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Conversion of food waste into hydrogen by thermophilic acidogenesis

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Abstract

Conversion of food waste into hydrogen by thermophilic acidogenesis was investigated as a function of organic loading rate (OLR), hydraulic retention time (HRT) and pH in a continuous stirred tank reactor. In order to identify hydrogen-producing microorganisms, denaturing gradient gel electrophoresis (DGGE) of the polymerase chain reaction (PCR) – amplified V3 region of 16S rDNA analysis was conducted at each tested pH. The conversion of food waste into hydrogen was strongly influenced by the operational conditions. The hydrogen production was increased as OLR increased up to 8 $gVSl^{-1}$ d^{-1}, but drastically decreased at 10 $gVSl^{-1}$ d^{-1}. The yield of hydrogen was decreased from 2.2 to 1.0 mol-H_2/mol-hexose consumed as HRT decreased from 5 to 2 days. More carbohydrates in the food waste were decomposed at longer HRT, 76–90%, at HRT of 2–5 days. The hydrogen production peaked at pH 5.5 ± 0.1 and significantly decreased at pH 5.0 ± 0.1. The biogas produced was composed of hydrogen and carbon dioxide, but no methane was detected at all tested conditions. The hydrogen contents in the gas produced were more than 55% (v/v) and not sensitive to all tested conditions. The optimum operational condition for continuous hydrogen production from the food waste was obtained at 8 $gVSl^{-1}d^{-1}$, 5 days HRT and pH 5.5 ± 0.1 where the hydrogen production rate, content, yield and the efficiency of carbohydrate decomposition were 1.0 l H_2/l-d, 60.5% (v/v), 2.2 mol-H_2/mol-hexose consumed and 90%, respectively. The hydrogen production was related with the concentration of total organic acids (TOA) which was strongly dependent on that of butyrate indicating that the reaction was mainly butyrate fermentation. The hydrogen-producing microorganism of *Thermoanaerobacterium thermosaccharolyticum* that involved in acetate/butyrate fermentation, was detected with strong intensity at all tested pHs by denaturing gradient gel electrophoresis (DGGE) of the polymerase chain reaction (PCR) – amplified V3 region of 16S rDNA analysis and sensitive to the tested pHs. The experimental results indicated that effective hydrogen production from the food waste could be obtained continuously by thermophilic acidogenesis at proper operational condition.

Introduction

Hydrogen is a promising alternative to fossil fuels due to its clean, renewable and high energy yield. Biological hydrogen production by anaerobic fermentation is an environmentally friendly and energy saving process. Anaerobic acidification of organic wastes produce various organic acids, H_2, CO_2, and other intermediates. The reactions involved in hydrogen production are rapid and do not require solar radiation, making them useful for treating large quantities of organic wastes.

Fermentative microorganisms, such as *Clostridium* and *Thermoanaerobacterium*, are able to

produce hydrogen from carbohydrates (Lay 2000; Ueno et al. 2001a, b; Zhang et al. 2003). During anaerobic acidification of organic wastes, methanogenesis or sulfate-reducing bacteria consume hydrogen produced by acidogenesis, contributing negatively to biohydrogen production (Mizuno et al. 2000). Therefore, most continuous and batch experiments conducted under mesophilic conditions on fermentative hydrogen production have been carried out at the inhibitory conditions of hydrogen consumers such as short hydraulic retention time (HRT) and/or low pH (Fang et al. 2002; Horiuchi et al. 2002; Yu et al. 2002).

To date, carbohydrate-rich organic solid wastes those need HRT longer than 3 days for acidification in which hydrogen consumers such as methanogenesis could be proliferated, were focused on efficient production of methane which had only about one third energy yield that of hydrogen (Mata-Alvarez et al. 2000). However, if hydrogen consumers were kept from growing at acidification, hydrogen can be obtained effectively from organic solid wastes. Although hydrogen productions from carbohydrate-rich organic solid wastes by batch experiments have been published (Lay et al. 1999; Noike et al. 2000; Okamoto et al. 2000), continuous experiments have not been reported. It was reported that more hydrogen could be produced at thermophilic condition than mesophilic condition (Yu et al. 2002; Zhang et al. 2003), and thermophilic acidogenesis such as *Thermoanaerobacterium thermosaccharolyticum* had nearly equivalent hydrogen production ability to that of *Clostridium butyricum* (Ueno et al. 2001b). In addition, thermophilic condition was reported as inhibitory effect to methanogenesis (Ueno et al. 1996).

This study, therefore, was attempted to investigate continuous hydrogen production from organic solid waste by inhibiting the growth of hydrogen consumers under thermophilic condition. Food waste, a carbohydrate-rich organic solid waste, was used in this study as a substrate. To find the optimum operational condition, the influence of OLR, HRT and pH on the performance of continuous hydrogen production were studied. The responsible hydrogen-producing microorganisms and their dynamic behavior in response to pH shift were examined by denaturing gradient gel electrophoresis (DGGE) of the polymerase chain reaction (PCR) – amplified V3 region of 16S rDNA.

Materials and methods

Seed sludge and substrate

The seed sludge was obtained from an anaerobic digestion tank in a municipal sewage treatment plant. The sludge had total suspended solid (TSS), volatile suspended solid (VSS) and pH of 23.6 gl^{-1}, 14.1 gl^{-1} and 7.3, respectively. The raw seed sludge was acclimated to food waste without heat-pretreatment in a continuous stirred tank reactor (CSTR) for 3 months at pH, OLR, HRT and temperature of 5.5 ± 0.1, 3 $gVSl^{-1}$ day^{-1}, 5 d and 55 ± 1 °C, respectively. The hydrogen content in the reactor at steady-state was 54% (v/v), but no detectable methane was produced. Butyrate and acetate were the main organic acids.

