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Fermentative hydrogen production from tofu-processing waste and anaerobic digester sludge using microbial consortium

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ABSTRACT

The combination of tofu-processing waste and anaerobic digester sludge was studied for its fermentative H_2 production capacity in batch and continuous modes using a thermophilic mixed culture. Heat-treatment (110 °C, 30 min) in the presence of 0.5% HCl increased the soluble carbohydrate content of the tofu waste from 2.2 to 10.4 g/l. Anaerobic digester sludge was added to the tofu waste for supplementary nutrients with the optimal mixing ratio of 20% (v/v) under batch conditions. In continuous experiments, the effects of HRT(hydraulic retention time) and pH were investigated for the ranges of 2–6 h and 5.0–6.0, respectively. The maximal H_2 production rate (12.0 l H_2/l/day) and yield (2.3 mol H_2/mol glucose equivalent) were obtained at HRT 4 h and pH 5.5 while maintaining the head space gas at 50–60% (v/v) of H_2 without CH_4. This study indicates that the combination of tofu-processing waste and digester sludge can be considered to be one of the most promising forms of organic waste for continuous H_2 production.

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1. Introduction

Among the various microbial hydrogen (H_2) production technologies, photosynthetic and fermentative processes are the most commonly used. Photosynthetic H_2 production is carried out by photosynthetic microorganisms under anaerobic conditions, whereas the fermentative process involves facultative or obligate anaerobes. Fermentative H_2 production generally exhibits higher cell-growth and H_2 production rates than the photosynthetic process, and is well known as a cutting edge technology. The economic feasibility of fermentative H_2 production largely depends on the availability of organic waste/wastewater as raw material as well as the H_2 productivity of that material (Benemann, 1998). However, so far, most studies of biohydrogen production have been limited to the use of pure carbohydrates such as glucose, sucrose and starch (Lin and Chang, 1999; Lay, 2000; Nguyen et al., 2008).

Soy bean curd (tofu) processing is a traditional oriental food. The typical process consists of soy bean grinding, cooking (boiling), filtration, protein coagulation, preservation, and packaging (Chai et al., 1999). During processing (especially in the filtration step), up to 30% of the soy bean is lost, becoming waste (in amounts of about 8×10^5 tons per year in Korea and Japan). However, only a small percentage of tofu waste is utilized as nutritious feed for livestock, the remainder being incinerated and/or reclaimed as industrial waste, thereby contributing to serious pollution problems (Chai et al., 1999; Mizuno et al., 2000; Noike et al., 2005).

There have been some efforts to use tofu-processing waste in the form of organic substrates for H_2 production. Noike et al. (2002) investigated H_2 fermentation from tofu waste using *Clostridium acetobutylicum* under mesophilic conditions, but the H_2 production could not be maintained longer than 5 days, due to the significant inhibitory effect of lactate-producing bacteria. After heat-treatment of the tofu waste at 70 °C for 30 min, however, a relatively stable H_2 production of 1.2 l H_2/l/day could be achieved, using a co-culture of three different facultative anaerobic bacteria (Noike et al., 2005). However, the H_2 production yield was very low: 0.52 mol H_2/mol hexose. It should also be noted that it can be difficult to use pure cultures for industrial applications, considering the problem of contamination by various H_2 utilizers such as methane (CH_4)-producing bacteria, and sulfate-reducing bacteria.

In the present study, we investigated the feasibility of high-rate H_2 production from a combination of tofu-processing waste and anaerobic digester sludge, with an inoculum of mixed cells. In order to enhance the solubilization of organic substrates from tofu waste, various pre-treatments including heat-treatment, acid/alkali treatment, homogenization and ultrasonication were examined alone or in combination. Anaerobic digester sludge was added to the tofu waste in the form of nutrient supplementation. The fermentation was conducted under a thermophilic condition at 60 °C, since at high temperatures, H_2 is less soluble, and lactate-forming bacteria, which inhibit H_2-forming bacteria, can be repressed. The bioreactor was operated at short retention times

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of 2–6 h, and the H_2 production performance of the tofu waste was compared with those of other organic wastes.

2. Methods

2.1. Feedstocks

Tofu-processing waste was obtained from a local tofu company (Daejeon, Korea) as a by-product of the filtration of boiled (95–105 °C) bean curd. The tofu waste was stored at −30 °C before subsequent use. Anaerobic digester sludge was collected from the second anaerobic digester at the local municipal wastewater treatment facility (Daejeon, Korea). The sludge was sieved through a 1 mm mesh, and stored at 4 °C before use. The characteristics of the tofu-processing waste and digester sludge are listed in Table 1.

2.2. Pre-treatment of tofu-processing waste

The stored tofu waste was thawed, diluted with tap water at a 1:4 ratio (w/w), and homogenized using a blender (Model HGBRWTG4, Waring Commercial Co., Ltd., USA) for 1 min. Before H_2 fermentation, the tofu waste was further treated using various pre-treatment methods alone or in combination. The tofu waste was heated at 120–130 °C using a cylindrical 304 stainless steel column (inner diameter, 13 cm; length, 30 cm; working volume, 1.5 l) equipped with an electric heating cover, a temperature controller, a temperature sensor, a pressure gauge, and a relief valve. During the thermal treatment, the waste was continuously mixed at 100 rpm using a Teflon magnetic bar. As an acid/alkali treatment, HCl and NaOH were added to the thermal reactor above mentioned, at ratios of 0.1% (w/v) and 0.5% (w/v) of tofu waste. The tofu waste was also treated using an ultrasonic processor (VCX 750, Sonics and Materials, Inc., USA) or a homogenizer (HMG2400, Misung Scientific Co., Korea).

