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Simultaneous saccharification and fermentation of steam exploded wheat straw pretreated with alkaline peroxide

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ABSTRACT

The cellulose content of substrate is one of the most important factors for ethanol production from lignocellulose. To increase the cellulose content of substrate and ethanol yield in simultaneous saccharification and fermentation (SSF), a pretreatment method coupling steam explosion with alkaline peroxide for wheat straw was studied. After the complex pretreatment, the cellulose content in wheat straw increased from 31.5% to 67.2%. In the hydrolysate of wheat straw pretreated with the complex method and steam explosion, the glucose concentration was 110.9 g/L and 67.8 g/L, respectively. The optimal conditions for SSF were 40 °C, 120 h, cellulase loading 40 FPU/(g wheat straw), yeast inoculum 10% (v/v) and substrate concentration 16.7% (w/v). Under the optimal conditions, the total ethanol concentration in SSF of wheat straw pretreated with steam explosion and alkaline peroxide reached 51.5 g/L, and an overall yield of 81.1% was obtained.

1. Introduction

Lignocellulose such as wheat straw is an abundant renewable resource in the biosphere [1]. Cellulose is the major component of lignocellulose, and can be hydrolyzed to glucose, which can be fermented to bioethanol. Therefore, bioethanol from lignocellulose has been of interest in recent decades [2–4]. Enzymatic hydrolysis of cellulose to glucose is of central importance in conversion of lignocellulose to bioethanol [5,6]. Since enzymatic hydrolysis of native lignocellulose usually results in lower cellulose solubilization, pretreatments are generally applied to enhance substrate accessibility [7]. Steam explosion is one of the most effective methods, which causes hemicellulose degradation and lignin transformation, and improves hydrolytic reaction rates [8].

Accordingly to the economic evaluation of bioethanol fermentation, higher ethanol concentration in the reaction mixture is required. The cellulose content of substrate is one of the most important factors for ethanol production from lignocellulose [9]. High glucose concentration after hydrolysis is preferable for the fermentation process, thereby decreasing the energy demand in the subsequent distillation step. Steam explosion pretreatment could improve the enzymatic hydrolysis rate in some degree. However steam explosion has some limitations, such as only destruction of partial xylan, incomplete disruption of the lignin-carbohydrate matrix, and lignin is removed only to a limited extent but rather redistributed on the cellulose surfaces [10]. Compared with native wheat straw, the cellulose component of the substrate after steam explosion is not improved notably. Therefore, to enhance the cellulose content of substrate and the ethanol concentration in fermentation mixture, the non-cellulose components need to be further decreased by others pretreatments. Alkaline peroxide has been successfully developed for lignocellulose pretreatment, and the agents are effective for hemicellulose and lignin removal [11,12]. However, the complex pretreatment of wheat straw coupling steam explosion with alkaline peroxide has not been reported.

To further enhance the accessibility and cellulose content of solid substrate, a pretreatment method coupling steam explosion with alkaline peroxide was investigated in this paper. To improve the glucose concentration in the hydrolysis process, the effect of solid substrate concentration on glucose concentration and cellulose conversion were studied. Taking wheat straw pretreated with different methods as substrates, the ethanol production in simultaneous saccharification and fermentation (SSF) was studied under the optimized conditions.

2. Materials and methods

2.1. Materials and enzymes

Wheat straw was obtained from local farmer in the suburb of Beijing, China, air-dried and stored in a dry and cool room. Commercial ethanol instant active dry

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yeast (*Saccharomyces cerevisiae*) was obtained from Angel Yeast Co., Ltd., Wuhan, China. Commercial *Trichoderma viride* cellulase, with filter paper activity (FPA) of 110 FPU/mL, was purchased from Shanghai Boao Biotech. Corp., China. All other chemicals used in this study were of analytical grade, and obtained from Beijing Chemicals & Reagent Corp., China.

