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Novel pretreatment of steam explosion associated with ammonium chloride preimpregnation

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HIGHLIGHTS

- Pretreatment of steam explosion with ammonium chloride preimpregnation is studied.
- The nitrogen incorporated in rice straw can be used for ethanol fermentation.
- Fermentability of pretreated rice straw is improved.

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ABSTRACT

Improving nitrogen content and enhancing enzymatic hydrolysis are key processes involved in cellulosic ethanol production. Steam explosion (SE) associated with NH_4Cl preimpregnation was carried out to investigate effects of the pretreatment on nitrogen content, enzymatic digestibility, and ethanol production. Results showed that nitrogen content in pretreated samples increased, which can be used as nitrogen resource for ethanol fermentation. The highest glucose yield of sample pretreated by 1.4 MPa SE with 90 g/l NH_4Cl preimpregnation was 62.64%, which was 2.1 and 0.2 times higher than that of untreated sample and 1.4 MPa SE pretreated sample, respectively. Ethanol yield of sample pretreated by 1.1 MPa SE with 135 g/l NH_4Cl preimpregnation resulted in 1.93 and 0.69 times higher than that of untreated sample and 1.1 MPa SE pretreated sample, respectively. This novel pretreatment improved nitrogen content and enhanced enzymatic digestibility under mild conditions, and could be recommended to further industrial application.

1. Introduction

Depletion of fossil fuels and warming of global climate have led to an increased interest in alternative, renewable energy sources to reduce dependence on fossil-fuel reserves (Moriarty and Honnery, 2012). Bio-ethanol production, the most promising biofuel, has attracted substantial interest from both government and private sectors due to the abundance of lignocellulosic biomass (Sarkar et al., 2012). Ample researches and demonstration plants have been conducted, providing a foundation for further investigation and optimization using lignocellulosic biomass for ethanol production (Chen and Li, 2013). Commonly, the conversion of lignocellulosic biomass to ethanol contains two steps: converting polysaccharides into monosaccharides by hydrolysis and converting monosaccharides into ethanol by fermentation (Limayem and Ricke, 2012). The natural lignocellulosic biomass is highly resistant to enzymatic hydrolysis because of its recalcitrance (Jeoh et al., 2007). Efforts have been made by various pretreatments to destroy the complex recalcitrant structure. The growth and fermentability of *Saccharomyces cerevisiae* are obviously affected by the assimilable nitrogen levels (Casey and Ingledew, 1986). However, rice straw is perceived to be nutrient-deficient (Lau et al., 2008.). Hence, extra nutrients supplement are deemed unavoidable. Yeast extract, peptone, ammonium and urea are always used in industrial media. Although the organic nitrogen sources are rich in peptides and free amino acids, the cost is also high. The ammonium is often substituted for organic nitrogen source, but the nutrient is poor.

Steam explosion (SE) is one of the most efficient pretreatment methods and has been developed into commercial scale (Chen and Liu, 2007), owing to its low chemicals use and energy consumption. Steam explosion combines effects of mechanical tearing and chemical degradation. The saturated steam can penetrate the pores of stalks and tear lots of pores in rapid decompression process, and chemical high-temperature cooking plays a

Abbreviations: SE, steam explosion; SERS, steam-exploded rice straw; URS, untreated rice straw.

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role in degradation of hemicellulose and lignin. As a result, the ammonium might be introduced into the rice straw by the physical/chemical effects. Consequently, ammonium chloride preimpregnation before steam explosion is proposed. It is known that the ammonium chloride is strong acidic-weak basic salt, and the pH value of its solution is weak acid. Previous studies have been demonstrated that the acidic condition can enhance the depolymerization of hemicellulose at high temperature (Martín et al., 2002.). Compared with SO_2 and H_2SO_4, the NH_4Cl is less corrosive and irritant. What's more, the incorporated ammonium might react with lignocellulosic materials by ammonolysis of ester crosslinks of some uronic acids with the xylan at high temperature (Wang et al., 1967; Barton et al., 1986). Compared with the addition of ammonium chloride after steam explosion, it is likely that the ammonium chloride preimpregnation can enhance the pretreatment effects and the ammonium can be transformed into other types of nitrogen resource, which are beneficial for ethanol fermentation.

To our knowledge, very little research has been done into integrating nitrogen addition process with steam explosion, so we assessed the potential of steam explosion associated with ammonium chloride preimpregnation for the first time. The composition profile changes in nitrogen, hemicellulose, cellulose, and lignin after pretreatment were measured. The effects of the pretreatment on the enzymatic hydrolysis and ethanol production were also carried out to evaluate pretreatment effectiveness.

2. Methods

2.1. Raw materials

Rice straw (*Oryza sativa*) was collected from the rural area of Chengdu, Sichuan Province of China. The rice straw was manually cut into pieces with the size of 15 cm, washed by damp cloth to remove dust, and then air-dried at room temperature. Cellulase was produced by *Trichoderma reesei* YG3, the average filter paper activity was 50–60 FPU/ml. All chemicals used in this study were of analytical grade and purchased from Beijing Chemical Reagent Corp., China.

