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Physical and chemical characterizations of corn stalk resulting from hydrogen peroxide presoaking prior to ammonia fiber expansion pretreatment

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ABSTRACT

To effectively improve enzymatic digestibility of carbohydrate in lignocellulosic biomass, hydrogen peroxide presoaking prior to ammonia fiber expansion (H-AFEX) was applied as pretreatment to corn stalk. Enzymatic hydrolysis using cocktail enzymes including cellulase, β-glucosidase and xylanase at 72 h after pretreatment under optimal conditions, the glucan and xylan conversions of 88.9% and 86.3% were achieved, respectively. It was about 3.31-fold in sugar yield for H-AFEX-treated corn stalk compared with untreated material. The results of composition analysis and enzymatic hydrolysis showed that H-AFEX pretreatment was effective to remove lignin and improve glucan digestibility. The characteristics of biomass surface and cell wall, biomass crystallinity, and chemical structure changes of H-AFEX-treated corn stalk were determined by digital microscope system, X-ray diffraction and Fourier transform infrared spectroscopy (FTIR), respectively. The results demonstrated that H-AFEX pretreatment induced some morphology changes including partial damages in vascular bundle and deconstruction of cell wall, and the modified biomass structure could increase surface area accessibility and create favorable conditions for enzymatic hydrolysis obviously. Due to removal amorphous substances, the crystallinity index of H-AFEX-treated corn stalk increased comparing with that of raw material. FTIR data showed that H-AFEX process induced changes in chemical structure and cross linking such as removal/dissolution of lignin and hemicelluloses, cleavage of bonds linkage, and decrystallization of cellulose. As a result, H-AFEX pretreatment effectively reduced recalcitrance of corn stalk, and promoted subsequent enzymatic hydrolysis.

1. Introduction

Biomass pretreatment is a critical processing step for bio-ethanol and bio-based material production with high sugar yield via bioconversion route. The role of pretreatment is to overcome the recalcitrance of lignocellulosic biomass through altering or removing structural and composition impediments to enzymatic hydrolysis and making high sugar yield possible (Mosier et al., 2005; Yang and Wyman, 2008). Over last few decades, a variety of physical, chemical, thermochemical and biological methods pretreating lignocellulosic biomass have been suggested for technical and/or economical effectiveness (Alvira et al., 2010). Among these different existing methods, thermochemical pretreatments are promising to industrialization and commercialization due to less chemical requirements, shorter pretreatment time, lower energy consumption, and higher sugar yields.

Thermochemical pretreatment, such as ammonia fiber expansion (AFEX) has been shown to be promising method to overcome the recalcitrance of lignocellulosic biomass for enzymatic hydrolysis. However, the AFEX pretreatment represents somewhat unique in that it without removing lignin or hemicelluloses (Bals et al., 2012; Teymouri et al., 2005). Some studies reported that lignin and/or hemicellulose are the main factors which affect enzymatic hydrolysis, especially lignin removal is emphasized as a basic requirement for pretreatment. Some researchers showed that the addition of hydrogen peroxide can modify cell wall structure and improve lignin removal toward favoring enzymatic digestibility (Studer et al., 2011; Yu et al., 2014; Zeng et al., 2014). Therefore,

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the pretreatment of presoaking in hydrogen peroxide solution prior to ammonia fiber expansion (H-AFEX) has been used for bamboo and corn stover in our laboratory, which leads to enhance lignin removal and achieve high enzymatic digestibility (Shao et al., 2013; Zhao et al., 2014a). Though significant strides have been made in elucidating the mechanism of AFEX (Chundawat et al., 2011a,b), such data is still lacking for H-AFEX pretreatment. It is vital to understand how H-AFEX process changes treated biomass characteristics and their impacts on following enzymatic hydrolysis. Examining the H-AFEX-treated substrate in tandem with physical and chemical features should switch light on fundamental mechanisms which contributing to the recalcitrance of lignocellulosic biomass. Some researchers using SEM micrographs and XRD crystallinity measurements reported that improving the surface area accessible to cellulase is more important than lignin removal for achieving a high sugar yield (Rollin et al., 2011). Fourier transform infrared spectroscopy (FTIR) was used to investigate the changes of cellulose structures and characteristics of lignin during pretreatment (Gabov et al., 2014; Ma et al., 2015).

In this study, H-AFEX pretreatment of corn stalk was investigated. The effects of reaction temperature and chemical loading on solid recovery, composition changes, and enzymatic hydrolysis were evaluated. To examine fundamental mechanism of H-AFEX pretreatment which contributing to recalcitrance of lignocellulosic biomass, physical and chemical characterizations of H-AFEX-treated substrate were assessed by digital microscope system, XRD and FTIR spectroscopy.

2. Methods

2.1. Materials and chemicals

Corn stalk was kindly provided by Shi'an farm in Mianyang (31.23, 104.43), Sichuan Province, China. It was planted in April 2013 and harvested in August 2013 (about 120 d from sowing to harvesting). Following harvest, corn stalk was stripped from leaves, husks, and ears. Air dried raw material was cut to 3–5 cm in length, and then further drying by a 40 °C oven for about 24 h. Afterwards, the samples were milled through 40 mesh screen sieve using a small mill machine (FZ102, Taisite Instrument Co., Ltd., Tianjin, China), and then kept at −20 °C in plastic bags until further use.

