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Hydrogen fermentation of food waste without inoculum addition

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ABSTRACT

A novel batch process that produces H_2 without inoculum addition was devised based on two facts: (1) the abundant indigenous microflora found within organic solid wastes and (2) batch H_2 production completion times being in the same range with hydraulic retention times at continuous processes. Food waste successfully served not only as a substrate but also as a source of H_2-producing microflora when heat (90 °C for 20 min), acid (pH 1.0 for 1 d), or alkali (pH 13.0 for 1 d) treatment was applied. Among the three pretreatments, the heat treatment showed the best performance. The role of the pretreatment was the selection of microbial population rather than the enhancement of hydrolysis. Polymerase chain reaction-denaturing gradient gel electrophoresis analysis showed that lactic acid bacteria were the most abundant species in untreated food waste while H_2-producing bacteria were dominant in the pretreated food wastes. The increase of pretreatment temperature depressed the lactate production while increased the H_2/butyrate production. Repeated batch operation performances were impressive and reliable, achieving a very high H_2 yield of 2.05 mol H_2/mol $hexose_{consumed}$ with a margin of 17% error. As this invented method is simpler than those of existing continuous systems, and does not require a start-up period, this method is thought to be practically applicable.

1. Introduction

The current energy system based on fossil fuels has raised a lot of concerns due to its lack of sustainability and its increasing greenhouse gas emissions. Today, 86% of the world energy consumption constitutes of fossil fuels, with the world energy demand tripling since 1949 and still increasing by 2% per annum [1,2]. It is assumed that the world's accessible oil reservoirs will be close to depletion within this century. Also, fossil fuel combustion has been the main cause of increased CO_2 concentration in the atmosphere, which is the highest ever recorded, exceeding pre-industrial concentration by 100 ppm [3].

Among many possible successors to fossil fuels, hydrogen is recognized as a permanent alternative since it is the most common element in the universe, produces only water when combusted, has 2.75 times higher energy content than hydrocarbon fuels, and is easily converted to electricity by fuel cells [4,5]. Many scholars have agreed that the 'Hydrogen Energy System' has already arrived, and the sooner this process begins, the less the economic burden will be on future generations [2,5]. Currently, over 90% of H_2 is now formed by steam reforming of hydrocarbons and coal gasification for their cost advantages. However, these methods are shunned from public and specialists because they rely on fossil fuels, emitting significant greenhouse gases [6]. Thus, a number of innovative hydrogen production paths including biological ways are being developed based on renewable energy sources.

Biological production of hydrogen (biohydrogen) is an exciting new area that utilizes bacteria which freely and efficiently produce H_2 as a by-product during their metabolisms. Largely, there are photosynthesis and dark fermentation, but the latter is known to have more superior aspects in practical points, i.e. no need of light, fast H_2 production, and low operating cost [7]. This cost advantage could be maximized when H_2 is produced from problematic actual wastes such as food waste.

In Korea, the generation of food waste reached about 13,373 tons/d, accounting for 39.7% of municipal solid wastes in 2006. It is the main source of decay, odor, and leachate in the collection and transportation due to its high volatile solids (VS; 85–95%) and moisture content (75–85%). About 93% of food waste is recycled, mostly to animal feed and compost, but the demand for these products is very rare due to its low quality. However, biogas production, especially H_2 from food waste seems possible as it has high energy content. Previously, several researchers attempted to generate H_2 from food waste and found it feasible [8,9].

The main purpose of continuous process in organic waste treatment is to retain active biomass at high concentration for the stable and fast treatment without any lag period. Introduction of high-rate anaerobic digesters including anaerobic sequencing batch reactor (ASBR) and up-flow anaerobic sludge blanket (UASB) reactor has dramatically improved the conventional anaerobic digestion

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Table 1
Comparison of H_2 production completion time in batch process and the HRT used in continuous operation treating organic solid wastes.

Operation mode	Substrate	H_2 production completion time or optimal HRT (h)	Maximum H_2 yield		Maximum H_2 production rate (mL/(L h))	Reference
			mol H_2/mol hexose	mL H_2/g VS		
Batch	Carbohydrate-rich food	50	NA[a]	96	NA[a]	[11]
	Rice bran	50	1.29	61	NA[a]	[12]
	Food waste + sewage sludge	72	1.05	NA[a]	NA[a]	[8]
	Refuse-derived fuel	72	NA[a]	NA[a]	NA[a]	[13]
	Beer less biomass	120	NA[a]	69	NA[a]	[14]
	Food waste	135	1.80	92	76	[15]
Continuous	Garbage slurry + paper	28.8	2.40	46	225	[16]
	Food waste	36	1.22	81	114	[17]
	Household solid waste	48	NA[a]	43	67	[18]
	Food waste + sewage sludge	72	1.05	62	42	[19]
	MSW[a] + slaughterhouse waste	72	NA[a]	34	71	[20]
	Food waste	120	2.20	125	42	[21]

[a] MSW = municipal solid waste; NA = not available.

process by maintaining high biomass concentration [10]. These processes may be applied to methanogens that have a low growth rate, but the merits obtained through these advanced reactor applications would be alleviated when using microorganisms that have a high growth rate such as H_2-producing bacteria.

