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A two-stage bioprocess for hydrogen and methane production from rice straw bioethanol residues

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ABSTRACT

This study evaluates a two-stage bioprocess for recovering hydrogen and methane while treating organic residues of fermentative bioethanol from rice straw. The obtained results indicate that controlling a proper volumetric loading rate, substrate-to-biomass ratio, or *F*/*M* ratio is important to maximizing biohydrogen production from rice straw bioethanol residues. *Clostridium tyrobutyricum*, the identified major hydrogen-producing bacteria enriched in the hydrogen bioreactor, is likely utilizing lactate and acetate for biohydrogen production. The occurrence of acetogenesis during biohydrogen fermentation may reduce the *B*/*A* ratio and lead to a lower hydrogen production. Organic residues remained in the effluent of hydrogen bioreactor can be effectively converted to methane with a rate of 2.8 mmol CH_4/gVSS/h at VLR of 4.6 kg COD/m^3/d. Finally, approximately 75% of COD in rice straw bioethanol residues can be removed and among that 1.3% and 66.1% of COD can be recovered in the forms of hydrogen and methane, respectively.

1. Introduction

Considering the energy security and the global environment, there is an urgent need in developing a clean and renewable energy source. Bioenergy is considered an important form of renewable energy because of its sustainable feature, by growing energy crops from sunlight, carbon dioxide, and water. Currently, production of bio-ethanol and bio-diesel from different energy crops is technologically feasible, although its impacts on global economic and food security issues are debatable. Hydrogen is a clean energy carrier, generating only water when it burns. However, for hydrogen production to meet sustainability requirements, it must be produced from renewable resources. One way to produce hydrogen renewably is through fermentative biohydrogen production from potential renewable materials such as carbohydrate-containing biomass and organic wastes (Li and Fang, 2007).

Hydrogen production from anaerobic waste treatment potentially benefits both organic wastes reduction and renewable energy production at the same time, but it also creates challenges because the waste materials usually are composed of a variety of substrates that can be used by different species of microorganisms (Whang et al., 2006; Li and Fang, 2007; Li et al., 2010). Fermentative biohydrogen has been studied for the organic fraction of municipal solid wastes (Okamoto et al., 2000), spoiled wheat grains (Kalia et al., 1993), cellulose (Lay, 2001), municipal wastewater and sludge (Kim et al., 2004; Van Ginkel et al., 2005; Cavinato et al., 2011), and food wastes (Kim et al., 2004; Van Ginkel et al., 2005; Lee et al., 2008), but results suggest that hydrogen production is more efficient from carbohydrates than other materials (Li and Fang, 2007), indicating that high-carbohydrate wastewaters will bethe most suitable ones for industrial production of hydrogen (Van Ginkel et al., 2005). Besides wastewater compositions and characteristics, another difficulty to the practical application of fermentative biohydrogen production is that conversion yields by known metabolic pathways appear to be limited to a maximum of 4 mol of hydrogen per mol of glucose, representing a maximum conversion efficiency of 33% (Gottschalk, 1986; Ljungdahl et al., 1989).This also indicates that the majority of the COD of waste streams remains untreated and other processes, such as methanogenesis, would be necessary.

In this study, a two-stage bioprocess was investigated for treating organic residues generated during ethanol fermentation production from rice straw. Current technologies for ethanol fermentation from energy cropscan attain a conversion efficiency of 75–80%, remaining about 20–25% of organic wastes as residues. The main objective of this study was to recover bioenergy in the form of hydrogen and methane while treating ethanol fermentation residues through the

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two-stage bioprocess. Two bioreactors were continuously operated at different organic loadings to evaluate their performance on reduction of organic wastes and production of hydrogen and methane, respectively. In this article, batch experiments on fermentative biohydrogen production were conducted to evaluate effects of substrate concentration on hydrogen production and metabolism of fermentative biohydrogen from rice straw ethanol fermentation residues. Furthermore, molecular methods were applied to investigate microbial ecology of hydrogen-producing bacteria in the hydrogen bioreactor. Finally, overall bioenergy recovery from the two-stage bioprocess treating rice straw bioethanol residues was evaluated.

