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Aerobic and anaerobic sequential culture fermentation (AASF) to produce bio-hydrogen from steam-exploded cornstalk

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ABSTRACT

A novel aerobic and anaerobic sequential culture fermentation (AASF) method was designed to improve the conversion efficiency of steam-exploded cornstalk during bio-hydrogen production. The enzyme activities of cellulase and β-glucosidase produced by *Trichoderma viride* ACCC 30169 were 76.79 FPU g^{-1} dry weight and 45.23 IU g^{-1} dry weight after 6-days steam-exploded cornstalk fermentation, respectively. The aerobic fermentation residue was used as the substrate for bio-hydrogen production by *Clostridium butyricum* AS1.209 anaerobic fermentation. The optimum solid-to-liquid ratio of the anaerobic fermentation substrate was 1:5. The maximum bio-hydrogen yield was attained on the medium with addition of 0.1 g g^{-1} substrate urea after 2 days of aerobic fermentation. Compared with simultaneous saccharification and fermentation (SSF), AASF for bio-hydrogen production could shorten the fermentation period by at least 66% and the hydrogen yield reached 83% of the total volume after 24 h of anaerobic fermentation. AASF from steam-exploded cornstalk was an effective way for bio-hydrogen production without additional commercial cellulase.

Introduction

Overuse of fossil fuels has led to crises in natural resources and environment, which forces people to find new energy sources, especially renewable ones to avoid the greenhouse effect resulted from the combustion gases of fossil fuels [1–4]. Hydrogen is usually regarded as an ideal alternative energy source due to its high energy density (122 kJ/g) and clean combustion product [5,6]. Hydrogen gas is also a valuable energy carrier due to its potential to be used to power chemical fuel cells [7,8]. Currently, hydrogen is mainly produced from fossil fuels and water while these methods need to consume a large amount of energy and result in environmental pollution. In this case, utilizing the renewable and abundant lignocellulosic biomass to obtain various kinds of clean energy has attracted much attention by researchers worldwide [9–14]. In China alone, the annual yields of

Abbreviations: AASF, aerobic and anaerobic sequential culture fermentation; SSF, simultaneous saccharification and fermentation.

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cornstalk, wheat straw, and straw wastes are around 220, 110, and 180 million tons, respectively, hence the biomass residues can be a valuable and vast source for bio-hydrogen production [15]. Hydrogen production from lignocellulosic biomass shows distinct advantages over other energy intensive methods that are used by humans [16–18]. Despite the situation, comparatively cheap hydrogen cannot be attained till nowadays due to the restriction of bio-hydrogen production technologies [17].

Although there are reports of bio-hydrogen production from cellulose, which have achieved fine results, they cannot represent the practical situation of natural cellulose application [19,20]. Fan and de Vrije used sugar juice derived from dilute acid treated and alkali treated stalk to produce hydrogen by fermentation, respectively [21,22]. While such pretreatment methods have pollution on the environment and the acid or alkali used in these processes should lead to equipment corrosion. Compared with these methods, steam explosion is a clean and economically feasible pretreatment method [23,24]. Steam explosion pretreatment could remove most of the hemicellulose within biomass, and thereby promoting the enzymatic digestion. More importantly, steam explosion could satisfy the requirement of pretreatment process and its energy cost is relatively moderate [25]. Our previous work adopted the clean steam explosion method as pretreatment for cornstalk and then used *Clostridium butyricum* for simultaneous saccharification and fermentation (SSF) to produce bio-hydrogen. The results showed that the bio-hydrogen productivity of steam-exploded cornstalk was 12 folds higher than that of untreated stalk, and the maximal specific hydrogen rate was increased by 60% [3]. However, the hydrogen production process still needed additional commercial cellulase during anaerobic fermentation, which increased the production cost of bio-hydrogen. Based on our previous researches, aerobic and anaerobic sequential culture fermentation (AASF) was considered to produce hydrogen with the intention to decrease the additive amount of commercial cellulase [3].