Interestingly, the H_2 production completion time, including the lag period in batch process, was in the same range with the hydraulic retention time (HRT) used in the continuous process that treated organic solid wastes as shown in Table 1 [8,11–21]. Moreover, the stable continuous operation of H_2 fermentation was impeded by indigenous non-H_2-producing bacteria in organic wastes [17,22]. These facts created a doubt about the need of continuous operation in the H_2 fermentation of organic solid waste. In fact, the H_2 yield in the batch operation was five times higher than that of the continuous one in treating olive pulp waste [23]. Valdez-Vazquez et al. [24] also doubted the general concept that batch and semi-continuous operations are less efficient than continuous one in H_2 fermentation of organic solid wastes. However, there is a critical defect in the practicality of the batch process: the need to prepare inoculum every time, which is cumbersome, as well as the possible addition of pollution itself.

Food decomposes by itself if it is left for a long time at an ambient temperature due to the presence of many kinds of indigenous microorganisms, including lactic acid bacteria (LAB), propionic acid bacteria (PAB), fungi, and coliform [25]. Therefore, when food waste was used as a substrate for continuous H_2 production, alkali pretreatment at pH 12.5–13.0 was applied to kill non-H_2-producing bacteria when [17]. Spore-forming bacteria, such as *Clostridium* sp. and *Bacillus* sp., would survive during the pretreatment and then germinate if suitable circumstances were provided. The existence of *Clostridium* sp., the main H_2-producing genus, in several kinds of food has been studied in the food and medical microbiology [26]. Thus, it was hypothesized that food waste could serve not only as a substrate but also as a source of H_2-producing microflora when a proper pretreatment is applied. In this case, it would simplify the operation and the device required for H_2 production in practical application and it could be a suitable process for decentralized hydrogen production system which would facilitate the settlement of 'Hydrogen Society' [27,28].

With this idea and research backgrounds, this study was aimed to test the feasibility of H_2 production from food waste without inoculum addition. Pretreatment using heat, acid, or alkali was applied to inhibit the activity of non-H_2-producing microorganisms in food waste.

Table 2
Characteristics of food waste.

Item	Unit	Batch test		
		I	II	III
TCOD	g COD/L	131.2	143.5	120.5
SCOD	g COD/L	50.5	54.8	48.3
TS (total solid)	g TS/L	117.1	122.6	111.8
VS (volatile solid)	g VS/L	109.3	121.1	106.1
Carbohydrate	g COD/L	62.1	84.4	74.1
TKN (total Kjeldahl nitrogen)	mg N/L	2513	2723	2518
pH	–	5.4	5.1	5.0

2. Materials and methods

2.1. Feedstock preparation

Food waste, collected from a cafeteria, was shredded by a grinder to smaller than 5 mm in diameter. As all of the food waste was not collected at the same time, the characteristics of food waste used were different at each batch test (Table 2). The acidic condition of food waste indicates the presence of organic acids by its natural decomposition.

2.2. Batch test

In batch test I, food waste was pretreated in three different ways: (1) heat treatment at 90 °C for 20 min, (2) acid treatment at pH 1.0 for 1 d, and (3) alkali treatment at pH 13.0 for 1 d. In order to investigate the effects of heat treatment in detail, the pretreatment temperature varied from 60 to 90 °C (10 °C interval) with the same heating time of 20 min in batch test II. Heating temperatures over 90 °C was excluded because it requires enormous energy in evaporating water. In batch test III, comparison of heat-treated food waste (90 °C for 20 min) with untreated food waste was repeated as an additional test.

For the batch tests I and II, 635-mL batch fermenters with pH sensors were used to examine H_2 production and microbial communities. As the H_2 production potential of carbohydrate is much higher than that of lipid and protein, the initial food waste concentration in the batch test was set at 30 g carbohydrate COD/L [24]. A predetermined amount of pretreated or untreated food waste was added to the fermenter and filled with tap water up to 200 mL. Neither external inoculum nor basal medium was added. The large headspace volume (435 mL) alleviated the adverse effects of high H_2 partial pressure during fermentation. The initial pH was adjusted to 7.0 using 6N HCl or 6N KOH. Then, the fermenters were purged with nitrogen gas, capped, and placed in a water bath with a magnetic stirrer. The mixing speed and temperature were kept at 100 rpm and at 35 ± 1 °C, respectively. The pH during fermentation was controlled at higher than 5.0 ± 0.2 by adding 3N KOH solutions. Biogas production and its constituents were monitored at 2–10 h intervals. When batch test I was finalized, the microbial communities at each case were analyzed using polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) analysis.

In batch test III, two 7.5-L batch fermenters (3 L working volume) with pH sensors were used. From the two fermenters, 20 mL of mixed liquor samples were taken using a syringe at 5–30 h intervals to be analyzed for organic acids, and aliphatic alcohols.

2.3. Analytical methods

Measured biogas production was adjusted to the standard conditions of temperature (0 °C) and pressure (760 mmHg) (STP). Hydrogen content in the biogas was determined by a gas chromatography (GC, Gow Mac series 580) using a thermal conductivity detector and a 1.8 m × 3.2 mm stainless-steel column packed with molecular sieve 5A with N_2 as a carrier gas. The contents of CH_4, N_2, and CO_2 were measured using a GC of the same model noted previously with a 1.8 m × 3.2 mm