For better illuminating the influence of H-AFEX pretreatment on micro-structural changes in different tissues of corn stalk, air-dried corn stalk was manually fractionated into three different tissues: rind, pith and vascular bundle. Then, the different tissues were individually cut to 0.3–0.5 cm in length. The drying and storage of different tissues were in accordance with corn stalk.

The cellulase and β-glucosidase were purchased from Novozymes Investment Co. Ltd. (China). The average activity of cellulase and β-glucosidase were 75 filter paper unit (FPU)/mL and 250 cellobiase units (CBU)/mL, respectively. Xylanase was purchased from Shandong Zesheng Bioengineering Technology Co. Ltd. (China).

Sulfuric acid (98 wt%) using for acid hydrolysis of composition determination was purchased from Minxing Chemical Co. Ltd. (Zhejiang, China). Two pretreatment reagents, hydrogen peroxide solutions (30 wt%) and ammonia (99 wt%) were purchased from Tongsheng Chemical Co. Ltd. (Jiangsu, China) and Longsan Chemical Co. Ltd. (Zhejiang, China), respectively. Glucose, xylose and arabinose were purchased from Sigma-Aldrich Chemical Co. Ltd. (Shanghai, China), using as reference substances for high performance liquid chromatographic (HPLC) analysis. All chemicals used were of standard analytical grades and used as received without further purification.

2.2. H-AFEX pretreatment

The H-AFEX pretreatment of corn stalk was performed in 1-L high-pressure reactor as outlined by Shao et al. (2013). Corn stalk (20 g dry biomass) was presoaked using hydrogen peroxide solution (30 wt%) at room temperature, then premixed with deionized water until to 0.7 water loading (the mass ratio of water to dry biomass). After equilibrating for 30 min, the wet stalk was placed into the high-pressure reactor and sealed. The amount of 1.0 ammonia loading (the mass ratio of ammonia to dry biomass) was charged containing biomass, then the reactor was heated rapidly to desired temperature and held for 10 min. Afterwards, the treated corn stalk was taken out from reactor, and then dried by an oven at 40 °C for about 24 h. Then, the samples were kept at −20 °C in plastic bags until further use.

The pretreatment variables were reaction temperature and hydrogen peroxide loading. The temperature was set at 90, 110, and 130 °C, respectively; and the H_2O_2 loading was set at 0.1, 0.4, and 0.7 (the mass ratio of 30 wt% hydrogen peroxide solution to dry biomass), respectively. The selection and range of variables were determined by previous study results from our laboratory.

The solid recovery of treated biomass was calculated as the following equation:

$$S_r(\%) = \frac{m_1}{m_2} \times 100\% \quad (1)$$

where S_r is solid recovery (%), m_1 is the total mass of pretreated biomass (g, dry basis), m_2 is the total mass of feedstock (g, dry basis).

Based on experimental results of corn stalk pretreatment, the H-AFEX pretreatments of different tissues were processed using optimal conditions. The collection and drying of treated different tissues were in identical to corn stalk mentioned above.

2.3. Composition analysis

The carbohydrates (sugars) and lignin were determined using two-step acid hydrolysis of standard protocol created by NREL (National Renewable Energy Laboratory) (NREL, 2010). Sugars were quantified by HPLC (high performance liquid chromatography) as described in Section 2.5. Biomass moisture content was measured by a moisture analyzer (Sartorius, Model MA35; Beijing, China). The average of duplicate runs was used in reporting.

2.4. Enzymatic digestibility tests

H-AFEX-treated biomass was hydrolyzed in 20 mL screw-cap vials at a total volume of 15 mL. All hydrolysis were started at 1% glucan loading, using 0.05 M citrate buffer solution to adjust pH 4.8. To prevent bacteria growth during enzymatic hydrolysis, tetracycline and cycloheximide were loaded at 40 mg/L and 30 mg/L, respectively. For all hydrolysis experiments, the standard enzyme loading was used: 15 FPU/(g glucan) of cellulase, 64CGU/(g glucan) of β-glucosidase, and 1000 IU/(g glucan) of xylanase. The time which adding enzymes were marked at the beginning of enzyme hydrolysis (0 h). Enzymatic hydrolysis was conducted in a shaking incubator (ZHWY-111B, Zhicheng Co. Ltd., Shanghai, China) at 50 °C and 150 rpm. Samples were taken at 72 h for monosaccharide analysis by HPLC as detailed in Section 2.5. The supernatant was filtered into HPLC vials using a polyethersulfone syringe filter (25 mm, 0.2 μm), then frozen at −20 °C. All the experiments were performed in duplicate.

2.5. HPLC analysis

Sugars in hydrolyzates from composition analysis and enzymatic hydrolysis samples were determined using a chromato-