Food waste collected from a dining hall was ground by garbage disposer (Anahiam MFG, Co., USA) after sorting out animal bones and clamshells. It was mixed with deionized water at the ratio of 1:2 and stored 4 °C to prevent pre-acidification. Table 1 shows the physical and chemical characteristics of food waste such as moisture content, VS/TS and C/N.

Table 1. Characteristics of food waste

Item	Unit	Value
Physical characteristics		
Moisture content	%	82.5 ± 3.0
Bulk density	kg/m^3	892.5 ± 22.5
VS/TS	%	0.94 ± 0.02
pH		5.0 ± 0.5
Composition		
Grains	% TS	35.7 ± 6.5
Vegetables	% TS	47.1 ± 7.4
Meat	% TS	17.2 ± 5.3
Chemical characteristics		
Elementary analysis		
Carbon, C	% TS	51.2 ± 6.5
Hydrogen, H	% TS	7.2 ± 1.3
Oxygen, O	% TS	38.1 ± 5.1
Nitrogen, N	% TS	2.8 ± 0.6
Sulfur, S	% TS	0.7 ± 0.1
C/N		18.3 ± 2.4
Total carbohydrate	gl^{-1}	25 ± 4.8

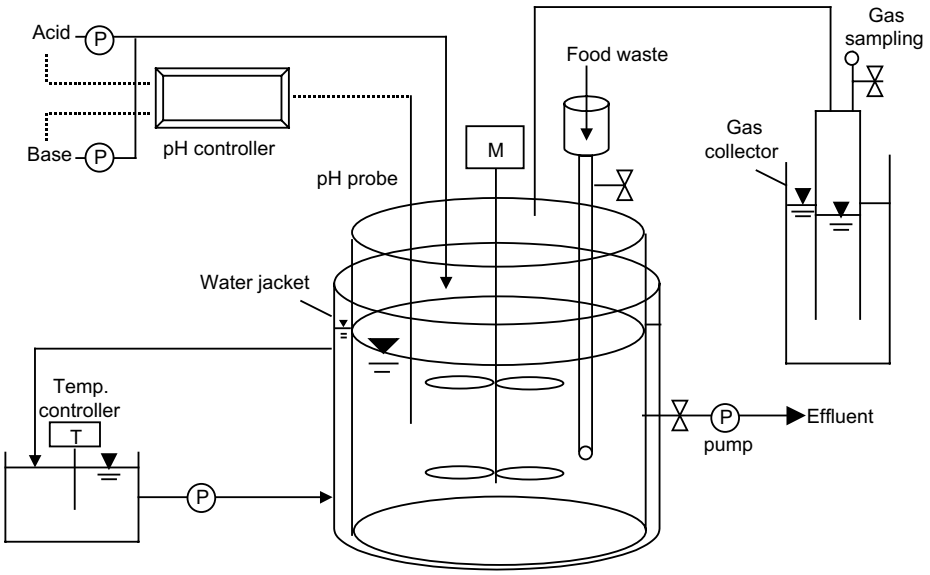

Figure 1. Schematic description of the continuous stirred tank reactor for continuous hydrogen production.

Experimental apparatus and operation

Figure 1 describes the lab-scale continuous stirred tank reactor (CSTR) with working volume of 3 l (internal diameter 13 cm, height 30 cm) that was fed with the food waste by draw and fill mode once every 24 h. Temperature was maintained at 55 ± 1 °C by the external water jacket. Constant pH was adjusted by 2N KOH and 2N HCl, and the mixing speed was 50 rpm. The biogas produced was collected by the downward displacement of acidified water (0.05 M H_2SO_4), and the gas volumes were corrected to a standard temperature (0 °C) and pressure (760 mmHg) (STP).

Three series of experiments were conducted to investigate the effect of different operational parameters on continuous hydrogen production. Table 2 shows the operational parameters and conditions used in this study.

In Series I, the OLR was increased stepwise as 6, 8 and 10 $gVSl^{-1}$ day^{-1}, while keeping HRT and pH at 5 days, 5.5 ± 0.1, respectively; in Series II, the HRT was decreased stepwise as 5, 3 and 2 days, while keeping OLR and pH at 8 $gVSl^{-1}$ day^{-1}, 5.5 ± 0.1, respectively; in Series III, the pH of the mixed liquor was changed from 5.5 ± 0.1 to 5.0 ± 0.1 and then 6.0 ± 0.1, while keeping HRT and OLR at 5 days, 8 $gVSl^{-1}$ day^{-1}, respectively. At each OLR, HRT and pH, the reactor was operated for 3 weeks to reach steady-state condition. Steady-state condition was defined when the variation range of product concentrations was less than 10% at least for 1 week.