2.2. Pretreatment of wheat straw

2.2.1. Steam explosion pretreatment of wheat straw (SPWS)

About 1 kg samples was cut to 3–4 cm, soaked in water overnight and steam exploded in a 7.5-L batch reactor (Weihai Automatic Control Reactor Ltd., China) under pressure 1.5 MPa (198 °C) for 10 min [13]. After pretreatment, the material was separated into solid residue and liquid by filtration. The solid residue was dried in a forced-air oven at 55 °C for 24 h, and stored in sealed plastic bags at 4 °C.

2.2.2. Alkaline peroxide pretreatment of SPWS

Alkaline peroxide pretreatment method was adapted from others' studies [14–16]. To obtain the optimal H_2O_2 concentration for pretreatment, different H_2O_2 concentration (v/v, 2%, 4% and 6%) was studied in preliminary experiment. The result showed that there is no significant difference between 4% and 6% concentration. The 4% H_2O_2 concentration therefore was applied in the alkaline peroxide pretreatment. The dried SPWS was slurried in distilled water containing H_2O_2 (4.0%, v/v) and NaOH (1%, w/v). The solids substrate concentration is 10% (w/v), and pH of the reaction mixture is 13.1. To analysis the effectiveness of alkaline peroxide on SPWS delignification, two other experiments were performed in parallel. One was solid SPWS slurried in distilled water with 10% (w/v) solid concentration. The other was SPWS slurried in distilled water containing NaOH (1%, w/v) with 10% (w/v) solid concentration. The slurried samples were shaken in an incubator at 150 rpm at 25 °C for 120 h. The pretreated samples were filtered, and dried in a forced-air oven at 55 °C for 24 h, and stored in sealed plastic bags at 4 °C. For compositions analysis, representative samples of the pretreated slurries were withdrawn. The solid residue was thoroughly washed with water to remove all soluble substances, and then filtered to remove excess moisture.

2.3. Optimization of wheat straw concentration for enzymatic hydrolysis

The hydrolytic experiments were conducted in sodium acetate buffer (100 mM, pH 4.5), at 50 °C with gentle agitation in water bath for 72 h, with *T. viride* cellulase loading of 30 FPU/(g substrate). Different SPWS concentrations (33.3%, 20%, 16.7%, 14.3% and 12.5%, w/v) were applied respectively. Samples were taken from the reaction mixture periodically during incubation, and boiled for 10 min to terminate the reaction and stored at −20 °C for glucose analysis.

2.4. Enzymatic hydrolysis of wheat straw with different pretreatments

The hydrolytic experiments were conducted according to Section 2.3, at the water-insoluble solids substrate concentration of 16.7% (w/v), SPWS, alkaline peroxide pretreated SPWS and alkali pretreated SPWS were applied as substrate, respectively. The dry materials were soaked in sodium acetate buffer (100 mM, pH 4.5) for 1 h, cellulase (30 FPU/g substrate) was then added to initiate the hydrolysis.

2.5. SSF of wheat straw to ethanol

For preparation of yeast inoculum, 1 g dry yeast was added to 20 mL sterile water containing 2% glucose, and incubated at 37 °C for 1 h. The inoculum concentration was about 2.0×10^9 yeast cells/mL and the amount of inoculum added was 10% (v/v) of the SSF medium.

2.5.1. Optimization of SSF process through orthogonal experiments

An orthogonal experiment was first carried out to optimize the SSF process in terms of ethanol yield. Medium was used in the SSF experiments, the composition consists of 2 g/L $(NH_4)_2SO_4$, 5 g/L KH_2PO_4, 0.4 g/L $MgSO_4 \cdot 7H_2O$, 0.2 g/L $CaCl_2$ and 2 g/L yeast extract. The pH of the medium is about 5.0. SPWS was taken as the substrate in orthogonal experiments, and SSF were conducted at water-insoluble substrate concentration of 16.7%. The "$L_9(3^4)$" orthogonal table was chosen, factors and their levels were designed as follows, temperature (35, 40, 50 °C), reaction time (72, 96, 120 h), yeast inoculum (8, 10, 12%) and cellulase loading (20, 30, 40 FPU/g substrate).

