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Enhanced biohydrogen production from cornstalk wastes with acidification pretreatment by mixed anaerobic cultures

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Abstract

Biohydrogen production from the cornstalk wastes with acidification pretreatment was reported in this paper. Batch tests were carried out to analyze influences of several environmental factors on biohydrogen production from cornstalk wastes. Two predominant bacterial morphologies, namely spore-forming rod shape bacteria and micrococcus were screened, purified, and identified after enriched from a hydrogen-producing fermentor with cow dung composts. The maximum cumulative H_2 yield of 149.69 ml H_2 g^{-1} TVS was obtained at initial pH 7.0 and substrate concentration 15 g l^{-1}, the value is about 46-fold as compared with that of raw cornstalk wastes. The maximum hydrogen production rate was 7.6 ml H2 h^{-1}. The hydrogen concentration in biogas was 45–56% (v/v) and there was no significant methane observed in the biogas throughout this study. In addition, biodegradation characteristics of the substrate by microorganisms were also discussed. During the conversion of cornstalk wastes into hydrogen, the acetate, propionate, butyrate, and the ethanol were main by-products in the metabolism of hydrogen fermentation. The test results showed that the acidification pretreatment of the substrate plays a crucial role in conversion of the cornstalk wastes into biohydrogen gas by the cow dung composts generating hydrogen.

Keywords: Biohydrogen production; Acid pretreatment; Alkali pretreatment; Cornstalk; Mixed culture

1. Introduction

Considering global environmental impacts, such as greenhouse effect and resource recovery, there is a pressing need to develop non-polluting and renewable energy source. Compared with fossil fuels as traditional energy sources, hydrogen is a promising candidate as a clean energy carrier in the future because of its high-energy yield (122 kJ g^{-1}) and producing only water instead of greenhouse gases on burning [1–5].

Compared with traditional hydrogen-production process by physical and chemical methods, microbial conversion of biomass, such as agricultural and industrial wastes and residues, into biohydrogen gas using fermentative bacteria, is an environmentally friendly and energy-saving process, and is attracting increasing interest as a useful way of converting biomass to hydrogen [5–7]. Most studies of biohydrogen production so far, however, have been limited to using pure carbohydrates, such as glucose, sucrose and starch [5–10]. Little information is available on the feasibility of using the biomass containing crude cellulose such as corn stover. Recently, the conversion of wheat straw and beer into hydrogen gas by mixed anaerobic culture was carried out in our lab [11,12]. However, bioconversion of agricultural residues into biohydrogen gas by microorganisms is surprisingly lacking because of their complex chemical composition, e.g., cellulose, hemicellulose, lignin, protein and fat.

It is well known that biomass residues, such as crop stalks, are persistent in the environment and remains as environmental pollutants. It is reported that the annual

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yields of biomass residues exceed about 220 billion tons in the world, the value are equivalent to the energy of 60–80 billion tons crude oil. In China alone, the annual yield of biomass wastes exceed about 0.7 billion tons, among them, the annual yields of cornstalk, wheat straw and straw wastes are around 220, 110 and 180 million tons, respectively [11,12]. Except that some of them were used to make paper or as feedstuff for livestock, most of them were set on fire or discarded as environmental pollutants. The biomass residues can, however, be a valuable and vast renewable resource.

Based on this background, our research interest is to explore the feasibility of conversion of corn stalk residues with acidification pretreatment into biohydrogen gas by the mixed culture. A expected hydrogen yield of 149.69 ml H_2 g^{-1} TVS from corn stalk residues with acidification pretreatment was obtained by mixed anaerobic cultures in this study. To the best of our knowledge, this is the first report of biohydrogen production from cornstalk residues by mixed culture so far.

2. Experimental methods

2.1. Hydrogen-producing microflora

Hydrogen-producing microflora was taken from cow dung compost in the suburb of Zhengzhou City, which had been composting for 3 months.