Analyses

The gas composition was analyzed using a gas chromatograph (Gow Mac series 580, USA) with a thermal conductivity detector and two columns. The methane and carbon dioxide were detected with a column packed with porapak Q (80/100 mesh), and the hydrogen was detected with a

Table 2. Operational parameters and conditions used in this study

	OLR ($gVSl^{-1}$ d^{-1})			HRT (day)			pH		
Series I	6	8	10		5			5.5 ± 0.1	
Series II		8		5	3	2		5.5 ± 0.1	
Series III		8			5		5.5 ± 0.1	5.0 ± 0.1	6.0 ± 0.1

column packed with molecular sieve 5A. The temperature of injector, detector and column were kept at 80, 90 and 50 °C, respectively. Helium was used as a carrier gas. Organic acids were quantified by a high-performance liquid chromatography (Spectrasystem P2000, USA) with an ultraviolet (210 nm) detector and an Aminex HPX-97H (300 × 7.8 mm) column after pretreatment with 0.45 μm membrane filter. H_2SO_4 of 0.005 M was used as a mobile phase. Carbohydrate was measured using the calorimetric ferric-cyanide method (Dubois et al. 1956). Measurements of total solid (TS), volatile solid (VS) and pH were performed according to the Standard Methods (APHA 1992).

In order to identify the responsible hydrogen-producing microorganisms and investigate their dynamic behavior in response to pH shift, DNA in the microbial community at each tested pH was extracted at steady-state by using the Ultraclean DNA Kit (Cat # 12800-50; Mo Bio Laboratory Inc., USA). For the amplication of 16S rDNA fragments, EUB 357f(5′-CCTACGGGAGGCAGCAG-3′) and UNIV518r(5′-ATTACCGCGGCTGCTGG-3′) with a GC clamp were used as a forward and reverse primer, respectively. PCR products were purified using MultiScreen Vacuum Manifold (MILLIPORE com., USA), and search of the GenBank database was conducted using the BLAST program.

Results and discussion

Effect of OLR

For the performance of OLR experiments, the food waste stored at 4 °C was mixed with deionized water again to adjust OLR to 6, 8 and 10 $gVSl^{-1}$ d^{-1} before feeding. Figures 2 and 3 show the effect of OLR conducted at pH 5.5 ± 0.1 and 5 days HRT on continuous hydrogen production and key organic acids production, respectively.

In anaerobic thermophilic acidification of the food waste, the biogas produced contained hydrogen and carbon dioxide, but no detectable methane at all tested OLRs. The hydrogen production rate and content were increased from 0.7 l H_2/l-d and 58.2% (v/v) at 6 $gVSl^{-1}$ d^{-1} to 1.0 l H_2/l-d and 60.5% at 8 $gVSl^{-1}$ d^{-1}, but significantly decreased from the operation time of 52 days at 10 $gVSl^{-1}$ d^{-1} when the concentration of total organic acids was exceeded 20,000 mgl^{-1} as COD. The yield of hydrogen was decreased as OLR increased from 2.4 mol-H_2/mol-hexose consumed at 6 $gVSl^{-1}$ d^{-1} to 2.2 mol-H_2/mol-hexose consumed at 8 $gVSl^{-1}$ d^{-1}. Previous studies also reported that the yield of hydrogen was decreased as the concentration of substrate increased (Yu et al. 2002; Zhang et al. 2003). The decrease in hydrogen yield as increase of substrate concentration could be resulted from the increase of total organic acids,

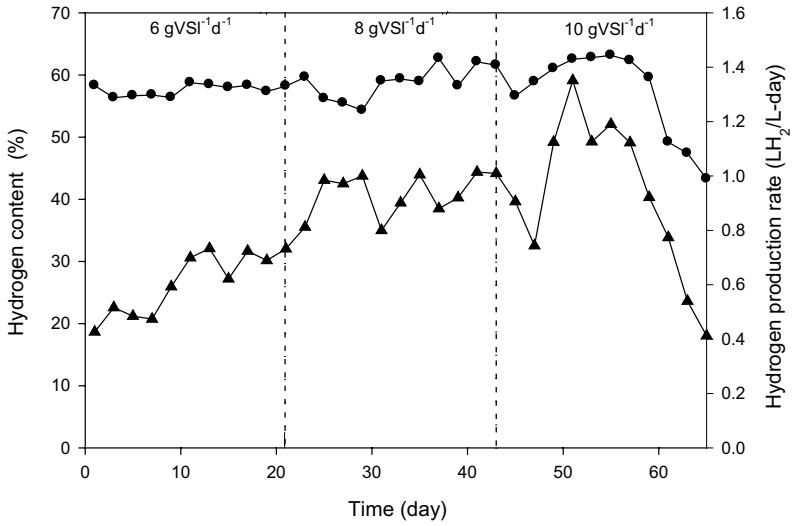

Figure 2. Effect of OLR on continuous hydrogen production. (●) Hydrogen content, (▲) hydrogen production rate.

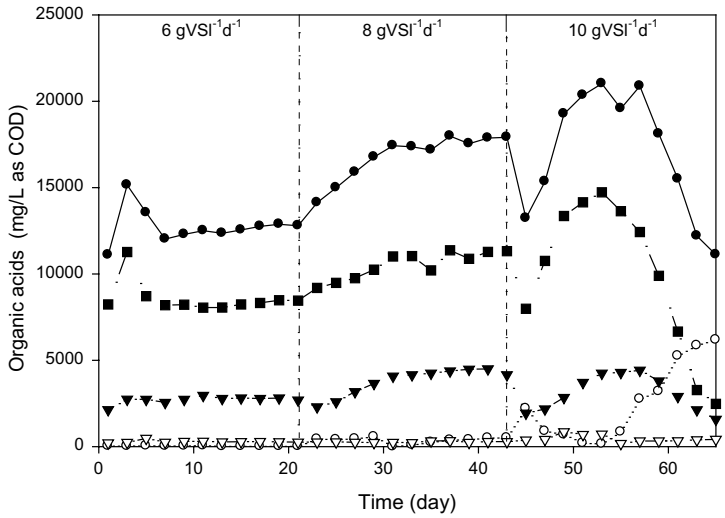

Figure 3. Effect of OLR on organic acids production. (●) Total organic acids, (■) *n*-butyrate, (▼) acetate, (○) lactate, (▽) propionate.

which may inhibit the further production of hydrogen (Ginkel et al. 2001). The decomposition efficiencies of carbohydrates in the food waste were decreased as OLR increased from 92% at 6 $gVSl^{-1}$ d^{-1} to 90% at 8 $gVSl^{-1}$ d^{-1}.

