Bioresource Technology 114 (2012) 327–333

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Coproduction of hydrogen and methane via anaerobic fermentation of cornstalk waste in continuous stirred tank reactor integrated with up-flow anaerobic sludge bed

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ARTICLE INFO

Article history:
Received 12 January 2012
Received in revised form 8 March 2012
Accepted 11 March 2012
Available online 19 March 2012

Keywords:
Cornstalk
Hydrogen and methane fermentation
Continuous stirred tank reactor
Up-flow anaerobic sludge bed
Clostridium thermocellum

ABSTRACT

A 10 L continuous stirred tank reactor (CSTR) system was developed for a two-stage hydrogen fermentation process with an integrated alkaline treatment. The maximum hydrogen production rate reached 218.5 mL/L h at a cornstalk concentration of 30 g/L, and the total hydrogen yield and volumetric hydrogen production rate reached 58.0 mL/g-cornstalk and 0.55–0.57 L/L d, respectively. A 10 L up-flow anaerobic sludge bed (UASB) was used for continuous methane fermentation of the effluents obtained from the two-stage hydrogen fermentation. At the optimal organic loading rate of 15.0 g-COD/L d, the COD removal efficiency and volumetric biogas production rate reached 83.3% and 4.6 L/L d, respectively. Total methane yield reached 200.9 mL/g-cornstalk in anaerobic fermentation with the effluents and alkaline hydrolysate. As a result, the total energy recovery by coproduction of hydrogen and methane with anaerobic fermentation of cornstalk reached 67.1%.

1. Introduction

With rising energy demands and increasing environmental pollution, interest in renewable hydrogen and methane production from organic wastes has grown (Cheng and Liu, 2010; Demirel et al., 2010; Park et al., 2010). Recently, bio-hydrogen production from various organic wastes has been the focus of research due to its advantages such as a high gravimetric energy density, a high efficiency of conversion to usable power, and the fact that it is environmentally-friendly (Das and Veziroglu, 2008). Lignocellulosic waste is an ideal resource for renewable hydrogen production due to its high annual production; however, it is quite difficult to produce hydrogen from lignocellulosic waste due to its recalcitrant structure (Levin et al., 2009; Lu et al., 2009).

Acid, alkaline, and steam explosion pretreatments are efficient ways to breakdown the structure of lignocellulosic biomass and enhance hydrogen production (Datar et al., 2007; Fan et al., 2008). The addition of external cellulase or cellulose-degrading microorganisms such as *Clostridium thermocellum* can augment the hydrolysis of lignocellulose (Li and Chen, 2007; Lo et al., 2008; Lu et al., 2009; Wang et al., 2010). Hydrogen can be produced by direct microbial conversion with *C. thermocellum* using natural lignocellulosic biomass as the substrate, but the overall conversion efficiency is significantly decreased due to the physical barrier created by lignin (Cheng and Liu, 2011; Liu et al., 2008; Magnusson et al., 2008). An alkaline pretreatment, which has advantages over steam explosion and acid treatments because steam explosion requires high temperatures and pressure and acid treatments require the use of acid-resistant vessels, has been regarded as an efficient method for the complete delignification and destruction of the lignocellulose microstructure (Rani et al., 1998). However, in the alkaline pretreatment process, a significant portion of the carbohydrate fraction is degraded to saccharinic acids, which are often toxic to microorganisms and may diminish substrate utilization ratios (Avgerinos and Wang, 1983).

In a two-stage system for the coproduction of hydrogen and methane, the production of hydrogen and methane from diluted molasses reached 27 L-H_2/L-molasses/d and 342 L-CH_4/L-molasses/d respectively, corresponding to energy recovery value of 0.3 and 12.9 MJ/L-molasses for the first hydrogen and second methane fermentation stage, respectively (Park et al., 2010). It is clear that the energy recovery ratio of single anaerobic hydrogen fermentation was quite low, and coproduction of hydrogen and methane from two-stage dark fermentation produced a remarkable increase in energy recovery (Park et al., 2010). The objective of the current study was to develop a two-stage thermophilic fermentation system of cornstalk by *C. thermocellum* integrated with alkaline treatment for improving hydrogen production in a continuous stirred tank reactor. The effluents of the two-stage hydrogen fermentation were used for continuous methane production in an up-flow anaerobic sludge bed (UASB), and the total energy recovery

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http://dx.doi.org/10.1016/j.biortech.2012.03.038

efficiency of the coproduction of hydrogen and methane from cornstalk was evaluated.

