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Efficient hydrogen gas production from cassava and food waste by a two-step process of dark fermentation and photo-fermentation

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ARTICLE INFO

Article history:
Received 14 December 2008
Received in revised form
26 May 2009
Accepted 13 June 2009
Published online 8 July 2009

Keywords:
Bio-hydrogen production
Biomass
Manihot esculenta Crantz
Food waste
Anaerobic consortia
Rhodobacter sphaeroides

ABSTRACT

A two-step process of sequential anaerobic (dark) and photo-heterotrophic fermentation was employed to produce hydrogen from cassava and food waste. In dark fermentation, the average yield of hydrogen was approximately 199 ml H_2 g^{-1} cassava and 220 ml H_2 g^{-1} food waste. In subsequent photo-fermentation, the average yield of hydrogen from the effluent of dark fermentation was approximately 611 ml H_2 g^{-1} cassava and 451 ml H_2 g^{-1} food waste. The total hydrogen yield in the two-step process was estimated as 810 ml H_2 g^{-1} cassava and 671 ml H_2 g^{-1} food waste. Meanwhile, the COD decreased greatly with a removal efficiency of 84.3% in cassava batch and 80.2% in food waste batch. These results demonstrate that cassava and food waste could be ideal substrates for bio-hydrogen production. And a two-step process combining dark fermentation and photo-fermentation was highly improving both bio-hydrogen production and removal of substrates and fatty acids.

1. Introduction

Bio-hydrogen production from renewable sources, which is considered a ''green technology,'' has received attention in recent years as an approach to promote the sustainable development of global prosperity [1]. The development of bio-hydrogen production faces a contentious problem: To seek suitable biomass for bioenergy producing. Cassava powder, from the root of the cassava plant, is mainly composed of starch, with small amount of cellulose, hemicellulose, and lignin. Because of its high yield and ability to grow well in arid areas, cassava is regarded as an ideal energy crop [2,3]. The United Nations Food and Agriculture Organization (FAO) evaluated that the annual worldwide production of cassava reached 200 Mt in 2005, with an average yield of 25–30 t ha^{-1}. Cassava was reported to be an optimal biomass for ethanol fermentation [2]. Cassava might also be suitable biomass for bio-hydrogen production.

Because of a shortage of food in the world, to produce bioenergy by using waste biomass was getting more and more attention. Some organic substrates such as beer lees [4], cornstalk wastes [5], starch-manufacturing wastes [6], sweet sorghum [7] and wastewater [1] have been used to produce hydrogen. Food waste, consisting mainly of starch, protein, and fat, with a small amount of cellulose and semi-cellulose, also constitutes a possible source for bioenergy production.

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doi:10.1016/j.biombioe.2009.06.008

A large amount of food waste is produced each day in the world. For example, only in China, the daily production of food wastes reaches to approximately 20,000 kt in 2006 [8]. There have been some studies of bio-hydrogen production from food wastes by anaerobic fermentation [9–11], approximately 0.9–2.2 mol H_2 mol^{-1} hexose was produced.

A variety of organic substrates including simple and complex carbohydrates can be fermented to fatty acids and hydrogen at a relatively high rate by facultative or anaerobic bacteria including mixed *anaerobic consortia* [4,7,12], which was usually designated as dark fermentation. Because dark fermentation usually produces different fatty acids, its maximum theoretical hydrogen yield is relatively low, ranging from 2 to 4 mol mol^{-1} hexose according to the composition of produced fatty acids [1]. Besides, the produced volatile fatty acids in the effluent of dark fermentation may carry a potential threat to the environment. To eliminate this potential pollution problem, many efforts have been made to decompose the fatty acids and to decrease chemical oxygen demand (COD) in the fermentation effluent. Converting the fatty acids to methane in a second reactor has been proposed [13], and hydrogen–methane mixtures were produced from glucose in a two sequential stages of hydrogen and methane fermentation [13]. A more efficient two sequential steps were tested by using purple non-sulfur (PNS) photosynthetic bacteria in the second reactor [6]. Photo-fermentation by PNS bacteria produces hydrogen from fatty acids at high efficiency, including the metabolites produced during hydrogen producing by dark fermentation, such as acetate and butyrate [14–16]. Several studies have reported the two-step hydrogen production process of sequential dark- and photo-fermentation achieved higher hydrogen yields from various substrates compared to hydrogen yields by dark fermentation or photo-fermentation alone [6,17]. In our previous study, the total hydrogen yield from sucrose increased from the maximum of 3.67 mol H_2 mol^{-1} sucrose in dark fermentation to 6.63 mol H_2 mol^{-1} sucrose by using the two-step process [17].

