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Photo-fermentative hydrogen production from enzymatic hydrolysate of corn stalk pith with a photosynthetic consortium

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ABSTRACT

Corn stalk pith is a waste product of papermaking from corn stalk, and is easy to digest, which makes it a promising feedstock for biofuel production. The main aim of this study is to evaluate utilization of corn stalk pith for hydrogen production. Corn stalk pith was hydrolyzed with commercial cellulase at 50 °C, and the hydrolysate was used as a carbon source for hydrogen production with a photosynthetic consortium. Effect of initial pH on sugar consumption, hydrogen production and byproducts accumulation was evaluated with initial reducing sugar concentrations of about 11 g/L. Single-factor experiments were conducted to determine optimal conditions. Substrate conversion efficiencies (82–94%) during photo-fermentation at initial pH of 6–9 were higher than those (40–80%) achieved in previous studies on hydrogen production from glucose using the same photosynthetic consortium. Hydrogen yield (2.6 ± 0.3 mol H_2/mol sugar consumed) obtained at an initial pH of 7 was higher or comparable to those reported in literatures. During the photo-fermentation process, hydrogen was mainly produced *via* acetic acid production pathway in the first 24 h, and the butyric acid production pathway dominated the hydrogen production for the next 72 h. It was for the first time that corn stalk pith has been used for hydrogen production.

Introduction

The global community depends heavily on crude oil for energy and materials, causing increasing concerns on depletion of this non-renewable source and deterioration of the environment due to greenhouse gas emission [1,2]. Developing energy and materials from low cost renewable sources, such as crop residues, can improve energy sustainability by reducing dependence on crude oil.

Hydrogen is well-known for its high mass based energy density. It is also the cleanest fuel since no greenhouse gas is released during its combustion [3–7]. Compared to commonly used thermochemical methods, such as pyrolysis and

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gasification, biological methods are attractive because of their mild operating conditions. Currently, biological hydrogen production methods can be classified into four groups: (1) biophotolysis with cyanobacteria or green algae, (2) dark fermentation with anaerobic bacteria, (3) photo-fermentation with photosynthetic bacteria, and (4) microbial electrolysis. Photo-fermentation is considered more feasible for application in the near future than others, due to its high substrate conversion efficiencies and capability in utilizing variable substrates [4,8,9]. Photo-fermentative hydrogen production can be conducted using either pure photosynthetic strains or photosynthetic consortia. Compared with pure strains, photosynthetic consortia have several advantages, such as no sterilization requirements, less secondary pollution, adaptive capacity due to microbial diversity and capacity to use mixed substrates [10,11]. Recently, a photosynthetic consortium was selected and augmented from a mixed substrate of silt sewage, pig manure and cow dung. This consortium has shown high conversion and hydrogen production efficiencies in studies using enzymatic hydrolysate of agricultural residue as a substrate [12,13].

China is the second largest corn producer, contributing about 21% of global production (1017 million tonnes) based on 2013 data [14]. For every tonne of corn grain produced, about 0.94 tonnes of corn stover is generated. As a result, about 200 million tonnes of corn stover is estimated to be produced annually in China. Corn stalk rind and pith account for about 40% of corn stover by weight. Corn stalk rind is rich in fibers, making it a promising source for papermaking [15]. Corn stalk pith, in contrast, is mainly composed of non- or short-fiber carbohydrates (oligosaccharides and polysaccharides of arabinose, xylose, mannose, galactose and glucose), and can result in high chemical consumption for pulp preparation, difficult washing and dewatering, and reduced sheet opacity. Therefore, corn stalk pith is usually removed from corn stalk as a waste product before papermaking. Corn stalk pith is easy to digest by enzymes compared to other parts of corn stover, which makes corn stalk pith a suitable feedstock for biofuel production. However, corn stalk pith has only been utilized for animal feed or methane production [16]. Up to date, there have been no reports on utilization of corn stalk pith for production of hydrogen.