2.5.2. Comparison of SSF processes with different pretreated wheat straw

SSF runs were performed in the optimum conditions, according to standard methods described by NREL [17]. SPWS, alkaline peroxide pretreated SPWS and alkali pretreated SPWS were applied as substrate, respectively. The substrates were autoclaved for 15 min at 121 °C before adding enzymes and inoculum. The amount of inoculum added was 10% (v/v) of the SSF medium, cellulase loading was 40 FPU/(g substrate). All SSF were performed at 40 °C for 120 h on an orbital shaker agitated at 150 rpm. Samples were withdrawn periodically and stored at −20 °C for ethanol determination.

2.6. Analytical methods

The compositions of the dry materials were determined according to the method of Van Soest [18]. The amount of glucose and ethanol in the reaction mixture was quantified by a high performance liquid chromatography system (Agilent technology 1200 series, Palo Alto, CA). Samples were filtered through a 0.45 μm filter and diluted properly by eluent (5 mM H_2SO_4). The separation was performed on an Aminex Hpx-87H ion exclusion column (Bio-Rad, Sunnyvale, CA) with 5 mM H_2SO_4 as eluent at a flow rate of 0.6 mL/min.

3. Results

3.1. Components of wheat straw with different pretreatments

The chemical compositions of wheat straw with different pretreatments were shown in Fig. 1. The percent hemicellulose content of native wheat straw is 33.3%, and decreased to 13.6% after steam explosion pretreatment. In contrast, after steam explosion pretreatment, the percent of lignin and cellulose increased to 23.2% and 38.7% from 11.6% and 31.5%, respectively. To further decrease the non-cellulose components, SPWS was treated with alkali and alkaline peroxide, respectively. Different from steam explosion pretreatment, the alkali or alkaline peroxide treatment decreased the hemicellulose and lignin content significantly. After treatment of alkali or alkaline peroxide, the percent of hemicellulose decreased to 6.3% and 5.3%, respectively. Similar with hemicellulose content changes, the percent of lignin decreased to 14.6% and 5.6% with alkali and alkaline peroxide treatment, respectively. Compared with that of native wheat straw, the lignin content of the alkali treated SPWS increases by 25.9%. With the decrease of hemicellulose and lignin content, the percent of cellulose increased to 47.7% and 67.2% with alkali and alkaline peroxide treatment, respectively. It could be seen from the results that the change of hemicellulose content treated by alkali was similar to that of alkaline peroxide treatment. Alkaline peroxide treatment was more effective for lignin solubilization than alkali treatment.

3.2. Optimization of wheat straw concentration for enzymatic hydrolysis

To optimize of the water-insoluble solid substrate concentration in enzymatic hydrolysis and ethanol fermentation, SPWS was applied as the material. The time courses of SPWS hydrolysis at

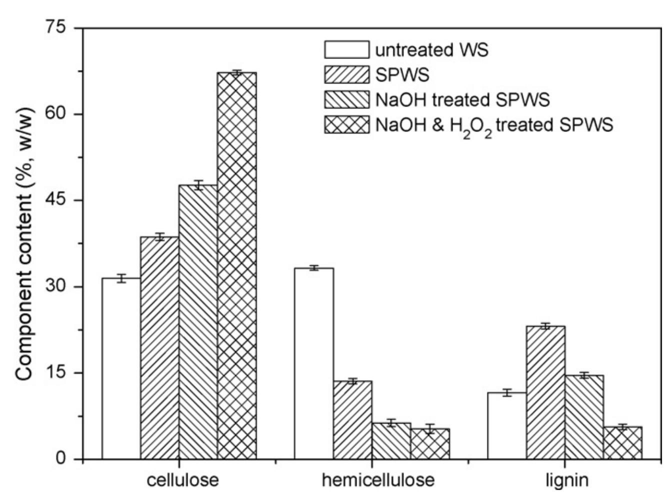

Fig. 1. Chemical compositions (%, w/w) of wheat straw with different pretreatments. The experiment was performed in triplicate and each value is expressed as the mean value ± standard deviation.