In this study, a two-step process of sequential dark- and photo-fermentation was established to produce hydrogen from cassava and food waste. And the removal of chemical oxygen demand (COD during the two-step hydrogen producing process was also investigated in order to address how much waste could be removed through the hydrogen producing system.

2. Materials and methods

2.1. Microbial inocula for dark fermentation and photo-fermentation

Cattle dung compost was collected from the suburb of Xi'an City, China, mixed with water (1:10, w/v), boiled for 15 min, and used as the dark-fermentation inoculum. *Rhodobacter sphaeroides* (*R. sphaeroides*) ZX-5, isolated from wastewater in our laboratory [18], was used as the inoculum for photo-fermentation. *R. sphaeroides* ZX-5 cells were pre-cultured aerobically in RCVB medium [17] in a shaker at 200 rpm at 30 ± 2 °C for approximately 24 h ($OD_{660} = 1.8$–2.0). After centrifugation, the concentrated cells were suspended with the same volume RCVB medium without carbon and nitrogen source.

2.2. Hydrogen production in dark fermentation

Cassava powder was suspended in distilled water and boiled with stirring until clear. Food waste was collected from our institute's cafeteria. The refuse was crushed into homogenate before use.

Dark fermentation was performed in 38-ml tubes in batch tests. Tubes were filled with 29 ml of modified dark-fermentation medium and 1 ml of pretreated cattle dung compost. 1 l of fermentation medium contained 1.5 g KH_2PO_4, 4.2 g $Na_2HPO_4 \cdot 12H_2O$, 0.18 g $MgCl_2 \cdot 6H_2O$, 2 g yeast extract, 0.1 g $FeSO_4 \cdot 7H_2O$ and 1.5 g glutamate as nitrogen source as well as 2 ml trace element solution (per litter containing 28.6 g H_3BO_3, 12 g $CoCl_2 \cdot 6H_2O$, 1.66 g $FeCl_3 \cdot 6H_2O$, 16 g $MnSO_4 \cdot H_2O$). The concentrations of carbon source were 18, 20, and 18 g l^{-1} cassava, food waste (dry weight), and sucrose, respectively. The initial pH value was adjusted to 6.8. To check whether the inocula solution or yeast extract would serve as carbon source for hydrogen production, two series of controls were carried out: Control A, without yeast extract and extra carbon source; and Control B, without extra carbon source, but containing the same amount of yeast extract used in the dark-fermentation medium. Test tubes sealed with rubber stoppers were kept at 37 ± 1 °C in a dark biochemical incubator and 60-ml syringes were used to collect and measure the biogas production. All batch tests were performed in triplication.

2.3. Hydrogen production in photo-fermentation

Dark-fermentation effluent from the batch test was centrifuged at 8000 rpm for 10 min, and then diluted to 50% (v/v) and adjusted as pH 7.0 for the optimal conditions for photo-fermentation [17]. Whereas, the photo-fermentation medium had the same composition as the modified RCVB medium, except for the carbon and nitrogen sources. Photo-fermentation with 32 ml of medium and 2 ml of pre-cultured ZX-5 cells suspension was performed in 38-ml anaerobic tubes at 30 °C under 4000 lux illumination. The tubes were sealed with rubber stoppers, and 60-ml syringes were used to collect and measure the gas produced.

2.4. Analyses

The produced gas (mainly H_2 and CO_2) was analyzed by gas chromatography (GC) using a GC7900 (Techcomp, China) equipped with a thermal conductivity detector. A 5-m stainless transformer oil analysis column was packed with acidic ethyl acetate (AE), with nitrogen as the carrier gas at a flow rate of 20 ml min^{-1}. The temperature of the injector, column, and detector was kept at 80, 80, and 130 °C, respectively.

Volatile fatty acids (VFAs) and alcohols were quantified by GC (Shimadzu 17A, Kyoto, Japan) using a flame ionization detector and a 30-m FFAP capillary column. The temperature of the injector and detector was 250 and 260 °C, respectively. The column was held at 80 °C for 2 min, heated to 200 °C at 5 °C min^{-1}, and then maintained at 200 °C for 1 min. The carrier gas was nitrogen at a flow rate of 70 ml min^{-1}.