Normal butyrate and acetate were the main organic acids, and the contents of normal butyrate and acetate in the total organic acids ranged 62–65% and 22–25%, respectively. Lactate and propionate considered as hydrogen consumers were only 0.1–2.0% and 1.6–2.2%, respectively. Lactate, however, increased significantly at 10 $gVSl^{-1}$ d^{-1}, while other organic acids decreased where the acidification process failed. It was found that the desirable OLR was 8 $gVSl^{-1}$ d^{-1}, and the total organic acids concentration of 20,000 mgl^{-1} as COD was the threshold in the thermophilic acidification process.

Effect of HRT

The volume of mixed liquor in the reactor required for the adjustment of HRT was pumped out daily. Since acidification in the reactor was drastically decreased at 10 $gVSl^{-1}$ d^{-1}, feeding was stopped for 5 d and then OLR was decreased to 8 $gVSl^{-1}$ d^{-1}. The experiments for HRT were performed when steady-state was achieved at pH 5.5 ± 0.1, 8 $gVSl^{-1}$ d^{-1} and 5 days HRT after 3 weeks. Figures 4 and 5 show the effect of HRT conducted at pH 5.5 ± 0.1 and OLR of 8 $gVSl^{-1}$ d^{-1} on continuous hydrogen production and key organic acids production, respectively.

When HRT decreased 5, 3 and 2 days, the hydrogen production rate was also decreased as 1.0, 0.6 and 0.5 l H_2/l-d, respectively. Hydrogen contents ranged from 54.9% to 60.5%, while peaked at 5 days HRT, and the biogas produced was free of methane at all tested HRTs. The yield of hydrogen decreased from 2.2 to 1.0 mol-H_2/mol-hexose consumed as HRT decreased from 5 to 2 days. More carbohydrates in the food waste were decomposed at longer HRT, 76–90%, at the HRT of 2–5 days. This is in agreement with the result of Ueno et al. (1996) in which the efficiencies of carbohydrates decomposition in the sugary wastewater was increased from 70% to 97% as the HRT increased from 0.5 to 5 days. Chang et al. (2004) also reported that long HRT favored sucrose degradation. However, the hydrogen yield in this study was also increased as HRT increased up to 5 days. This result was discrepant from the findings of Ueno et al. (1996) in which hydrogen yield was decreased as HRT increased, and Chang et al. (2004) in which higher sucrose degradation at longer HRT did not enhance hydrogen production. The discrepancy could be explained that hydrogen utilising methanogenesis were proliferated and increase of methane production with increasing HRT resulted in decreasing hydrogen yield as HRT increased (Ueno et al. 1996), while

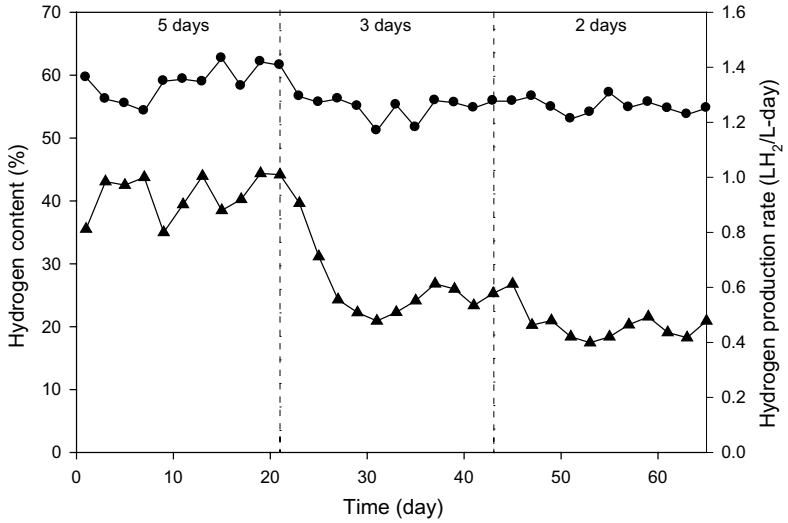

Figure 4. Effect of HRT on continuous hydrogen production. (●) Hydrogen content, (▲) hydrogen production rate.

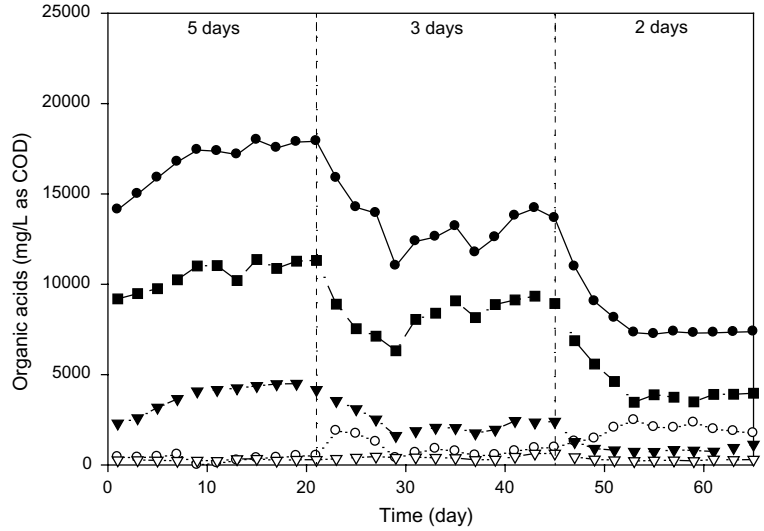

Figure 5. Effect of HRT on organic acids production. (●) Total organic acids, (■) *n*-butyrate, (▼) acetate, (○) lactate, (▽) propionate.