In this study, photo-fermentative hydrogen production with the photosynthetic consortium was conducted using enzymatic hydrolysate from corn stalk pith as a carbon source. The photo-fermentation was first carried out at different biomass concentrations at an initial pH of 7, in order to determine the optimal biomass concentration. Effect of initial pH on reducing sugar consumption, hydrogen production, and byproduct accumulation was further evaluated to determine the optimal initial pH. The hydrogen production performance obtained under optimal conditions was also compared with those reported in literatures.

Materials and methods

Photosynthetic consortium and corn stalk pith

The photosynthetic consortium originally isolated from a mixed substrate of silt sewage, pig manure, and cow dung, contains 5 stains (*Rhodospirillum rubrum*, *Rhodopseudomonas capsulata*, *Rhodopseudomonas pulastris*, *Rhodobacter sphaeroides*, and *Rhodobacter capsulatus*), based on 16S rDNA sequencing [17]. The photosynthetic consortium was maintained in a medium containing 1 g/L of NH_4Cl, 2 g/L of $NaHCO_3$, 1 g/L of yeast extract, 0.2 g/L of K_2HPO_4, 4 g/L of CH_3COONa, 0.2 g/L of $MgSO_4 \cdot 7H_2O$, and 2 g/L of NaCl, and incubated at 30 °C with light intensity of 2000 Lux for 3–5 days. The culture grown in exponential period with a biomass concentration of 0.8 g/L was used as an inoculum for photo-fermentation.

Corn stalk obtained from a local farmer (Zhengzhou, Henan Province, China) was air-dried and split into rind and pith with a shell/pith separator. The corn stalk pith was ground by a ball mill (Tai Chi Ring Nano Products Co., Ltd., Qinhuangdao), and stored in sealed plastic bags at room temperature before enzymatic hydrolysis.

Enzymatic hydrolysis

Enzymatic hydrolysis of corn stalk pith was conducted in 200-mL glass reactors based on methods described in a previous study [18]. Briefly, each reactor was loaded with 5 g of corn stalk pith powder, 0.75 g cellulase and 100 mL citric acid buffer (0.05M, citric acid/sodium citrate, pH 4.8). The cellulase was purchased from Yuanye Corp. (Shanghai, China), with a filter paper activity (FPA) of 10 FPU/mg. The glass reactors were incubated at 50 °C with shaking at 150 rpm for 72 h. After enzymatic hydrolysis, reducing sugar concentration in the hydrolysate was about 21 g/L.

Batch photo-fermentation

Photo-fermentation for determination of optimal initial biomass concentration was conducted in 350-mL glass reactors in duplicate. Each reactor was loaded with 100 mL of enzymatic hydrolysate at pH of 7 (adjusted with 5M KOH), 100 mL of nutrient solution (0.4 g/L of NH_4Cl, 0.5 g/L of K_2HPO4, 0.2 g/L of $MgCl_2$, 0.1 g/L of yeast extract, 2 g/L of NaCl, and 3.5 g/L of sodium glutamate), and different amounts of inoculum to reach biomass concentrations of 0.07, 0.13, 0.18, 0.23, 0.27, and 0.3 g/L, respectively. The total volumes were 220, 240, 260, 280, 300, and 320 mL, respectively. The concentration of NH_4Cl in the nutrient solution has been optimized in a previous study, because NH_4Cl at high loadings could negatively affect hydrogen production [19]. After loading, the reactors were purged with pure argon (Ar) gas, sealed with rubber stoppers, and incubated at 30 °C for 96 h under light exposure with a light intensity of 2000 Lux provided by filament lamps. The reactors were static without mixing, since agitation may cause negative effects on hydrogen production during photo-fermentation according to a previous study [13]. A 60-mL syringe was attached to the outlet on each reactor to collect biogas. Every 12 h or when the syringe was full, 30 mL of gas sample (at 30 °C and 1 atm) was taken for analyzing the hydrogen content with gas chromatography. Optimal initial biomass concentration was determined in terms of hydrogen productivity and yield.

In order to determine the optimal initial pH for photo-fermentation, hydrolysates with different levels of pH (4, 5, 6, 7, 8, 9 and 10) were prepared with 5M KOH or 5M HCl. Photo-