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Review

Characteristics of hydrogen and methane production from cornstalks by an augmented two- or three-stage anaerobic fermentation process

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ABSTRACT

This paper presents the co-production of hydrogen and methane from cornstalks by a two- or three-stage anaerobic fermentation process augmented with effective artificial microbial community. Two-stage fermentation by using the anaerobic sludge and DGGE analysis showed that effective and stable strains should be introduced into the system. We introduced *Enterobacter aerogens* or *Clostridium paraputrificum* into the hydrogen stage, and *C. paraputrificum* was proven to be more effective. In the three-stage process consisting of the improved hydrolysis, hydrogen and methane production stages, the highest soluble sugars (0.482 kg/kg cornstalks) were obtained after the introduction of *Clostridium thermocellum* in the hydrolysis stage, under the thermophilic (55 °C) and acidic (pH 5.0) conditions. Hydrolysates from 1 kg of cornstalks could produce 2.61 mol (63.7 l) hydrogen by augmentation with *C. paraputrificum* and 4.69 mol (114.6 l) methane by anaerobic granular sludge, corresponding to 54.1% energy recovery.

1. Introduction

Biomass-based biorefinery will be a platform for producing different biofuels from renewable biological feedstocks (Kamm and Kamm, 2004). As gas biofuels, hydrogen and methane are two important energy carriers, which can be produced by anaerobic fermentation from various organic wastes (Borkris, 1973; Chynoweth, 2005). Because hydrogen fermentation from organic substrates is incomplete, the integration of hydrogen with methane fermentation will benefit the total energy recovery from the renewable biomasses (Han and Shin, 2004). Moreover, compared with ethanol or other liquid biofuels, hydrogen and methane are easily separated from liquid phase, which can contribute to the reduction of the process costs.

Most studies reported so far on the two-stage fermentation processes for hydrogen and methane production, have been focused on organic solid wastes rich in carbohydrates, such as food waste (Han and Shin, 2004), household solid waste (Liu et al., 2006), a mixture of pulverized garbage and shredded paper wastes (Ueno et al., 2007a), artificial organic solid waste (Ueno et al., 2007b) and wastewater sludge (Ting and Lee, 2007). A Japanese research group has also established a hydrogen/methane fermentation semi-pilot plant to decompose kitchen waste (Sawayama, 2004). However, until now, few researches have been carried out on the co-production of hydrogen and methane from lignocellulosic biomasses. The highest yield of hydrogen and methane per kg of sorghum biomass was reportedly 10.4 and 29.0 l by several microorganisms (Antonopoulou et al., 2008).

For the lignocellulosic biomasses, the major difficulties are that many structural and compositional factors hinder the cellular enzymatic digestibility. Among many biomass pretreatment methods, such as acid or alkali, refining, fungi, and steam explosion, the steam explosion is an attractive choice. The steam explosion pretreatment could improve methane productivity of anaerobic fermentation (Take et al., 2006). By the steam pretreatment under different operating conditions, 20–50% (w/w) of the corn stalks were dissolved (Datara et al., 2007). In order to further promote the dissolution of lignocellulosic biomasses and avoid destroying the sugar fragments, combination of biological and steam explosion pretreatments will be necessary.

Because of the complexity of the components in biomass, it is clear that the pretreated biomass is hard to be utilized completely only by a single bacterial strain. A microbial community such as anaerobic sludge is always used as inoculums for hydrogen or methane production (Han and Shin, 2004; Mitani et al., 2005; Liu et al., 2006). However, with this approach, it is difficult to control and optimize the hydrogen-producing microbial consortium, and the hydrogen production rate and yield are always not high. Therefore, construction of a stable and effective microbial community capable of enhancing the hydrolysis of the biomass and hydrogen production will be indispensable. But until now, few studies have

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been carried out on the augmentated hydrogen production from the lignocellulosic biomasses with the efficient hydrogen producers (Wang et al., 2008).

