	*Clostridium beijerinckii* AM21B	Taguchi (1994)
	221.6	batch	*Clostridium pasteurianum* DSM 525	Dabrock (1992)
Sucrose	379.4	batch	*Clostridium beijerinckii* AM21B	Taguchi (1994)
Starch	230.3	batch	*Clostridium beijerinckii* AM21B	Taguchi (1994)
Cellulose	52.3	batch	digested sludge	Lay (1998)
	56.7	batch	*Clostridium thermocellum* YS	Lamed (1988)
	72.8	batch	*Clostridium thermocellum* AS-39	Lamed (1989)
	85.1	batch	*Clostridium thermocellum* LQRI	Lamed (1990)
cabbage	31.8–74.7 (26.3–61.7[(2)])			
carrot	8.8–61.4 (50.4–79.7[(2)])			
rice	16.8–83.3 (19.3–96.0[(2)])			
chicken skin	1.43–4.08 (3.56–10.2[(2)])	batch	digested sludge	this study
fat	1.56–3.95 (4.41–11.14[(2)])			
egg	1.31–3.57 (2.60–7.07[(2)])			
lean meat	1.75–5.43 (2.47–7.68[(2)])			

[(1)] Cellulose, Starch was calculated as hexose $[(C_6H_{10}O_5)_n]$, [(2)] (mL/mg-VS)

predominant by-products were organic acids under the low hydrogen partial pressure, on the other hand, those were solvent under the high hydrogen partial pressure. Operational factors such as stirring speed influenced on hydrogen partial pressure may affect on the metabolic pathway selection (Lamed, 1988; Doremus, 1985; Yerushalmi, 1985). Because *Clostridium* species could change metabolic pathway by environmental conditions, hydrogen gas, one of the metabolites could not be obtained if suitable control was not performed.

Lipids

Glycerol and long chain fatty acids mainly produce from anaerobic hydrolysis of lipids. The degradations of long chain fatty acids (β-oxidation) are thermodynamically unfavorable reactions unless the hydrogen partial pressure is maintained to an extremely low level. Moreover, it takes a long time to degrade long chain fatty acids by anaerobic bacteria, because long chain fatty acids are one of the inhibitors for anaerobic bacteria (Hanaki, 1981). Therefore, it may be difficult to produce hydrogen from long chain fatty acids. When lipid was used as a substrate, a long lag time was observed in this study. For example, 200 hours of lag time was observed on fat (TS4%). Glycerol could be a substrate for hydrogen production and for solvent production with saccharolytic clostridia (Heyndrickx, 1991). Among *Clostridium butyricum* and *Clostridium pasteurianum* strains, there are strains

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which can grow with glycerol as carbon and energy sources. *Clostridium butyricum* mainly produces butanol and *Clostridium pasteurianum* mainly produces 1,3-propandiol (Heyndrickx, 1991). Heyndrickx *et al.* (1991) suggest that glycerol is not a suitable substrate for hydrogen production, but an excellent substrate for solvent production. Dabrock *et al.* (1992) also cultivated *Clostridium pasteurianum* on glycerol under the limited condition of iron and phosphate, and *Clostridium pasteurianum* produced ethanol, butanol, lactate and 1,3-propandiol, on the other hand acetate and butyrate were hardly produced. Biebl *et al.* (1991) cultivated *Clostridium butyricum* on glycerol, and *Clostridium butyricum* produced 1,3-propandiol as a predominant metabolite. It is apparent that glycerol may be suitable substrate for solvent production rather than hydrogen production. Because a large portion of chemical oxygen demand (COD) of lipid is converted to long chain fatty acids during the hydrolysis reaction, even if glycerol could have high hydrogen production potential, hydrogen production from lipid may not be high. In fact, hydrogen production yields of fat and chicken skin were very low. From the above mentioned, it seems that it may be very difficult to produce hydrogen from substrate which includes a large quantity of lipids.

Proteins

Protein is hydrolyzed to various amino acids by extracellular enzymes. There are two types of anaerobic amino acid degradation reactions by clostridia. One is sole degradation of amino acid, the other is degradation by Stickland reaction. Degradation of amino acids involves production of volatile fatty acids and ammonia. The concentration of ammonia produced correlated with the amount of amino acids (proteins) degraded (Breure, 1984). Therefore, degree of protein degradation can be known by observing ammonia concentration. In this study, when egg and lean meat were used as substrate, ammonia was produced and the concentration had been increased during the experimental period. It was apparent that amino acids were produced from protein degradation, and moreover the amino acids were degraded by hydrogen producing bacteria. Protein degradation is suitable on neutral pH range (Breure, 1984). Because pH was over 6.3 in this study, protein might be efficiently degraded. Biogas was produced from protein degradation and major portion of the biogas was carbon dioxide. The hydrogen percentage of the biogas produced from protein degradation was much lower than that produced from carbohydrate and lipid. There may be some reasons why hydrogen percentage was low. Hydrogen is not produced by Stickland reaction of clostridia. Amino acids such as glycine, which was degraded by reductive deammination can consume molecular hydrogen as an electron donor (Nagase, 1982). Therefore, even if hydrogen is produced from sole amino acid degradation, the hydrogen can be utilized. From the above, it seems that it may be difficult to produce hydrogen from substrate which includes a large quantity of protein.

Conclusions

1. Among the OFMSW, carbohydrate produced the most hydrogen through biological hydrogen fermentation compared with proteins or lipids.
2 The biological hydrogen production potentials of some individual carbohydrates were measured: cabbage, 26.3–61.7 mL/g-Vs; carrot, 44.9–70.7 mL/g-VS; and rice, 19.3–96.0 mL/g-VS.
3 The hydrogen percentages of the total biogas produced from cabbage, carrot and rice were 33.9–55.1%, 27.7–46.8% and 44.0–45.6%, respectively.

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