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Effects of green liquor pretreatment on the chemical composition and enzymatic digestibility of rice straw

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HIGHLIGHTS

- Green liquor (GL) was used as an effective pretreatment on rice straw.
- Most polysaccharides retained in pretreated solid with a high delignification.
- Most silica was kept in the residue as a potential high value-added byproduct.
- GL pretreatment can significantly improve the efficiently of enzymatic hydrolysis.
- The maximum sugar yield after GL pretreated and enzymatic hydrolysis was 78%.

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ABSTRACT

Green liquor ($Na_2S + Na_2CO_3$, GL) pretreatment is a proven pathway to improve the enzymatic saccharification for the production of bioethanol. In this work, the effects of GL pretreatment on the chemical composition and enzymatic digestibility of rice straw at various total titratable alkali (TTA) charge and temperature were investigated. The GL pretreatment showed excellent performance in high polysaccharides retention and delignification selectivity. Under the optimized GL pretreatment condition (4% TTA charge, 20% sulfidity and 140 °C), 92.5% of glucan, 82.4% of xylan and 81.6% of arabinan in rice straw were recovered with a delignification of 39.4%. The maximum sugar yields of 83.9%, 69.6% and 78.0%, respectively for glucan, xylan and total sugar, were achieved at the same GL pretreatment condition with an enzyme loading of 40 FPU/g-substrate. The results suggested that GL pretreatment is a practicable method for rice straw to enhance enzymatic saccharification for bioethanol production.

1. Introduction

Fossil fuel, including petroleum, coal, and natural gas, is the most popular energy resource. Over 85% of the energy demands are met by the combustion of fossil fuels, and it is estimated that these non-renewable resources will be used out in the next 40–150 years (Shafiee and Topal, 2009). Meanwhile, as a result of fossil fuel consumption, CO_2, NO_x, SO_x and other pollutants will cause considerable damage to the environment. Exploiting the renewable resources to replace the depleting energy is the only way to realize the sustainable development of society. There are various new energy resources on the earth, such as energy from the sun, the wind, the water and the biomass. Compared with other alternative energy, biomass resources can be converted into liquid fuel which providing the most convenient storage mode (Szczodrak and Fiedurek, 1996). So the utilization of biomass as a solution to substitute fossil fuel has attracted much attention.

Rice straw (*Oryza sativa* L.) offers great potential as a raw material for bioethanol production. According to FAO (Food and Agriculture Organization of the United Nations) statistics, the production of rice straw is about 650–975 million tons per year globally, which is calculated according to the ratio of straw to grain (Binod et al., 2010). Until now, the rice straw has not been used reasonably or just burned in field resulting air pollution and greenhouse gas emission. Efficient utilization of rice straw resource is the best choice for both sides of providing bioenergy and releasing risk of environmental pollution (Chen et al., 2009).

Cellulose, hemicellulose and lignin are the major cell wall components in lignocellulosic materials. Rice straw containing 50–80% carbohydrates is viewed as a potential alternative to our current reliance on fossil fuels (Saha, 2003). However, cellulose is high-crystalline and closely associated with hemicellulose and lignin, which is the major obstacle restricting polysaccharides

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degradation by hydrolytic enzymes, thereby limiting the bioconversion of lignocellulosic materials into liquid fuels (Rahikainen et al., 2011). Therefore, development of practical and environmentally friendly pretreatment methods for reducing cellulose crystallinity, disrupting the association and partially removing lignin and hemicellulose is of great importance to bioethanol production from lignocellulosic biomass.

Pretreatment is an essential step towards the development and industrialization of efficient 2nd generation lignocellulosic ethanol processes (Chiaramonti et al., 2012). However, it has been recognized as a technological bottleneck for the cost-effective development of bioprocess from biomass (Mosier et al., 2005). Green liquor (GL) pretreatment on woody biomass has shown effective performance in improving enzymatic digestibility (Jin et al., 2010; Wu et al., 2010). All the equipment and processes in GL pretreatment system have been industrially practiced for many decades in dozens of kraft pulp mills in the world, and the chemicals of green liquor, sodium carbonate and sodium sulfide, can be completely recovered from a proven chemical recovery system. Meanwhile, GL pretreatment keeps as much polysaccharides as possible in the substrate for enzymatic hydrolysis, and all fermentable sugar can be recovered in one step. It avoids fermentable sugars collection from both pretreatment and enzymatic hydrolysis steps such as steam explosion pretreatment, and retains a higher sugar concentration for fermentation to ethanol. An important benefit of GL pretreatment is that no toxic byproducts such as furfural, acetic acid (from hemicellulose degradation) are produced to affect the fermentation step and cause corrosion in the equipment. An interest possibility that may reduce the process cost is to integrate ethanol production with a pulp mill or to repurpose a kraft pulp mill to a bioethanol plant. Considering the capital costs, investment risk, technical feasibility, especially the efficiency for bioethanol production, the GL process provides an effective pretreatment method for bioethanol production from lignocellulosic biomass.

