Industrial Crops & Products 109 (2017) 391–397

Contents lists available at ScienceDirect

Industrial Crops & Products

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

Research paper

Comparison of alkaline and acid pretreatments for enzymatic hydrolysis of soybean hull and soybean straw to produce fermentable sugars

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ARTICLE INFO

Keywords:
Soybean hull
Soybean straw
Dilute acid pretreatment
Alkaline pretreatment
Enzymatic hydrolysis

ABSTRACT

The specific characteristics of biomass structure and differences in chemical composition of soybean hull (SBH) and soybean straw (SBS) may result in different behavior of pretreatment and enzymatic hydrolysis. In this work, dilute sulfuric acid (DA) pretreatment and alkaline sodium hydroxide (AL) pretreatment in a range of pretreatment temperatures and durations were investigated to improve the enzymatic digestibility of SBH and SBS. Satisfactory enzymatic digestibility was observed in the hydrolysis of both pretreated soybean fractions, but SBH showed higher digestibility than that of SBS, no matter which pretreatment technology was applied. Furthermore, DA pretreatment was more effective than AL pretreatment in conversion of soybean fractions into fermentable sugars, if both pretreatment and enzymatic hydrolysis stages were considered. The highest total sugar yield of SBH and SBS using DA pretreatment and subsequently hydrolyzed with 30 FPU/g-DM cellulase were 86.9% and 70.3%, respectively.

1. Introduction

Soybean is an important source of food in many countries, especially in many Asian countries (Cabrera et al., 2015). The oil and protein constituents of soybeans are regarded as valuable products in soybean processing, but little attention has been paid to the producing residues, such as soybean hulls and straws (Cassales et al., 2011). These residues are rich in cellulose and do not require an extra grinding process prior to pretreatment as some other lignocellulosic material. As a major food and energy crop in China, soybean has an annual production of 13 Tg. Soybean straws and hulls, collected after soybean harvest, are partially used for animal feed and the main part of these residues are burnt directly in the fields, causing serious environmental pollution. Therefore, exploiting the potential utilization of soybean carbohydrates can not only increase the added value of soybean industry, but also benefit to environmental protection (Corredor et al., 2008).

Soybean hull and straw are basically lignocellulosic material composed of fermentable hextose and pentose sugars, polymerized as cellulose and hemicellulose, in addition to a small proportion of lignin that is composed of phenolic compounds. Therefore, the efficient hydrolysis of cellulose and hemicelluloses in soybean residues to liberate monomer sugars is of potential interest for conversion into biofuels and chemicals with economic interest. However, the complex network of lignocellulosic biomass presents a major resistance for degradation of polysaccharide fractions into soluble monomeric sugars, which is commonly referred to as the recalcitrance barrier (Himmel et al., 2007; Behera et al., 2014). Consequently, the saccharification efficiency is extremely low unless a suitable pretreatment conducted before enzymatic hydrolysis to break down the lignin and hemicelluloses network and to disrupt the crystalline cellulose structure. Among existing pretreatment technologies, dilute acid pretreatment (DA) and alkaline pretreatment (AL) are believed as the most mature ones that are ready for commercialized application (Lee et al., 2015; Alvira et al., 2010), which also have shown high effectiveness on several agricultural residues (Dagninoa et al., 2013).

In DA pretreatments, substantial amounts of sugars, mainly from hemicellulose and partially from cellulose, could be solubilized into the liquid phase of the hydrolysis slurry (Jung et al., 2013). Dilute sulfuric acid, which has been extensively investigated for pretreatment using experimental and theoretical approaches, can effectively degrade hemicellulose in the cell wall network by the catalytic effect of proton H^+ (Chen et al., 2015). It was reported that over 90% of hemicellulose could be successfully removed during DA pretreatment under moderate severities, remaining highly digestible residue that mainly composed of cellulose with small part of lignin (Singh et al., 2015). However, due to the strong protonation effect, the monomer sugars obtained during acid pretreatment are ready to be further decomposed into their degradation forms, such as furfural, 5-hydroxymethyl furfural (5-HMF), levulinic acid, and even humins, leading to strong inhibition to enzymes and

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http://dx.doi.org/10.1016/j.indcrop.2017.08.051
Received 11 May 2017; Received in revised form 14 August 2017; Accepted 28 August 2017

fermentation microorganisms.

On the other hand, AL pretreatment is considered as the most effective one with less sugar degradation, lower energy consumption, more lignin removal, and less furan derivatives (Behera et al., 2014; Whitfield et al., 2012). Compared to DA pretreatment, AL methods exhibit higher capacity in breaking the linkages between lignin and carbohydrates, and disrupting lignin structures, with a minor cellulose and hemicelluloses alteration. Acetyl groups and various uronic acid substitutes, which have lower susceptibility to hydrolytic enzymes, are also eliminated by AL pretreatment (Mosier et al., 2005; Zheng et al., 2009). More importantly, with the salvation reactions, the AL pretreatment could swell the material, thus increase the internal surface area of the recovered solids and the accessibility of the enzymes (Jin et al., 2013).

