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Biological hydrogen and methane production from bagasse bioethanol fermentation residues using a two-stage bioprocess

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HIGHLIGHTS

- A two-stage bioprocess recovers H_2 (0.3%) and CH_4 (78.9%) from bagasse bioethanol residues.
- H_2 formation from the lactate/acetate utilization accompanied with butyrate production.
- Acetogenesis may potentially reduce the B/A ratio and lead to a lower H_2 production.
- The B/A ratio has a positive relationship with H_2 yield.

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ABSTRACT

This study investigated the recovery of H_2 and CH_4 from bagasse bioethanol fermentation residues (bagasse BEFR) using a two-stage bioprocess. In the hydrogen fermentation bioreactor (HFB), carbohydrate removal efficiency was maintained at 82–93% and the highest hydrogen yield was 8.24 mL/g COD at volumetric loading rate (VLR) of 80 kg COD/m^3/day. The results indicated a positive correlation between hydrogen yield and butyrate-to-acetate ratio, which might be due to the mechanisms of lactate/acetate utilization for hydrogen production and acetogenesis occurring in the HFB. Remaining volatile fatty acids and alcohols in the HFB effluent were further utilized for methane production in methane fermentation bioreactor (MFB), in which the highest methane yield of 345.2 mL/g COD was attained at VLR of 2.5 kg COD/m^3/day. Overall, the two-stage bioprocess achieved a maximum COD removal of 81% from bagasse BEFR, and converted 0.3% and 72.8% of COD in the forms of H_2 and CH_4, respectively.

1. Introduction

To alleviate the current trend in air pollution and global warming due to the combustion of fossil fuels, the development of alternative clean energy has become a critical issue in our society. Bioenergy is considered to be a promising strategy of renewable-energy form because of its sustainability through growth of energy crops from sunlight, carbon dioxide, and water. Biological hydrogen is recognized to be an ideal bioenergy, because it has a high specific energy content (122 kJ/g), generates only water when burned, and may be produced biologically via fermentation of potential renewable materials such as carbohydrates-containing biomasses and various organic wastes.

Because the waste reduction and renewable energy production can be achieved at the same time, fermenting biohydrogen from organic wastes has been investigated with many source materials, e.g. municipal waste fractions (De Gioannis et al., 2013), cellulose (Ratti et al., 2013), domestic wastewater (Van Ginkel et al., 2005), lipid-extracted microalgae (Yang et al., 2011), dairy wastewater, and food waste (Jo et al., 2008). The varied compositions and material characteristics in organic wastes and wastewaters pose challenges in investigation because a variety of substrates may be utilized by different species of microorganisms (Whang et al., 2006; Rafrafi et al., 2013). Another practical issue of biohydrogen fermentation is the limitation in the yield of 4 mol of hydrogen per mole of glucose (Thauer et al., 1977), which responds only 33% of the conversion as compared to the theoretical maximum yield of 12 mol of hydrogen per mole of glucose based on the complete conversion of glucose to H_2 and CO_2. The low yield is likely a consequence of the tendency of microorganisms to produce biomasses and of the competing reactions that consume

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hydrogen or divide it into other end-products (Hallenbeck and Benemann, 2002). In practice, hydrogen yield is related to the metabolic pathways and the end-products distribution; for example, the butyrate-to-acetate ratio (B/A ratio) and the lactate production are found to be related to the hydrogen yield. Unfortunately, detailed mechanisms were not clear at this moment. The other metabolites produced during hydrogen fermentation also indicates that a certain amount of COD in wastes or wastewaters remains not digested, and thus a post methanogenic process may be used to recover the remaining potential energy content (Hallenbeck, 2009).

The two-stage anaerobic bioprocess has been demonstrated for many advantages, including selection and enrichment of different functional microorganisms (Zhang and Noike, 1991), reduction of the effect of pH change (Anderson et al., 1994), higher overall degradation efficiency than single methane fermenter (Kubler and Schertler, 1994), and improved net energy production than single hydrogen fermenter (Ruggeri et al., 2010). In the present study, a two-stage bioprocess was employed to recover the bioenergy from bagasse bioethanol residues, since approximate 20–25% of COD may remain in residues after bioethanol fermentation (BEFR). The main objective of this study was to recover bioenergy in the forms of H_2 and CH_4 while treating bagasse BEFR through the two-stage bioprocess. Two bioreactors were operated at different HRT and substrate concentrations to evaluate the performance in H_2 and CH_4 production. Batch experiments were also conducted to further evaluate the effects of substrate concentration and biomass concentration on biohydrogen fermentation. The variation of metabolites under different operational conditions was found correlated to hydrogen production performance, and the possible mechanisms for the positive relationship between B/A ratio and hydrogen yield were discussed. Finally, the overall bioenergy recovery and COD removal efficiency from bagasse BEFR using the two-stage bioprocess were also evaluated.