2.3. Inoculum and H_2 fermentation

An inoculum for the H_2 fermentation was obtained from a 5 l CSTR (continuous-stirred tank reactor) operated for the production of H_2 at 60 °C, pH 5.5, and an HRT (hydraulic retention time) of 10.3 h for 3 months. The biomass concentration and volumetric H_2 production rate were 1.11 g VSS (volatile suspended solid)/l and 4.5 l H_2/l/day, respectively. It was transferred anaerobically by a sterile hypodermic disposable syringe and inoculated at the ratio of 10% (v/v).

Batch and continuous H_2 fermentations were conducted in a 1.5 l CSTR (working volume, 590 ml), made of Pyrex glass and equipped with a water jacket. The temperature was maintained at 60 °C and the pH at 5.5 by the automatic addition of 2.0 N NaOH. For the continuous experiments, initially the reactor was operated at HRTs of 12–6 h with a synthetic medium containing 1% (w/v) glucose. After the reactor had attained a steady state with respect to the glucose removal efficiency (95%) and the H_2 production rate (8.0 l H_2/l/day), the mixture of tofu waste and digester sludge began to be added. The mixture was stored at 4 °C and purged continuously with argon gas (99.999%). The mixing ratio of the waste mixture to the synthetic medium was gradually increased from 20% to 100% (v/v) while maintaining the HRT at 6 h.

2.4. Analytical methods

The volume of biogas produced was measured by the water displacement method. The H_2 and CH_4 contents in the biogas were analyzed using a gas chromatograph (Model 14-B, Shimadzu Co., Japan) equipped with a thermal conductivity detector. A stainless steel column packed with a Molecular Sieve 5A (80/100 mesh; Alltech, Deerfield, USA) was used for H_2, and one packed with a Hayesep Q (80/100 mesh; Alltech) was used for CH_4. VS (volatile solid), VSS and TS (total solid) were measured according to the analytical procedures listed in the standard methods (APHA, 1995).

Liquid samples were centrifuged at 10,000 rpm for 2 min, filtered through a 0.45 μm membrane and analyzed for various components. The protein concentration was determined by the Lowry method using a protein assay kit (Bio-Rad, USA). Bovine serum albumin was used as a standard. The carbohydrate content was measured by the dinitrosalicylic acid method using glucose as a standard (Miller, 1959). The COD (chemical oxygen demand), TN (total nitrogen), TP (total phosphorus), NH_3^+, SO_4^{2-}, and PO_4^{2-} concentrations were assayed by colorimetric methods according to the manufacturer's instructions (DR-2000; Hach Co., USA). Various organic acids and ethanol were analyzed using a high-performance liquid chromatograph (Model VP, Shimadzu Co., Japan) equipped with a sulfonated divinyl benzene-styrene copolymer column (300 mm × 7.8 mm, Aminex HPX-87H, BioRad, USA). A photometric detector (216 nm) was used to quantify the organic acids, a refractive index detector being used to quantify the ethanol. The organic acids analyzed included succinate, lactate, formate, acetate, propionate, *n*-butyrate, and iso-valerate. An aqueous solution of 10 mM H_2SO_4 was used as an eluting solution at 0.6 ml/min, and the column was maintained at 30 °C.

Table 1
Characteristics of digester sludge and tofu-processing waste used in this study.

	Raw tofu-processing waste	Diluted tofu waste[a]	Pre-treated tofu waste (0.5% HCl, 110 °C, 30 min)	Digester sludge waste
TS (%, w/w)	22.8 ± 0.2			
VS (%, w/w)	21.2 ± 0.7			
Water (%, w/w)	77.2 ± 0.2			
Ash (%, w/w)	1.6 ± 0.6			
TS (g/l)			23.5 ± 3.5	18.7 ± 3.7
VS (g/l)			22.4 ± 3.2	10.8 ± 4.3
pH		4.9	1.0	6.8
Soluble component[b]				
COD (g/l)		5.5 ± 0.8	37.3 ± 1.8	0.5 ± 0.1
Carbohydrate (g/l)		2.4 ± 0.7	10.4 ± 2.5	0.28 ± 0.27
Protein (g/l)			1.19 ± 0.41	0.01 ± 0.01
TN (mg/l)			640 ± 46	415 ± 41
TP (mg/l)			334 ± 41	56 ± 17
NH_3^+ (mg/l)			859 ± 119	414 ± 77
SO_4^{2-} (mg/l)			1081 ± 83	46 ± 11
PO_4^{2-} (mg/l)			577 ± 60	57 ± 2

[a] Diluted with tap water in 1:4 ratio (w/w) and homogenized using blender for 1 min.
[b] Centrifuged at 10,000 rpm for 2 min and filtered through 0.45 μm membrane.

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