2. Methods

2.1. Operation of the two-stage bioprocess

Two bioreactors were operated in this study as a two-stage bioprocess. The first bioreactor, hydrogen fermentation bioreactor, was fed with organic residues obtained from a bioethanol fermentation process using rice straw as substrate. Table 1 summarizes the wastewater characteristics of ethanol fermentation residues investigated in this study. As shown in Table 1, the residues contained a total COD of 23,200 mg/L, which consisted of total volatile solid (TVS) (620 mg/L), total carbohydrate (5060 mg/L), organic acids (3680 mg/L of lactate and 3120 mg/L of acetate) and alcohols (1760 mg/L of ethanol). The following chemicals as growth nutrients (in mg/L) were added to the residues before fed into the hydrogen bioreactor (Lin et al., 2007; Whang et al., 2011): $CaCl_2{\cdot}6H_2O$, 32.32; $MgCl_2{\cdot}6H_2O$, 232.26; KCl, 167.81; $MnCl_2{\cdot}4H_2O$, 63.87; $CoCl_2{\cdot}6H_2O$, 3.87; H_3BO_3, 0.74; $CuCl{\cdot}2H_2O$, 0.35; $Na_2MoO_4{\cdot}2H_2O$, 0.33; $ZnCl_2$, 0.27; $FeCl_2{\cdot}4H_2O$, 10.62; sodium thioglycolate, 217.35; KH_2PO_4, 119. Sodium ammonia (400 mg-N/L) and peptone (360 mg/L) were added as nitrogen source, and trace amount of resazurin (0.175 mg/L) was also added as the redox-status indicator. The second bioreactor, methane fermentation bioreactor, was fed with the effluent collected from the hydrogen fermentation bioreactor. The total volume of hydrogen and methane fermentation bioreactor was 5 and 12 L with a working volume of 2 and 8 L, respectively. Hydrogen fermentation bioreactor was equipped with a magnetic stirrer for mixing, as a mechanic propeller was installed in methane fermentation bioreactor. A completely-mixed condition was achieved for the hydrogen fermentation bioreactor while a gently-mixed condition was applied for the methane fermentation bioreactor at an agitation speed of 15 rpm, in order to retain granular sludge in the bioreactor without washout. Both bioreactors were kept in incubators in order to maintain an operational temperature at 35 °C. The influent feeds of both bioreactors were stored at 4 °C in a refrigerator and continuously fed into the bioreactors using a peristaltic pump. Oxidation–reduction potential (ORP) and pH were monitored for both bioreactors during operation. The pH values for hydrogen and methane fermentation bioreactors were controlled at 6 and 7, respectively, using a pH controller with addition of 45% H_3PO_4 and 50% NaOH throughout the experiments. The amount of biogases produced from both bioreactors was measured with a wet-gas flow meter (Shinagawa W-NK-0.5B, Tokyo, Japan).The seeding microorganisms for the hydrogen fermentation bioreactor and the methane fermentation bioreactor were obtained from a lab-scale bioenergy recovery process treating bioethanol residues (Juang et al., 2011).

The operational conditions of the hydrogen and methane fermentation bioreactors are summarized in Table 2, respectively. Based on predetermined operational conditions for hydraulic retention time (HRT) and feed concentration, the volumetric loading rates (VLR) for the hydrogen fermentation bioreactor increased gradually from 19.7 to 46 kg COD/m^3/day, while for the methane fermentation bioreactor, the VLR varied between 2.0 and 4.6 kg COD/m^3/day.

Table 1
Summary of wastewater characteristics of bioethanol fermentation residues investigated in this study.

Parameter	Value	COD percentage
Total COD (mg/L)	23,200	100%
Total suspended solid (mg/L)	760	
Total volatile solid(mg/L)	620	
Total carbohydrate (mg/L)	5060	23%
Soluble carbohydrate (mg/L)	4500	
Xylose (mg/L)	1310	6%
Lactate (mg/L)	3680	17%
Acetate (mg/L)	3120	14%
Ethanol (mg/L)	1760	16%
NH_4^+-N (mg-N/L)	28	

2.2. Batched fermentative biohydrogen tests

The batched fermentative biohydrogen tests conducted in this study was a modified version of biochemical methane potential test originally developed by Owen et al. (1979). The test was carried out in a series of 1 L glass bottles equipped with pH/ORP monitoring and gas collection systems. To each bottle with a liquid working volume of 800 mL, predetermined concentrations of bioethanol fermentation residues and biomass sludge taken from the fermentation bioreactor were added as fermentation substrate and seeding sludge, respectively. To prepare the seeding sludge for batch experiments, 1 L of examined sludge sample was centrifuged at 6000 rpm for 5 min. The supernatants were discarded and the solids were resuspended in a 1 L glass bottle containing 800 mL of the substrate. The bottles were flushed with oxygen free nitrogen gas before capped tightly with rubber septum stoppers. The bottles were incubated at 37 °Cin a water bath tank equipped with magnetic stirred machines at a rotational rate of 120 rpm. The pH of the mixed liquor was automatically controlled by feeding with either NaOH (10%) or H_3PO_4 (5%).The total volume of gases produced during fermentation was determined using a gas collection system with water displacement and gas composition was analyzed using a gas chromatography (GC). Samples were frequently taken throughout the batch experiments for the determination of alcohols and organic acids using a high-performance liquid chromatography (HPLC).

2.3. Analytical methods

Biogas collected from bioreactors and batches was analyzed using a gas chromatograph (China GC 8900, Taipei, Taiwan) equipped with a thermal conductivity detector (TCD). A 2 m

Table 2
Operational parameters of the hydrogen and methane fermentation bioreactor.

Operational parameter	Reactor	(A) H_2 fermentation bioreactor			
	Unit	Run1–1	Run1–2	Run2	Run3
HRT	h	28.0		18.0	12.0
Substrate Con.	g COD/L	23		23	23
VLR	kg COD/m^3/day	19.7		30.7	46
F/*M*	kg COD/kg VSS/day	20.6	15.3	19.7	29.5
		(B) CH_4 fermentation bioreactor			
		Run1	Run2	Run3	Run4
HRT	Day	10.9	4.8	5.3	13.3
Substrate Con.	g COD/L	22	22	22	22
VLR	kg COD/m^3/day	2.0	4.6	4.2	1.7
F/*M*	kg COD/kg VSS/day	2.99	6.52	3.62	1.40