2. Methods

2.1. Operation of the two-stage bioprocess

Two bioreactors, i.e. hydrogen fermentation bioreactor (HFB) and methane fermentation bioreactor (MFB), were operated in this study as a two-stage bioprocess. The total volume of the glass-made HFB and the MFB were 5 and 12 L with a working volume of 2 and 8 L, respectively. Both bioreactors were equipped with a magnetic stirrer for mixing. A complete-mix condition was achieved for the HFB at an agitation speed of 160 rpm, while a gentle mixing was applied for the MFB at an agitation speed of 30 rpm. Both bioreactors were kept in an incubator in order to maintain an operational temperature at 37 °C. The first bioreactor, HFB, was fed with organic residues obtained from a bioethanol fermentation process (i.e. bioethanol fermentation residues, BEFR) using bagasse as the substrate. Table 1 summarizes the wastewater characteristics of bagasse BEFR investigated in this study. As shown in Table 1, the residues contained a total COD of 31,000 mg/L, which consisted of carbohydrate (8700 mg/L), organic acids (3700 mg/L of lactate and 4500 mg/L of acetate) and alcohols (1800 mg/L of ethanol). The following chemicals as growth nutrients (in mg/L) were added to the BEFR before the latter was fed into the HFB (Whang et al., 2011): $CaCl_2{\cdot}6H_2O$, 32.3; $MgCl_2{\cdot}6H_2O$, 232; KCl, 168; $MnCl_2{\cdot}4H_2O$, 63.9; $CoCl_2{\cdot}6H_2O$, 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; $FeCl_2{\cdot}4H_2O$, 10.6; sodium thioglycolate, 217; KH_2PO_4, 119. Sodium ammonia (400 mg-N/L) and peptone (360 mg/L) were added as nitrogen sources, and a trace amount of resazurin (0.175 mg/L) was also added as the redox-status indicator. The second bioreactor, MFB, was fed with the effluent collected from the HFB. Both bioreactors were kept in incubators in order to maintain an operational temperature at 35 °C. The influent feeds to both bioreactors, including bagasse BEFR and effluent of the HFB, were stored at 4 °C in a refrigerator and fed from there into the bioreactors continuously using a peristaltic pump. The oxidation–reduction potential (ORP) and pH were monitored for both bioreactors during operation. The pH values for hydrogen and MFB were controlled at 6 and 7, respectively, throughout the experiments via a pH controller using 45% H_3PO_4 and 50% NaOH. 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 both the HFB and the MFB were obtained from a lab-scale bioenergy recovery process for treating cellulosic BEFR (Cheng et al., 2012).

The operational conditions of the HFB and MFB are summarized in Table 2A and B, respectively. Based on the predetermined operational conditions for hydraulic retention time (HRT) and feed concentration, the volumetric loading rates (VLRs) for the HFB increased gradually from 19 to 180 kg COD/m^3/day, while for the MFB, the VLR varied between 2.4 and 3.7 kg COD/m^3/day.

2.2. Batched fermentative biohydrogen tests

The batched fermentative biohydrogen tests conducted in this study was a modified version of the 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-controlling and gas-collection devices with the working volume of 800 mL. Predetermined concentrations of bagasse BEFR were firstly mixed with nutrients and added to the bottles as the

Table 1
Summary of wastewater characteristics of bagasse BEFR investigated in this study.

Parameter	Value (g/L)	COD percentage (%)
Total COD	30.6 ± 1.2	100
Total carbohydrate	8.7 ± 0.8	28
Xylose	4.7 ± 0.1	15
Lactate	3.7 ± 0.6	12
Acetate	4.5 ± 0.3	15
Ethanol	1.7 ± 0.2	5

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

Operational parameter	Reactor	(A) H_2 fermentation bioreactor				
	Unit	Run 1	Run 2	Run 3	Run 4	Run 5
HRT	h	23	18	13	11	9
Substrate con.	g COD/L	18	18	18	18	18
VLR	kg COD/m^3/day	19	24	33	39	48
Duration	day	6	35	14	19	25

Operational parameter	Reactor	(A) H_2 fermentation bioreactor			
	Unit	Run 6	Run 7	Run 8	Run 9
HRT	h	9	9	6	4
Substrate con.	g COD/L	24	30	30	30
VLR	kg COD/m^3/day	64	80	120	180
Duration	day	31	53	19	7

Operational parameter	Reactor	(B) CH_4 fermentation bioreactor			
	Unit	Run 1	Run 2	Run 3	Run 4
HRT	h	7.9	9.3	6	8.7
Substrate con.	g COD/L	20	22	22	22
VLR	kg COD/m^3/day	2.5	2.4	3.7	2.5
Duration	day	17	12	13	10