the high removal efficiencies of easily biodegradable sucrose ranged from 91.8% to 96.3% at tested HRTs from 4 to 24 h might not much increase the hydrogen yields as HRT increased (Chang et al. 2004). It can be postulated that increase of carbohydrates decomposition with increasing HRT and inhibition of methanogenesis at longer HRT may cause the higher yield of hydrogen at longer tested HRT in this study.

The concentration of total organic acids also decreased as HRT decreased from 17,641 mgl^{-1} as COD at 5 days to 7323 mgl^{-1} as COD at 2 days, but the components of organic acids changed. The concentration of butyrate and acetate were decreased, while that of propionate and lactate increased as HRT decreased. The content of butyrate and acetate were decreased from 62% to 52% and 25% to 12%, but that of propionate and lactate were increased from 1.6% to 3.6% and 2.0% to 27.4%, respectively as HRT decreased from 5 to 2 days. Lactate was significantly increased at 2 days HRT.

Effect of pH

Since the optimum OLR and HRT were revealed at 8 $gVSl^{-1}$ d^{-1} and 5 days, the HRT in the reactor was increased from 2 to 5 days after HRT tests to establish the steady-state at pH 5.5 ± 0.1, 8 $gVSl^{-1}$ d^{-1} and 5 days HRT. The steady-state was achieved after 2 weeks. To find the optimum pH, the pH in the reactor was decreased from 5.5 ± 0.1 to 5.0 ± 0.1, and then increased to 6.0 ± 0.1. Figures 6 and 7 show the effect of pH conducted at OLR of 8 $gVSl^{-1}$ d^{-1} and 5 days HRT on continuous hydrogen production and key organic acids production, respectively.

The hydrogen content was not sensitive to the each tested pH. It was just decreased from 60.5% at pH 5.5 ± 0.1 to 57.6% at pH 5.0 ± 0.1. The biogas produced was free of methane at all tested pHs. The maximum hydrogen production rate was 1.0 l H_2/l-d at pH 5.5 ± 0.1, but significantly

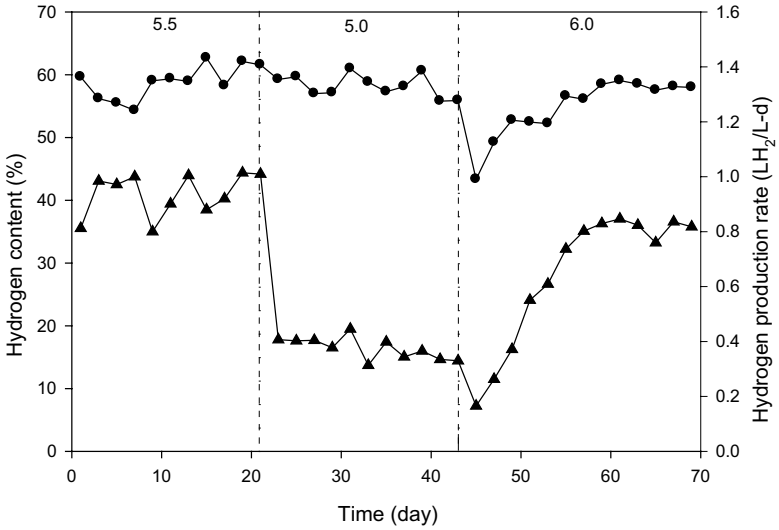

Figure 6. Effect of pH on continuous hydrogen production. (●) Hydrogen content, (▲) hydrogen production rate.

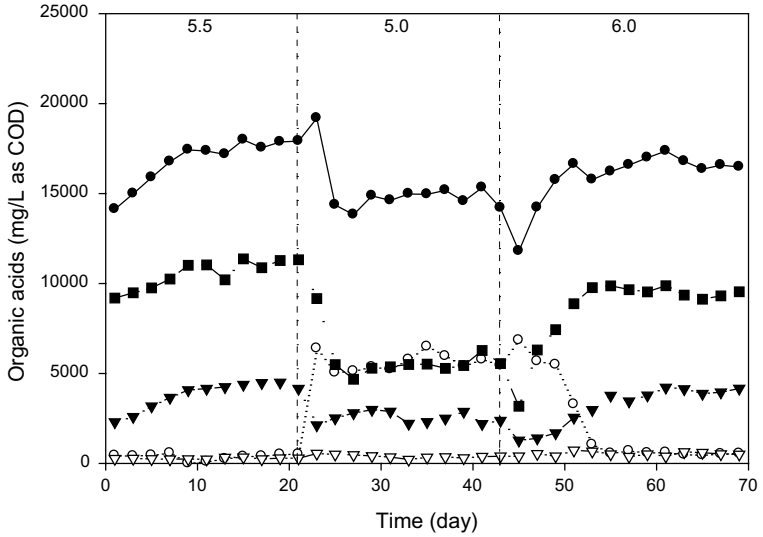

Figure 7. Effect of pH on organic acids production. (●) Total organic acids, (■) *n*-butyrate, (▼) acetate, (○) lactate, (▽) propionate.

