Contents lists available at ScienceDirect

Food and Bioproducts Processing

journal homepage: www.elsevier.com/locate/fbp

Fractional pretreatment of lignocellulose by alkaline hydrogen peroxide: Characterization of its major components

Yapeng Su[a,b,1], Ruoyu Du[a,b,1], Hong Guo[a,b], Ming Cao[a,b], Qinfeng Wu[a,b], Rongxin Su[a,b,*], Wei Qi[a,b], Zhimin He[a,b]

[a] State Key Laboratory of Chemical Engineering, Tianjin Key Laboratory of Membrane Science and Desalination Technology, School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, PR China
[b] Collaborative Innovation Center of Chemical Science and Engineering (Tianjin), Tianjin 300072, PR China

ABSTRACT

The biorefinery process for lignocellulose conversion generally requires fractionation of its three major components including cellulose, hemicellulose, and lignin. The present work demonstrates that the alkaline hydrogen peroxide (AHP) method for fractionating lignocelluloses, such as corncob, is advantageous due to its modest reaction conditions, low alkaline usage, and effective fractionation of three components. The removal ratio of lignin and hemicellulose after 6 h treatment reached 75.4% and 38.7%, respectively, leaving the recovery ratio of cellulose in the AHP residues at 81.3%. After 24 h enzymatic hydrolysis of the AHP residues, we found that the degree of cellulose conversion was approximately 80%. Through ethanol precipitation and desalination, 89% of dissolved hemicellulose was recovered as white solids, whereas 77.5% of soluble lignin was obtained as brown solids. The isolated three components were further characterized. The major pyrolysis temperature of the AHP residues increased. And the recovered lignin revealed an increase in carboxylic acid content and a decrease in phenolic hydroxyl content by oxidation of H_2O_2.

Keywords: Lignocellulose; Fractionation; Alkaline hydrogen peroxide; Pretreatment; Characterization

1. Introduction

The depletion of fossil fuel resource and the increasing demand for bioethanol are driving the surge in lignocellulose production; agricultural and forestry wastes are now viewed as potential sources of renewable carbohydrates for energy production (Hoogwijk et al., 2003; McKendry, 2002). Based on the concept of "biorefinery" (Ragauskas et al., 2006), three constituents (i.e., cellulose, hemicellulose, and lignin) are required to be converted into high margin products for the profitable utilization of lignocellulose (Zhang, 2008). In addition to producing bioethanol from cellulose, the remaining fractions in the lignocellulosic biomass can be economical sources of valuable products such as health food additives, polymeric materials, glue, and carbon fiber (Doherty et al., 2011; Himmel et al., 2007) based on their different physical and chemical properties after separation and purification. In China, corncob is one of the most abundant agricultural residues among the various sources of lignocellulose, yielding a total of 20 million tons of discarded waste per year (Bai et al., 2008).

Lignocellulose is made up of complicated natural plant cell wall materials, such as cellulose, hemicellulose, and lignin,

---

* *Corresponding author at*: State Key Laboratory of Chemical Engineering, Tianjin Key Laboratory of Membrane Science and Desalination Technology, School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, PR China.
Tel.: +86 22 27407799; fax: +86 22 27407599.
E-mail address: surx@tju.edu.cn (R. Su).

Received 5 March 2013; Received in revised form 14 February 2014; Accepted 7 April 2014
Available online 18 April 2014

[1] Both these authors contributed equally to this study.

http://dx.doi.org/10.1016/j.fbp.2014.04.001

which intertwine together to form a recalcitrant structure (Himmel et al., 2007). Effectively disrupting the stubborn structure of lignocellulose and releasing the locked polysaccharides are the most significant and urgent R&D priorities for the emerging cellulosic ethanol and bio-based chemical industries.

To date, various fractionation and pretreatment processes, including organosolv (Zhang et al., 2010a), ionic liquid (Yang et al., 2013), concentrated phosphoric acid (Kim and Mazza, 2008; Zhang et al., 2007) and liquid hot water (LHW) (Bobleter, 1994), are used to break up the cell wall macromolecular complexes for lignocellulose fractionation. Among these pretreatments, acid pretreatments are mostly popular, but their inevitable toxic byproducts will inhibit greatly subsequent enzymatic hydrolysis and fermentation. Moreover, acid can degrade hemicellulose into mono- and oligosaccharides, which impede the production of solid hemicellulose. The hydrothermal pretreatments offer the advantages of utilizing hemicellulose and avoiding the usage of chemicals (Garrote et al., 1999), but they require harsh operating conditions. Lignocellulose fractionation performed in this work by using alkaline hydrogen peroxide (AHP) treatment. It is widely used in pulp and paper industry to bleach lignin-rich pulp, showing effective for both the delignification and the solubilization of hemicelluloses (Gould, 1985), with modest reaction conditions and low usage of alkaline. It can also provide bleaching action on the final solid products.