The hydrolysis of steam-exploded biomass materials to various monomers that are available to microbes should be under the action of cellulase. However, the high cost of commercial cellulase restricts the development of enzymatic hydrolysis of lignocellulosic biomass. This paper was aimed to improve the bio-hydrogen production efficiency by AASF and tried to use the cellulase produced from aerobic fermentation for the subsequent bio-hydrogen production, and thereby decreasing the cost of hydrogen fermentation. For that purpose, we used steam-exploded cornstalk as the substrate to produce cellulase by *Trichoderma viride* ACCC 30169 aerobic fermentation, and then inoculated *C. butyricum* AS1.209 in the aerobic fermentation residue to produce bio-hydrogen via SSF process.

Materials and methods

Preparation of steam-exploded cornstalk

Cornstalk used in this research was purchased from Shandong, China. Steam-explosion vessel was designed by our own laboratory. Steam-exploded cornstalk was prepared by putting the chopped corn straw (3–4 cm) into the steam-explosion vessel at 1.5 MPa and maintaining the pressure for 10 min and then a valve on the wall of the vessel was opened suddenly to bring the reactor rapidly to atmospheric pressure [23]. The method described by Van Soest was adopted for the component analysis of cornstalk [26].

Microorganisms

T. viride ACCC 30169 purchased from China General Microbiological Culture Collection Center (CGMCC) was used for cellulase production. The strain was preserved in PDA medium, and saved in a 4 °C refrigerator after slant cultivation at 30 °C for 5 days. Seed medium contained (g/L): bran, 10; peptone, 1.5; $(NH_4)_2SO_4$, 1.4; $MgSO_4 \cdot 7H_2O$, 0.3; KH_2PO_4, 2.0; $CaCl_2$, 0.3; and urea, 0.3 (pH 5.5–6.0). Inoculum was cultured on an orbital shaker (150 rpm) at 30 °C for 48 h.

C. butyricum AS1.209 purchased from China General Microbiological Culture Collection Center (CGMCC) was used for bio-hydrogen production. The strain was preserved in 6.5% corn meal agar, and saved in a 4 °C refrigerator after anaerobic cultivation at 35 °C for 3 days. Seed medium contained (g/L): glucose, 20; yeast extracts, 2; KH_2PO_4, 0.2; K_2HPO_2, 1.6; $MgSO_4 \cdot 7H_2O$, 0.2; NaCl, 0.1; $CaCl_2$, 0.01; $Na_2S \cdot 9H_2O$, 0.25; $NaMoO_4 \cdot 2H_2O$, 0.01; $NaHCO_3$, 0.2; and $(NH_4)_2SO_4$, 3.0.

Cellulase preparation from aerobic fermentation

Substrate of cellulase aerobic fermentation contained 8.0 g steam-exploded cornstalk and 2.0 g bran. The solid-to-liquid ratio of substrate was 1:2. Inorganic salt addition to the substrate contained (%, w/w): $(NH_4)_2SO_4$, 1.5; $MgSO_4 \cdot 7H_2O$, 0.6; and KH_2PO_4, 0.3. The substrate was put into a 250 ml serum bottle and then the seed solution was inoculated after sterilization. Inoculation size was 1.5×10^5 spore g^{-1} substrate, and the eventual solid-to-liquid ratio was 1:2.5.

Enzyme activities of cellulase and β-glycosidase of commercial concentrated cellulase solution (Xiasheng Co. Ltd., Ningxia, China) derived from *T. viride* were 110 FPU ml^{-1} and 37 IU ml^{-1}, respectively.

Bio-hydrogen production by C. butyricum AS1.209 anaerobic fermentation

The *C. butyricum* AS1.209 strain in the logarithm growth period was blended with aerobic fermentation residue while passing through nitrogen gas to eliminate oxygen, and then cultured in a 35 °C water bath.

For comparison of the substrate with different pretreatment methods, a boiling water bath was used to deal with the cellulase fermentation residue in the contrast experiments, and then *C. butyricum* AS1.209 was inoculated on the substrate with additional commercial cellulase zymin. And at the same time, another test was done under the same conditions with the contrast experiments except that no additional commercial cellulase zymin was supplied during the fermentation process of bio-hydrogen production.

The anaerobic fermentation process was the same in all experiments.