decreased to 0.4 l H_2/l-d at pH 5.0 ± 0.1. At pH 6.0 ± 0.1, it was recovered to 80% of its maximum as 0.8 l H_2/l-d. The yields of hydrogen were 2.2, 1.0 and 2.0 mol-H_2/mol-hexose consumed at pH 5.5 ± 0.1, 5.0 ± 0.1 and 6.0 ± 0.1, respectively. The efficiencies of carbohydrates decomposition in the food waste ranged from 74% to 90% and peaked at pH 5.5 ± 0.1. The production of organic acids especially normal butyrate and lactate were strongly dependent on pH. The content of butyrate was decreased from 62% to 38%, while that of lactate was increased from 2.0% to 39% as pH decreased from 5.5 ± 0.1 to 5.0 ± 0.1. However, at pH 6.0 ± 0.1, the content of butyrate was increased to 57% while that of lactate was decreased to 3.3%. The results suggested that the optimum pH for continuous hydrogen production in this study was 5.5 ± 0.1 that is in accordance with the previous researches. Fang & Liu (2002) systematically investigated the pH optimum of hydrogen production from glucose at a 6 h HRT over the range 4.0–7.0 and found the optimum yield at pH 5.5. Ginkel et al. (2001) also studied hydrogen production as a function of pH and reported that a pH between 5.0 and 6.0 was suitable for hydrogen production, whereas the highest conversion efficiency occurred at a pH of 5.5. Yu et al. (2002) reported that an optimum hydrogen production was achieved at pH 5.5 and 55 °C from a high-strength rice winery wastewater.

Tables 3 and 4 show the steady-state analysis at each operational parameter and condition. The average values of gas contents and carbohydrate decomposition in Table 4 were estimated from 6 data points (operation in 12 days) those were in the range of steady-state.

Table 3. Steady-state analysis for hydrogen and organic acids production at each operational parameter and condition

		H_2 (%)	H_2 (LH_2/L-day)	H_2 yield (mol-H_2/mol-hexose)	Organic acids (mgl^{-1} as COD)				
					TOA	HLa	HAc	HPr	*n*-HBu
Series I									
OLR	6	58.2 ± 0.4	0.7 ± 0.04	2.4 ± 0.3	12637 ± 183	15 ± 13	2807 ± 81	280 ± 15	8263 ± 172
($gVSl^{-1}$ d^{-1})	8	60.5 ± 1.7	1.0 ± 0.06	2.2 ± 0.2	17641 ± 301	344 ± 144	4318 ± 140	284 ± 43	11012 ± 400
Series II									
HRT	5	60.5 ± 1.7	1.0 ± 0.06	2.2 ± 0.2	17641 ± 301	344 ± 144	4318 ± 140	284 ± 43	11012 ± 400
(day)	3	54.9 ± 1.5	0.6 ± 0.04	1.5 ± 0.3	13199 ± 818	739 ± 171	2158 ± 255	446 ± 144	8921 ± 373
	2	55.2 ± 1.1	0.5 ± 0.03	1.0 ± 0.2	7323 ± 47	2006 ± 182	863 ± 138	266 ± 30	3817 ± 160
Series III									
pH	5.5 ± 0.1	60.5 ± 1.7	1.0 ± 0.06	2.2 ± 0.2	17641 ± 301	344 ± 144	4318 ± 140	284 ± 43	11012 ± 400
	5.0 ± 0.1	57.6 ± 1.8	0.4 ± 0.03	1.0 ± 0.3	14844 ± 379	5799 ± 363	2410 ± 232	328 ± 60	5592 ± 318
	6.0 ± 0.1	57.7 ± 1.1	0.8 ± 0.05	2.0 ± 0.2	16714 ± 387	552 ± 79	3872 ± 226	315 ± 72	9568 ± 291

TOA – total organic acids; HLa – lactate; HAc – acetate; HPr – propionate; *n*-Bu – normal butyrate.

PCR-DGGE analysis

The microbial community and their dynamic behavior in response to pH shift were examined by PCR-DGGE analysis targeted at eubacterial 16S rDNA. The DGGE profiles and the results of sequence affiliation determined by the BLAST are shown in Figure 8 and Table 5, respectively.

Thermoanaerobacterium thermosaccharolyticum (bands A-1, A-2, A-3, A-4, A-5, B-2, C-3, C-4, C-5, and C-6) that was known as a hydrogen-producing microorganism, was detected with strong intensity at all tested pHs. *Thermoanaerobacterium thermosaccharolyticum* is a thermophilic saccharolytic

Table 4. Steady-state analysis for carbohydrate decomposition efficiency and biogas content at each operational parameter and condition

		Carbohydrate decomposition (%)	Gas content (%)		
			H_2	CO_2	CH_4
Series I					
OLR	6	92 ± 2.1	58.2 ± 0.4	41.8 ± 1.4	ND
($gVSl^{-1}$ d^{-1})	8	90 ± 2.7	60.5 ± 1.7	39.5 ± 2.2	ND
Series II					
HRT	5	90 ± 2.7	60.5 ± 1.7	39.5 ± 2.2	ND
(day)	3	81 ± 3.2	54.9 ± 1.5	45.1 ± 2.8	ND
	2	76 ± 3.0	55.2 ± 1.1	44.8 ± 2.4	ND
Series III					
pH	5.5 ± 0.1	90 ± 2.7	60.5 ± 1.7	39.5 ± 2.2	ND
	5.0 ± 0.1	74 ± 2.9	57.6 ± 1.8	42.4 ± 3.2	ND
	6.0 ± 0.1	87 ± 3.2	57.7 ± 1.1	42.3 ± 2.8	ND

ND – non-detectable; ND is gas content <0.1%.