This study proposes a new method for lignocellulose fractionation based on a revised AHP pretreatment technology to obtain solid cellulose, hemicellulose, and lignin products. The key treatment conditions are optimized, and the mass balance of the whole process is evaluated to obtain the most effective operating conditions and to observe the changes in major components during the AHP pulping and subsequent separation procedures. The structures and other properties of the fractionated components are characterized to evaluate the potential applications of these polymers.

2. Materials and methods

2.1. Materials

The corncob used in this study was obtained from a local farm in Tianjin, China. The fraction between 20 mesh and 80 mesh was used in the experiments after premilling and screening. The initial composition of the dried corncob was 34.77% cellulose, 30.32% hemicellulose, and 21.63% lignin. All the chemicals used in the subsequent experiments were of analytical grade. The celllulase (Spezyme CP, 117 FPU/mL) derived from *Trichoderma reesei* was a generous gift from Genencor International (Palo Alto, CA, USA). The β-glucosidase (Novozyme 188) was purchased from Sigma (St. Louis, MO, USA).

2.2. Lignocellulose fractionation

2.2.1. Recovery of AHP residues

Oven-dried corncob was placed in a screw-capped laboratory bottle (Pyrex glass) and then mixed with 2% (w/w) H_2O_2 solution at a ratio of 1 g solid per 20 mL liquid. The 2% H_2O_2 solution was prepared by diluting 33 mL 30% (w/w) hydrogen peroxide in 500 mL distilled water. We adjusted the pH to 11.5 with 5 M sodium hydroxide. The solid/liquid slurry was incubated in a shaking water bath at 120 rpm and 50 °C. After the desired reaction time, the mixture was filtered. The solid residues were thoroughly washed with distilled water until the pH of the washes became neutral. The residues were oven-dried at 60 °C, and then weighed. The resulting solid is referred to here as the AHP residue, which consists mainly of cellulose.

2.2.2. Recovery of hemicellulose and lignin

The yellow liquor filtrate obtained from the filtered mixer during the pretreatment procedure was adjusted to pH 5.5 with 6 M hydrochloric acid, after which three volumes of 95% ethanol were added and incubated statically for 8 h to precipitate the hemicellulose solid. The corresponding supernatant containing the majority of the soluble lignin was carefully transferred to another breaker and concentrated to four times the original concentration, and pH was adjusted to 1.5 to precipitate the acid-insoluble lignin. The acid-insoluble lignin was isolated by filtration and purified by washing three times with acidified water (pH 2.0). Afterward, the lignin preparations were freeze-dried and weighed. The collected hemicellulose pellets were washed with 70% aqueous ethanol to eliminate the salts produced during the neutralization process. The pellets were then air-dried and weighed. All solid samples were stored at −20 °C for further analysis.

The ethanol used in the whole process was recovered by distillation for reuse. Sodium chloride, as a byproduct, could also be obtained through the recycling operation. The scheme for the fractionation of lignocellulose using AHP is shown in Fig. 1.

2.2.3. Calculations

The fractionation process was evaluated using the following formulas:

$$R_{\text{removal},x}(\%) = 1 - \frac{W_{\text{AHP},x}}{W_{\text{raw},x}} \quad (1)$$

$$R_{\text{recovery},x}(\%) = \frac{W_{\text{AHP},x}}{W_{\text{raw},x}} \quad (2)$$

2.3. Enzymatic hydrolysis of AHP residues

Firstly, 3 g of AHP residues were mixed with 50 mM citrate buffer (pH 4.8) in 50 mL Erlenmeyer flask. Enzymatic hydrolysis was carried out in an air wave bed at 50 °C and 150 rpm. The enzyme loadings were cellulase 50 FPU/g glucan supplemented with β-glucosidase 30 CBU/g glucan. After enzymatic hydrolysis, the samples were collected and centrifuged (5000 rpm, 10 min). Then, the supernatant was drawn to determine the soluble sugars. The residual pellets were oven-dried to measure their constituents.

2.4. Analytical methods

The composition of raw materials, the solid residues after AHP treatment and enzymatic hydrolysis residues were analyzed according to the NREL procedure LAP-002. The soluble sugars (glucose, xylose) were measured by high-performance liquid chromatography (HPLC) system equipped with a refractive index detector and an Aminex HPX-87H column (Bio-Rad, USA) using 0.005 M H_2SO_4 as the mobile phase at 65 °C and at a flow rate of 0.6 mL/min.