2. Methods

2.1. Microorganisms and media

C. thermocellum 7072 was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ, i.e. German Collection of Microorganisms and Cell Cultures). 125 mL anaerobic bottles with a working volume of 50 mL were used for inoculum preparation. All bottles were air sealed with butyl rubber stoppers and screw caps. Each bottle was gassed and degassed with 100% nitrogen before sterilization. All bottles were autoclaved at 121 °C for 20 min. *C. thermocellum* 7072 was cultured at 55 °C in CM4 medium with slight modifications, the composition of the CM4 medium was as follows (per liter): KH_2PO_4 1.5 g, $K_2HPO_4{\cdot}3H_2O$ 3.8 g, $(NH_4)_2SO_4$ 1.3 g, $MgCl_2{\cdot}6H_2O$ 1.6 g, $CaCl_2$ 0.013 g, yeast extract 5.0 g, $FeSO_4{\cdot}7H_2O$ 1.25 mg, resazurin 1.0 mg, and L-cysteine 0.5 g (Avgerinos and Wang, 1983). Microcrystalline cellulose (Sinopharm Chemical Reagent Co. Ltd, China, 10 g/L) was used as the carbon source for inoculum cultures, and the final pH was adjusted to approximately 7.2 with sodium bicarbonate. Anaerobic bottles for inoculum preparation were inoculated with 10% (v/v) of exponentially growing *C. thermocellum* cultures (with an OD_{600} value of approximately 1.0) that were cultured at 55 °C.

2.2. Batch hydrogen fermentation in a continuous stirred tank reactor

Fresh raw cornstalks were collected, chopped and then dried in the sun. Sun-dried raw cornstalk (RC) were then dried at 60 °C for at least 24 h to a constant weight in an oven and milled to pass through a 15 mm sieve using a plant miller. The main components of cornstalk were as follows (on a dry weight basis): cellulose 30.1%, hemicellulose 28.7%, lignin 11.2%, and soluble sugar 13.0%.

A two-stage hydrogen fermentation process integrated with alkaline treatment consists of three steps: hydrogen fermentation I, alkaline treatment, and hydrogen fermentation II (Supplementary Materials-Fig. 1.). In hydrogen fermentation I, the raw cornstalk (RC) was directly fermented by *C. thermocellum* 7072 for hydrogen production. After hydrogen fermentation I, the solid cornstalk residue was treated with a solution of NaOH. The alkaline treatment was carried out according to the following procedure: 100 g solid residue, 10 g NaOH, and 1200 mL distilled water were mixed in a 4000 mL bottle, and the bottle was then treated at 120 °C for 20 min. The solid–liquid mixtures were filtered, and the solid residues were thoroughly washed with distilled water until they reached a neutral pH, and were then dried at 60 °C to a constant weight. The dried solid residues (ACC) were used as the substrate for hydrogen fermentation II.

Hydrogen fermentation I and II were carried out in a 10 L continuous stirred-tank reactor (CSTR) designed by our group (Fig. 1A). A micro-motor, controlled by a transducer at selected speeds ranging from 50 to 750 r/min, was installed below the bottom of the reactor. The pH value in the reactor was controlled by a pH sensor (Mettler Toledo 405-DPAS-SC-K8S) and a pH controller (Eutech Alpha-pH 800, USA). The temperature was controlled by a Pt 100 sensor and a PID temperature controller (Beijing Huibang XMT614, China). 6500 mL non-sterile CM4 medium with RC (10, 20, 30, 40, and 50 g/L) or ACC substrates (3.3, 6.9, 10.6, 14.8, and 19.3 g/L) were added into the reactor, followed by nitrogen sparging for 30 min. The reactor was inoculated with 10% (v/v) exponentially growing *C. thermocellum* 7072 cultures, and was then flushed with nitrogen gas for 15 min. The motor speed and temperature were adjusted to selected stirring speeds (60, 80, 100, and 150 r/min) and 55 ± 1 °C, respectively. Samples were collected at selected time points.