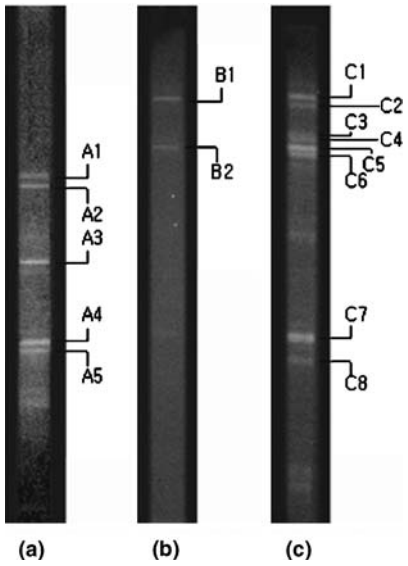

Figure 8. Denaturing gradient gel electrophoresis (DGGE) profiles of the PCR-amplified 16S rDNA extracted from the microbial community at tested pH under steady-state. (a) pH 5.5 ± 0.1; (b) pH 5.0 ± 0.1; (c) pH 6.0 ± 0.1.

microorganism involved in acetate/butyrate fermentation that leads to produce large amount of hydrogen from carbohydrates (Ueno et al. 2001a). From the characteristic study of *T. thermosaccharolyticum* (Ueno et al. 2001b), it was reported that the maximum growth of *T. thermosaccharolyticum* was at the pH range from 5 to 6, and the optimum temperature for growth was 60 °C. The yield of hydrogen production from *T. thermosaccharolyticum* was 2.4 mol-H_2/mol-glucose nearly equivalent hydrogen production ability to that of *Clostridium butyricum* which had hydrogen production yield of 2.4 mol-H_2/mol-hexose.

At pH 5.5 ± 0.1, only one species, *T. thermosaccharolyticum* was detected. It can be inferred from the result that other microorganisms in the reactor were inactivated at the thermophilic and acidogenic operational condition, but it was favor environment for the growth of *T. thermosaccharolyticum* resulted in the microorganism as a predominant species in the community. The performance of hydrogen production was deteriorated when the number of bands affiliated with the *T. thermosaccharolyticum* was decreased as pH decreased from 5.5 ± 0.1 to 5.0 ± 0.1, but recovered at pH 6.0 ± 0.1 when the number of bands increased. The results implied that this microorganism participated in the fermentation reactions and played key role for the hydrogen production.

Table 6 compares the yield of hydrogen obtained in this study with those in the literature.

The maximum hydrogen yield of 2.20 mol-H_2/mol-hexose consumed in this study was comparable to the yields reported. The distinguished result of this study was the high yield of hydrogen from the food waste by inhibition of methanogenesis at

Table 5. Affiliation of denaturing gradient gel electrophoresis (DGGE) fragments determined by their 16S rDNA sequence

pH	Band	Sequence determined (bp)	Affiliation	Similarity[a] (%)	Accession no.
5.5	A-1	1524	*Thermoanaerobacterium thermosaccharolyticum*	95	M59119
	A-2	1524	*Thermoanaerobacterium thermosaccharolyticum*	97	M59119
	A-3	1524	*Thermoanaerobacterium thermosaccharolyticum*	99	M59119
	A-4	1524	*Thermoanaerobacterium thermosaccharolyticum*	100	M59119
	A-5	1524	*Thermoanaerobacterium thermosaccharolyticum*	97	M59119
5.0	B-1	469	Uncultured actinobacterium	100	AF431551
	B-2	1524	*Thermoanaerobacterium thermosaccharolyticum*	97	M59119
6.0	C-1	469	Uncultured actinobacterium	100	AF431551
	C-2	136	Uncultured bacterium	92	AB059460
	C-3	1524	*Thermoanaerobacterium thermosaccharolyticum*	85	M59119
	C-4	1524	*Thermoanaerobacterium thermosaccharolyticum*	95	M59119
	C-5	1524	*Thermoanaerobacterium thermosaccharolyticum*	97	M59119
	C-6	1524	*Thermoanaerobacterium thermosaccharolyticum*	98	M59119
	C-7	1463	*Pseudomonas* sp. JPL-1	88	AY030314
	C-8	627	*Marine bacterium* HP42a	88	AY241569

[a] Percentage similarity to the closest relative according to the BLAST comparison.

Table 6. Comparison of hydrogen yield obtained in this study with those in the literature

Substrate	HRT	Temp. (°C)	pH	H_2 yield (mol-H_2/mol-hexose)	References
Food waste	5 days	55	5.5	2.20	This study
Sugary wastewater	3 days	60	6.8	1.91	Ueno et al. (1996)
Winery wastewater	2 h	55	5.5	1.90	Yu et al. (2002)
Glucose	8.5 h	35	6.0	1.43	Mizuno et al. (2000)
Sucrose	8 h	35	6.7	1.50	Chang et al. (2004)

long HRT. The reasons for the apparent complete inhibition of methanogenesis in this study could be inferred from previous researches. Ueno et al. (1996) conducted hydrogen production from sugary wastewater at pH 6.8 and 60 °C for various HRTs of 0.5, 1, 2 and 3 days and reported that methane was produced at all tested HRTs and increased with increasing HRT. It was speculated that the fermentation pattern might shift to methanogenic fermentation, if the HRT of the wastewater would be increased. The incomplete inhibition of methanogenesis in their study might be caused by the short period of cultivation which was less than 1 month and operation at relatively