2.2. Steam explosion associated with ammonium chloride preimpregnation pretreatment

The dry rice straw was impregnated by water or NH_4Cl solutions (90 g/l and 135 g/l), with ratio of 1:1, and maintained statically for overnight at room temperature. Then the presoaked rice straw was put into the self-designed steam explosion vessel with a volume of 4.5 L (Weihai Automatic Control Co. Ltd., Weihai, China). The steam explosion process was performed at 1.1 or 1.4 MPa for 4 min, and then the pressure was discharged immediately to get the steam-exploded rice straw (SERS). The dry rice straw was impregnated by water (with ratio of 1:1) and maintained statically for overnight at room temperature, without steam explosion, which was used as the untreated rice straw (URS).

2.3. Prehydrolysis and simultaneous saccharification and fermentation

The URS and SERS without washing were tested directly for simultaneous saccharification and fermentation. Prehydrolysis of URS and SERS was conducted in sodium acetate buffer (100 mM, pH 4.8) with gentle agitation in water-bath shaker at 50 °C for 36 h under cellulase loading of 20 FPU/ g (substrate). After prehydrolysis the temperature was reduced to 37 °C. The relative content of NH_4Cl (9 g/100 g substrate) was filled into the URS and SERS (steam explosion without ammonium chloride preimpregnation) before ethanol fermentation. The 10% (v/v) yeast (*S. cerevisiae*) inoculum was added. The bottles were mounted with fermentation locks in anaerobic conditions. The ethanol fermentation proceeded for 36 h. For preparation of yeast inoculum, 1 g dry yeast was added to 50 ml sterile water containing 20 g/l glucose and incubated at 37 °C for 1 h. To test the significance of differences among the pretreatments, results were subjected to one-way analysis of variance (ANOVA) test using SPSS. Significance in differences was indicated at $p \leqslant 0.05$ with number of replicates of 2 for all cases.

2.4. Analytical methods

2.4.1. Chemical composition analysis

The chemical compositions of URS and SERS were determined using the standard NREL method described by Sluiter et al. (Sluiter et al., 2008). The samples were treated by 72% H_2SO_4 in water bath, at 30 °C for 60 min, following by diluting the acid to 4% by adding distilled water, and then autoclaved at 121 °C for 1 h. Residual and hydrolysis filtrate were separated. The residual was dried at 105 °C overnight. Then the dried residual was heated to 550 °C for 3 h to determine the ash content. The acid-insoluble lignin was calculated by subtracting the ash content from the dried residual. The major structural components (glucan, xylan, and galactan) in the hydrolysis filtrate were determined by high-performance liquid chromatography (HPLC; Agilent 1200, American) equipped with a refractive index detector (G1362A) and Aminex HPX-87H column at 65 °C with 5 mM H_2SO_4 as the mobile phase at 0.5 ml/min (Zhang et al., 2013). The samples for acid-soluble lignin were determined by UV–Visible spectrophotometer. The concentrations of monosaccharides and ethanol in the supernatants after prehydrolysis or simultaneous saccharification and fermentation were also analyzed by HPLC. The cellulose conversions were calculated as follows:

$$\text{Cellulose conversion}\,(\%) = \frac{\text{amount of glucose}\,(\text{g}) \times 0.9 + \text{amount of cellobiose}\,(\text{g}) \times 0.95}{\text{amount of cellulose}\,(\text{g})} \times 100 \quad (1)$$

0.9 is the conversion factor for glucan to glucose and 0.95 is the conversion factor for cellobiose to glucose.

The ethanol yield was defined as follows:

$$\text{Ethanol yield}\ (\%) = \text{EtOH}/[0.511 \times (f[\text{biomass}] \times 1.11) \times 100 \quad (2)$$

EtOHt is the amount of ethanol, f is glucan fraction of dry biomass (g/g), biomass is dry biomass amount at the beginning of fermentation, 0.511 is the conversion factor for glucose to ethanol, and 1.11 is the conversion factor for glucan to glucose.

2.4.2. Determination the nature and content of nitrogen

The URS and SERS were washed three times using 10 times (v/w) tap water to determine the existence state of the nitrogen. The washed samples were air-dried. The total nitrogen and insoluble nitrogen in samples were evaluated by kjeldahl method (Jones, 1991). 0.5 g air-dried samples, 3.5 g K_2SO_4 and 0.4 g $CuSO_4$ and 10 ml H_2SO_4 were put into kjeldahl flask. The digestion was performed for 3 h. After that water was added to give a total volume of 100 ml. The digests were put into the distillation apparatus, and boric acid-mixed was used as indicator solution. After distillation N contents were determined by hydrochloric acid titration. The content of soluble nitrogen was calculated by subtracting the insoluble nitrogen from the total nitrogen.

2.4.3. Analysis of potential inhibitors

5 g pretreated samples were soaked in 25 ml distilled water for 3 h, and then centrifuged for 10 min at 8000 r/min in a Desk Centrifuge. The concentrations of furfural and HMF were determined by HPLC equipped with a UV detector (G1314B) at λ280 and Eclipse XDB-C18 column at 30 °C with methanol water acetic acid