2.3. Continuous methane fermentation in an up-flow anaerobic sludge bed

The 10 L up-flow anaerobic sludge bed (UASB) used in this study for methane fermentation had a 8.2 L working volume consisting of a 6.4 L reaction zone and a 1.8 L setting zone (Fig. 1B). Seed sludge for the start-up of the UASB was taken from the Lunan Wastewater Treatment Plant in Beijing, and contained 35,000 mg/L total suspended solids (TSS). The volatile suspended solids/total suspended solids (VSS/TSS) ratio was 0.6, and the VSS concentration in the reaction zone of the UASB was approximately 11.5 g-VSS/L at the start-up. To accelerate the sludge granulation process in the UASB, 132 g activated carbon with an average particle size of 0.5–1 mm was added. The reactor temperature was controlled at 35 ± 1 °C by a hot water bath. The hydraulic retention time (HRT) and organic loading rate (OLR) were gradually increased during the start-up process in the UASB.

The hydrogen fermentation effluents were diluted to a final COD concentration of 6.3 g-COD/L with tap water and then used as the influents for the UASB start-up and high OLR operation. The compositions of the hydrogen fermentation effluents after dilution are shown in Supplementary Materials-Table 1. Synthetic VFAs wastewater was used as the influent for the UASB to keep sludge activity after start-up and high OLR operation were finished. The composition of the synthetic VFAs wastewater was as follows (per liter): acetic acid 1.283 g, butyric acid 0.687 g, propionic acid 0.251 g, ethanol 0.612 g, *i*-butyric acid 0.035 g, *i*-valeric acid 0.095 g, yeast extract 0.2 g, glucose 0.7 g, NH_4Cl 0.4 g, KH_2PO_4 0.075 g, $MgCl_2{\cdot}6H_2O$ 0.0346 g, $CaCl_2{\cdot}2H_2O$ 0.0283 g, and trace metal solution 2 mL. The composition of the trace metal solution was as follows (per liter): $FeCl_3{\cdot}4H_2O$ 2000 mg, H_3BO_3 50 mg, $ZnCl_2$ 50 mg, $MnCl_2{\cdot}4H_2O$ 500 mg, $NH_4MoO_7{\cdot}24H_2O$ 50 mg, $AlCl_3$ 30 mg, $CoCl_2{\cdot}6H_2O$ 150 mg, $NiCl_2{\cdot}6H_2O$ 100 mg, and EDTA 500 mg.

2.4. Analysis

The initial sludge and the granular sludge samples after the start-up operation of the UASB were collected and their microbial compositions observed by scanning electron microscopy (SEM). The sludge samples were prepared according to the method reported previously (Tay et al., 2001), and all samples were then examined with a JEOL JSM-6700F scanning electron microscope (Tokyo, Japan).

The concentrations of volatile fatty acids (VFAs) and ethanol were determined using a gas chromatograph (Agilent GC 7890, USA) equipped with a flame ionization detector and a 30 m × 0.32 mm × 0.25 μm fused-silica capillary column (PB-INNOWax). The temperatures of the injector and detector were 200 °C and 250 °C, respectively. The oven temperature increased from 60 °C by a ramp-up of 20 °C/min for 6 min, and was held at a final temperature of 180 °C for 4 min. Nitrogen was used as the carrier gas with a flow rate of 2.6 mL/min.

The biogas composition was analyzed using gas chromatography (Agilent GC 7890, USA). The gas chromatograph was equipped with two columns separated by a switching valve as designed by the manufacturer. The first column was a Plot Q polymer column, to separate CO_2 and the compounds with a higher molecular weight, and the second was a molecular sieve column to separate the lower molecular weight gases (H_2, O_2, N_2, and CH_4). Helium was used as the carrier gas at a flow rate of 23 mL/min. The oven, injector, and thermal conductivity detector temperatures were 50 °C, 150 °C, and 250 °C, respectively. Calibration curves of the