Table 7. COD balance and the fraction of feed COD converted to hydrogen-COD

		$COD_{influent}$ (g d^{-1})	$COD_{effluent}$ (g d^{-1})	COD of H_2 (g d^{-1})	COD recovery (%)	COD of H_2 /$COD_{influent}$ (%)
Series I						
OLR	6	16.2	14.0	1.5	95.7	9.3
($gVSl^{-1}$ d^{-1})	8	21.6	18.6	2.1	95.8	9.7
Series II						
HRT	5	21.6	18.6	2.1	95.8	9.7
(day)	3	21.6	19.2	1.3	94.9	6.0
	2	21.6	19.1	1.1	93.5	5.1
Series III						
pH	5.5 ± 0.1	21.6	18.6	2.1	95.8	9.7
	5.0 ± 0.1	21.6	19.4	0.9	94.0	4.2
	6.0 ± 0.1	21.6	18.7	1.7	94.4	7.9

high pH of 6.5 even though the experiments were performed at thermophilic condition. It was reported that at pH values lower than 6.3, the methanogenesis rate decreased or stopped (Van Haandel and Lettinga 1994). Another study conducted at 55 °C and pH 5.5 ± 0.1 for the acidification of dairy wastewater reported that methane was produced and decreased as OLR increased from 4 to 24 $gCODl^{-1}$ d^{-1}, but the production of methane was ceased from OLR of 16 $gCODl^{-1}$ d^{-1} (Yu et al. 2000). The reason for the suspension of methane production at high OLRs was not clearly explained in the paper. However, methanogenesis might be still existed even though start-up was completed after operation of 67 days and complete inhibition of methanogenesis might be occurred at high OLRs as time passed.

In our study, the seed sludge was acclimated to food waste for 3 months at pH, OLR, HRT and temperature of 5.5 ± 0.1, 3 $gVSl^{-1}day^{-1}$, 5 days and 55 ± 1 °C, respectively. The biogas produced was free of methane after acclimation of seed sludge to the food waste for 3 months and then the experiments for OLR, HRT and pH were conducted. It could be postulated that thermophilic condition, operation at low pH and long period of acclimation of seed sludge to inhibit hydrogen utilising methanogenesis resulted in kept from the growth of methanogenesis even though at tested long HRT. In addition, *T. thermosaccharolyticum* that had high yield of hydrogen became a predominant species and the high efficiency of carbohydrates decomposition at long HRT might be caused the high yield of hydrogen in this study. The comparison indicated that food waste could be converted into hydrogen like the wastewaters and easily biodegradable pure substance such as glucose and sucrose. Since the fraction of feed COD converted to hydrogen-COD is probably more relevant in a practical sense, Table 7 shows COD balance and the fraction of feed COD converted to hydrogen-COD.

The influent COD value of food waste at 8 $gVSl^{-1}$ d^{-1} was 21.6 gCOD d^{-1} (that is, 8 $gVSl^{-1}$ d^{-1} × 3 L × 0.9 gCOD/gVS). The effluent COD included COD in organic acids, alcohol mainly ethanol and residuals. As 1 mol (22.4 L) of hydrogen was equivalent to 16 g of COD, the COD of hydrogen was calculated as: the volume (L) of $H_{2\ produced}$ × 0.714 $gCOD/LH_2$. The fraction of feed COD converted to hydrogen-COD ranged from 4.2% to 9.7% and peaked at OLR, HRT and pH of 8 $gVSl^{-1}$ d^{-1}, 5 days and 5.5 ± 0.1. The maximum conversion value of 9.7% is higher than previous studies. Yu et al. (2000) reported that only 2.5–8.8% of influent COD was converted to hydrogen and methane from thermophilic acidification of dairy wastewater. Another study reported that 4.0% of carrot as an example of carbohydrates was converted to hydrogen based on COD (Okamoto et al. 2000). The COD recoveries ranged from 93.5% to 95.8%.

Conclusions

From the experiments for conversion of food waste into hydrogen by thermophilic acidogenesis, the following conclusions were obtained.

(1) It was possible to harvest hydrogen-producing microorganism by acclimation of raw seed sludge to the food waste without heat-pretreatment and the growth of methanogenesis could be prevented at the thermophilic and acidogenic operational condition.
(2) *T. thermosaccharolyticum* was hydrogen-producing microorganism that involved in acetate/butyrate fermentation of carbohydrates in the food waste and sensitive to the tested pHs.
(3) Conversion of food waste into hydrogen was strongly dependent on the operational condition, thus proper operational condition control was essential to obtain efficient hydrogen production.
(4) The optimum operational condition for continuous hydrogen production from the food waste was obtained at 8 $gVSl^{-1}$ d^{-1}, 5 days HRT and pH 5.5 ± 0.1 where the hydrogen production rate, content, yield and the efficiency of carbohydrate decomposition were 1.0 l H_2/l-days, 60.5% (v/v), 2.2 mol-H_2/mol-hexose consumed and 90%, respectively.
(5) Continuous and stable hydrogen production from the food waste was feasible by thermophilic acidogenesis.
(6) It might be possible for the harvest of hydrogen at the acidification stage of anaerobic treatment, leaving the remaining acidification products such as acetate and butyrate for further methane production in a two-stage process from food waste.

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