E. aerogenes as a facultative anaerobe, can consume oxygen, for growth and produce hydrogen by the formate and NADH pathway under anaerobic conditions (Ren et al., 2005; Zhang et al., 2005, 2009; Zhang and Xing, 2008). *C. paraputrificum* can produce hydrogen at a high rate from cellobiose (Evvyernie et al., 2001). *C. thermocellum* is a thermophilic, anaerobic and cellulolytic bacterium capable of growing on cellulosic substrates, including avicel, filter paper, solka floc, and pretreated mixed hardwood (Zhang and Lynd, 2005). These three strains have their respective advantages, and can be used to augment co-production process of hydrogen and methane from the lignocellulosic biomass.

In this study, cornstalks were used to produce hydrogen and methane by a two- or three-stage fermentation process consisting of anaerobic sludge augmented with different microbial strains mentioned above. The anaerobic sludge was used as the primary inoculum under mesophilic (37 °C) conditions, and the effects of augmentation with *E. aerogenes* and *C. paraputrificum* on the hydrogen production. Effects of *C. thermocellum* on the hydrolysis of cornstalks were also examined. During the fermentation, the microbial community and its stability were analyzed by the denaturing gradient gel electrophoresis (DGGE) method.

2. Methods

2.1. Biomass preparation

Maize was cultivated in the spring in a suburb of Beijing, China. Pretreatment of the cornstalks was accomplished with high-pressure steam at 1.6 MPa for 5 min in a steam-explosion reactor (Chen et al., 2005). Then the pretreated cornstalks were collected and used in the fermentation experiment after air drying.

2.2. Inoculum and culture medium

The anaerobic sludge used as the primary inoculum was taken from an UASB reactor in an industrial wastewater treatment plant in Heibei Province of China. In the hydrogen production stage, the seedling anaerobic sludge was boiled for 15 min to inactivate the hydrogenotrophic bacteria prior to the inoculation (Han and Shin, 2004). 5% (w/v) of the boiled anaerobic sludge was inoculated in the hydrogen stage.

E. aerogenes IAM1183 purchased from the Institute of Applied Microorganisms of the University of Tokyo, Japan, was cultivated at 37 °C. The pure culture medium (1000 ml) consisted of glucose 15.0 g, tryptone 5.0 g, $(NH_4)_2SO_4$ 2.0 g, KH_2PO_4 14.0 g, $K_2HPO_4 \cdot 3H_2O$ 0.6 g and $MgSO_4 \cdot 7H_2O$ 0.2 g (Ren et al., 2005; Zhang et al., 2005). The strain used for augmentation was cultivated at 37 °C to reach OD_{600} of 2.0%, and 10% (v/v) of bacterial culture was inoculated in the hydrogen stage.

C. paraputrificum M-21, which was a gift from Professor Kunio Ohmiya of Mie University, Japan, was cultivated at 37 °C. The pure culture medium (1000 ml) contained glucose 10.0 g, tryptone 2.0 g, yeast extract 4.5 g, Na_2CO_3 4.0 g, 1-cysteine hydrochloride 3.0 g, solution A 75 ml, solution B 75 ml and solution C 3 ml. Solution A (1000 ml) included K_2HPO_4 6.0 g. Solution B (1000 ml) included NaCl 12.0 g, $CaCl_2$ 1.2 g, $(NH_4)_2SO_4$ 12.0 g, $MgSO_4 \cdot 7H_2O$ 2.5 g and KH_2PO_4 6.0 g. Solution C (1000 ml) included acetic acid 118 ml (Evvyernie et al., 2000). The strain used for augmentation was cultivated at 37 °C to reach OD_{600} of 2.0%, and 10% (v/v) of bacterial culture was inoculated in the hydrogen stage.