In this study, rice straw was pretreated by green liquor at various total titratable alkali (TTA) charge and temperature, to understand the effects of GL pretreatment on the chemical composition and enzymatic hydrolysis of rice straw for the production of bioethanol or sugar based chemicals.

2. Methods

2.1. Materials

Rice straw used as feedstock in this work was collected in Jiangsu, China. Air dried raw materials without classification, including stem, leaf and sheath was cut into a length of 3–5 cm and stored in sealed plastic bags at 4 °C in a refrigerator. Prior to composition analysis, the biomass was ground using a Wiley mill, and the particles between 40 and 80 mesh were collected. All weights and calculations were made on the basis of oven dry materials (DM).

Three enzymes, cellulase from *Trichoderma reesei* (NS-50013, 52.3 mg protein/mL, 84 FPU/mL), β-glucosidase from *Aspergillus niger* (NS-50010, 48.5 mg protein/mL, 350 CBU/mL) and xylanase (NS-50014, 50.2 mg protein/mL, 850 FXU/mL) were provided by Novozymes (Franklinton, NC, USA). All the chemicals used for pretreatment and enzymatic hydrolysis were analytical grade and purchased from Nanjing Chemical Reagent Co., Ltd. of China and used as received without further purification.

2.2. Green liquor pretreatment

Green liquor solution was prepared by mixing Na_2S and Na_2CO_3 with a sulfidity of 20%. The definition of sulfidity is the ratio of Na_2S–Na_2S and Na_2CO_3 (on Na_2O basis). The TTA charge as Na_2O on oven dry material was 0–16%. GL pretreatment was carried out in a ten-bomb (1 L) lab scale pulping system with oil bath. The ratio of pretreatment liquor to biomass was 6 (v/w). The raw materials were first impregnated with the pretreatment liquor at 60 °C for 30 min. After impregnation, the temperature was raised with the rate of 2 °C/min to the target temperature (100–160 °C) and maintained for 1 h. The pretreatment was terminated immediately by cooling the bombs to room temperature in cold water. The pretreated solid was collected and washed with water to remove residual chemicals and dissolved straw components. Solid recovery was calculated according to the wet weight and moisture content of the collected solid. The pretreatment spent liquor was collected for pH analysis.

2.3. Enzymatic hydrolysis

A laboratory refiner (Φ 300 mm, 3000 rpm, KRK, Jilin, China) was used to defiberize the pretreated solids to prepare substrates for enzymatic hydrolysis. Enzymatic hydrolysis of the substrates was carried out in a 150 mL Erlenmeyer flask at a consistency of 5% (w/w) in sodium acetate buffer (0.2 M, pH = 4.8) at 50 °C using a shaking incubator (DHZ-2102, Jinhong, Shanghai, China) at 180 rpm for 48 h. An enzyme cocktail mixed by cellulase (NS-50013), xylanase (NS-50044) and β-glucosidase (NS-50010) was used for the enzymatic hydrolysis of GL pretreated samples. The dosages of β-glucosidase and xylanase supplementation constituted 30% of the volume of cellulase added, according to the suggestion from the manufacturer. The enzyme loadings were 5, 10, 20 and 40 filter paper units (FPU) per gram of substrate based on cellulase activity. Sodium azide was charged at 0.3% (w/v), based on total volume of the pulp slurry as an antibiotic to inhibit microbial growth during the enzymatic hydrolysis. Enzymatic hydrolysis residue and hydrolysate was separated by centrifugation after boiled in water for 5 min. Hydrolysate was sampled for monomeric sugar (glucose, xylose and arabinose) analysis. Each data point was the average of duplicate experiments.

2.4. Analytical methods

The enzymatic hydrolysate was diluted 2000 times with the addition of L-(−)-fucose (F2252, Sigma, Saint Louis, MO, USA) as internal standard. Monomeric sugars were determined using an improved high performance anion exchange chromatography (ICS-3000, Dionex Corp., Sunnyvale, CA, USA) with pulsed amperometric detector (HPAEC-PAD). A CarboPac™ PA1 (2 × 250 mm) and a CarboPac™ PA1 (2 × 50 mm) (Dionex Corp., Sunnyvale, CA, USA) were used as analytical and guard column. An 18 mmol/L NaOH solution prepared with degassed super-purified deionized water was used as eluent at a flow rate of 0.25 mL/min. Aliquots (5 μL) were injected after passing through a 0.22 μm nylon syringe filter. The column was reconditioned by using 200 mmol/L NaOH after each three analysis. Monomeric sugars were quantified with reference to standards using the same analytical procedure. The concentration of monosaccharide was corrected by calibration curve of standard sugars. The average of duplicate runs was used in reporting. Data of monomeric sugar content were corrected to polymeric sugar for yield calculation.

The contents of hot water, 1% NaOH and benzene–ethanol extractives were determined according to Tappi Standard T207 cm-99, T212 om-98 and T204 cm-97, respectively. Lignin and carbohydrates of raw materials and pretreated solids were analyzed by Laboratory Analytical Procedures from National Renewable Energy Laboratory (NREL) (Sluiter et al., 2008). The Klason lignin (KL) content was taken as the ash free residue after acid hydrolysis. The hydrolysate from this determination was