2.2. Acidification pretreatment of the substrate

The cornstalk residues used as substrate were obtained from the suburbs of Zhengzhou City. Before they were degraded by microorganisms, the raw cornstalks were ground separately by a vegetation disintegrator (FZ-102, 250 kw, Beijing Yong Guang Ming Medical Appliance Factory, China) to pass 20-mesh screen. The grinding sample was employed as substrate of hydrogen production in the batch experiments, then the mixture of the ground cornstalks and dilute HCl were boiled in a beaker for 30 min and neutralized to pH 7 with either dilute NaOH or HCl solution. TVS value was determined as follows:

$$\mathrm{TVS} = \frac{W_{\text{dry corn stalk}} - W_{\text{ash}}}{W_{\text{dry corn stalk}}} \times 100\%.$$

Here, $\mathrm{TVS} = 0.8745\, W_{\text{dried cornstalk}}$.

2.3. Experimental procedure

The batch experiments were carried out with 250 ml serum vials as batch reactors filled with 150 ml comprising the mixture of the composts, the pretreated cornstalks, and 3 ml of nutrient stock solution. These vials were gassed with nitrogen gas to remove oxygen and the headspace of the reactors to keep the anaerobic environment. The bottles were incubated at 36 ± 1 °C and operated in an orbital shaker with a rotation speed of 90 rpm to provide better contact among substrates. Each liter of nutrient stock solution contained 80 g of NH_4HCO_3, 12.4 g of KH_2PO_4;0.1 g of $MgSO_4 \cdot 7H_2O$;0.01 g of NaCl;0.01 g of $Na_2MoO_4 \cdot 2H_2O$;0.01 g of $CaCl_2 \cdot 2H_2O$;0.015 g of $MnSO_4 \cdot 7H_2O$;0.0278 g of $FeCl_2$, which was slightly modified from Lay [5]. The volume of biogas was determined using glass syringes of 5–50 ml.

2.4. Analytical methods

The hydrogen gas percentage (H_2%) was determined by comparing the sample biogas with a standard of pure hydrogen using a gas chromatograph (GC, Agilent 4890D) equipped with a thermal conductivity detector (TCD) and 6-foot stainless-steel column packed with Porapak Q (80/100 mesh). The operational temperatures of the injection port, the oven and the detector were 100°, 80° and 150°, respectively. Nitrogen was used as the carrier gas at a flow rate of 20 ml min^{-1}. The concentrations of the volatile fatty acids (VFAs) and the alcohols were analyzed using another GC of the same model with a flame ionization detector (FID) and an 8-ft stainless-steel column packed with 10% PEG-20 M and 2% H_3PO_4 (80/100 mesh). The temperature of the injection port, the detector and the oven were 220, 240 °C and a programmed column temperature of 130–175 °C, respectively. Nitrogen was used as the carrier gas at a flow rate of 20 ml min^{-1}. The flow rate of hydrogen and air was 30 ml min^{-1}. The pH values inside the digesters were determined by a microcomputer pH-vision 6071.

3. Results and discussion

3.1. Seed hydrogen-producing microflora and microscopic observations

A mixture of the cow dung composts and water (v/v = 1:5) was continuously aerated by forced air for 3 h at 50° in order to inhibit the bioactivity of hydrogen consumers and to harvest high yield hydrogen-producing spore-forming anaerobes.

A close examination revealed two predominant bacterial morphologies, namely spore-forming rod-shaped bacteria Fig. 1(a) and micrococcus Fig. 1(b), that were screened, purified, and identified after enrichment from a hydrogen-producing fermentor with cow dung composts. Morphological, physico-biochemical characteristics and comparative sequence analysis of 16S rDNA indicated that two dominant strains belonged to Clostridium sp. Fig. 1 illustrated the micrographs of spore-forming rod-shaped bacteria and micrococcus. As illustrated in Fig. 1, the rod-shaped bacteria were in the size range of 2.5–5 μm in length and 0.4–0.9 μm in width, which were spore-forming obligate anaerobic bacteria with maximal hydrogen-producing potential of 2.088 mol H_2 mol^{-1} glucose. The micrococcus were in the range of 0.9–2 μm in size, which also