C. thermocellum ATCC31549 was cultivated at 55 °C. The pure culture medium (1000 ml) contained cellobiose 5.0 g, KH_2PO_4 1.5 g, $Na_2HPO_4 \cdot 12H_2O$ 4.2 g, NH_4Cl, 0.5 g, $MgCl_2 \cdot 6H_2O$ 0.18 g, yeast extract 2.0 g, vitamin solution 0.50 ml and mineral solution 1.00 ml. The vitamin solution contained the following (1000 ml): biotin 20 mg, *p*-aminobenzoic acid 50 mg, folic acid 20 mg, nicotinic acid 50 mg, thiamine 50 mg, riboflavin 50 mg, lipoic acid 50 mg and cyanocobalamin 10 mg. The mineral solution contained (1000 ml): trisodium nitrilotriacetate 20.2 g, $FeCl_3 \cdot 6H_2O$ 2.1 g, $CoCl_2 \cdot 6H_2O$ 2.0 g, $MnCl_2 \cdot 4H_2O$ 1.0 g, $ZnCl_2$ 1.0 g, $NiCl_2 \cdot 6H_2O$ 1.0 g, $CaCl_2 \cdot 2H_2O$ 0.5 g, $CuSO_4 \cdot 2H_2O$ 0.5 g, and $Na_2MoO_4 \cdot 2H_2O$ 0.5 g (Zhang and Lynd, 2005). The strain used for the augmentation were cultivated at 55 °C to reach OD_{600} of 1.0%, and 10% (v/v) of bacterial culture was inoculated in the separate biomass hydrolysis stage.

2.3. Anaerobic fermentation of cornstalks

The two-stage bioprocess consisting of hydrogen and the subsequent methane production was simulated by using two batchwise serum closed bottles with a total volume of 70 ml containing 20 ml medium. The pretreated cornstalks were added to a final concentration of 50 g/l in the 1st hydrogen production bottle. The liquid phase of the 1st bottle was then transferred into the 2nd bottle for methane production. The inoculum size for each bottle was 5% (w/v). Both bottles were air-sealed with butyl rubber stoppers and degassed with 100% nitrogen for 5 min prior to the inoculation. Unless otherwise stated, the cultivation was performed at 37 °C. The pH value was adjusted to 6.5 and 7.5 in the respective hydrogen and methane production stages at a one-day cycle.

2.4. Analytical methods

The optical density of the cells at 600 nm was measured by using an ultraviolet–visible spectrophotometer (UV757CRT, Shanghai Precision & Scientific Instrument Co., Ltd.). The pH of the culture was measured by a pH meter (Model 828, Orion).

The gas volume was quantitatively determined by a 100 ml of syringe with a needle. The syringe was inserted into the fermentation bottle sealed with the butyl rubber stopper, and after the pressure was balanced, the scale of syringe indicated the total gas volume produced during the fermentation.

The hydrogen and methane composition was analyzed by a gas chromatograph (GC112A, Shanghai Precision & Scientific Instrument Co., Ltd.) equipped with a thermal conductivity detector (TCD) and a 2 m × 3 mm (i.d.) stainless-steel column packed with TDX-01 (80–100 mesh). The temperatures of the injector, detector and column were kept at 120, 120, and 80 °C, respectively. Nitrogen was used as a carrier gas at a flow rate of 10 mL/min. Incidentally, in the present experiment, the gas phase consisted of H_2 or CH_4, and CO_2. Therefore, by determining the total gas volume and the H_2 or/and CH_4 composition, we could calculate the CO_2 volume and content.

The concentrations of individual organic acids were analyzed by a high-performance liquid chromatograph (Shimadzu 10A) equipped with a FID detector and a Shimadzu SCR-102H column after pretreatment with a 0.45 μm membrane filter. About 5 mM of $HClO_4$ was used as a mobile phase at a flow rate of 1 ml/min.

Soluble sugars were measured by the phenol–sulfuric acid method (Dubois et al., 1956). Cellulose, hemicelluloses and lignin in the pretreated and fermented cornstalks were evaluated according to the procedures reported by Goering and Van Soest (1970).

According to the stoichiometry of anaerobic metabolism for VFA production, the mole number of the glucose equivalent utilized by microbes was equal to carbon moles of the fermentation end-products, i.e., $(4 \times \text{pyruvate} + 4 \times \text{succinate} + 3 \times \text{lactate} + \text{formate} + 2 \times \text{acetate} + 2 \times \text{ethanol} + 4 \times \text{butyrate} + CO_2) \div 6$.