In this study, soybean residues including SBH and SBS, as renewable and low-cost lignocellulosic materials for the production of fermentable sugars were investigated. DA and AL pretreatments were performed separately on different soybean residue fractions. Then the digestibility of different pretreated soybean fractions was evaluated with various enzyme dosages, in addition to determination of the structural characteristics and composition changes of the pretreated solids. The impacts of the intrinsic structure differences between SBS and SBH, and different pretreatment methods on the digestibility of the pretreated solids were therefore compared and elucidated.

2. Materials and methods

2.1. Material

SBH and SBS were separated after soybean crops collected from the farmland nearby Changzhou (Jiangsu Province, China). The materials were washed with deionized water and dried at 45 °C until constant weight, then milled into size smaller than 3 mm. The composition of the raw and pretreated substrates were determined based upon National Renewable Energy Laboratory Analytical Procedure (Sluiter et al., 2008). Accellerase 1500 (96 FPU/mL) was generously provided by Genencor (Wuxi, Jiangsu province, China). Novozyme 188 (066K0676603) was purchased from Sigma (St. Louis, MO, USA). Sodium hydroxide (NaOH), sulfuric acid (H_2SO_4) and other chemical reagents were purchased from Sinopharm Group Chemical Reagent Co. Ltd. (Shanghai, China).

2.2. Pretreatment

The quantitative dried sample was mixed with 1 w/w% H_2SO_4 or NaOH solution in a 100 mL high-temperature and high-pressure stainless steel reactor (Zhenjiang Dantu Universal Electrical Equipment, China), equipped with a stirring apparatus. The solid to liquid ratio was set to 1: 20. Then the reactor was sealed and electrically heated to the desired temperature using a porcelain-heating jacket. After the reaction, the reactor was quenched in an iced water bath, then filtrated to separated the liquid from the solid. The collected solid was washed for several times until pH neutral. The pretreatment liquid and solid were collected for different analyses. The solid recovery, delignification, and hemicellulose removal of different pretreatments were calculated based on the following equations:

$$\text{Solid recovery}(\%) = \frac{\text{substrate recovered after pretreatment (g)}}{\text{substrate used for pretreatment (g)}} \times 100\%$$

$$\text{Delignification}(\%) = \left(1 - \frac{\text{lignin inpretreated samples (g)} \times \text{SR}\%}{\text{lignin in one gram native samples (g)}}\right) \times 100\%$$

$$\text{Hemicellulose removal}(\%) = \left(1 - \frac{\text{hemicellulose inpretreated samples (g)} \times \text{SR}\%}{\text{hemicellulose inone gram native samples (g)}}\right) \times 100\%$$

2.3. Enzymatic hydrolysis

Saccharification of the untreated and pretreated samples were performed following the NREL laboratory analytical procedure (Selig et al., 2008). A solid loading of 2% (w/v) with 0.05 M acetate buffer (pH 4.8) were added in 50 mL Erlenmeyer flasks. 800 μg of 20 mg/mL tetracycline antibiotic in DI water was also supplemented before adding enzymes to prevent possible microorganism contamination during hydrolysis. Enzymes were added once the mixture reached 50 °Cand the slurry was stirred at 160 rpm in a thermostated shaker (Model # THZ-072HT, Shanghai, China). Samples were taken after 1, 4, 24, 48, and 72 h of hydrolysis and immediately analyzed with a high performance liquid chromatography (HPLC) to determine the monomer sugar yields. All enzymatic hydrolysis samples were prepared in triplicates and run under parallel conditions.

2.4. Analytical method

2.4.1. Sugar analysis

Samples were analyzed for carbohydrates using a Waters Alliance HPLC (Model 2695, Waters Corporation, Milford, MA), equipped with an Aminex HPX-87H column (Bio-Rad Laboratories, Hercules, CA) run at 65 °C and a refractive index detector (Waters 2414). The sample injection volume was 20 μL and 0.005 M sulfuric acid at a flow rate of 0.6 mL/min was set for the mobile phase. The concentration of various monomeric sugars, furfural, HMF, and levulinic acid were quantified based on the calibration curves constructed by standards. The monomer sugar yields were calculated according to the following equations, and the total sugar yields were the sum of monomer sugars and cellobiose.

$$\text{Glucose yield}(\%) = \frac{\text{glucose released} \times 0.9\ \text{(g)}}{\text{intial glucan content in the substrate (g)}} \times 100\%$$

$$\text{Xylose yield}(\%) = \frac{\text{xylose released 3} \times 0.88\ \text{(g)}}{\text{intial xylan content in the substrate (g)}} \times 100\%$$

2.4.2. Sample characterization

Crystallinity index (CrI) was analyzed by X-ray diffraction (XRD) using a D/max 2500 PC diffractometer with Cu Ka radiation (Rigaku Corporation, Tokyo, Japan) and operated at a voltage of 60 kV and a current of 300 mA. The 2θ range was detected from 5 to 40°in a step of 0.02. CrI was calculated according to the following equation:

$$\text{CrI}\ (\%) = \frac{I_{002} - I_{am}}{I_{002}} \times 100\%$$

Where I_{002} is the intensity of the crystalline portion (crystalline cellulose) determined at 2θ = 22.2°and I_{am} is the peak for the amorphous portion (i.e. amorphous cellulose, hemicellulose and lignin) at 2θ = 16.4°.

The surface morphological features of different samples were imaged with a scanning electron microscope (SEM, Model # JSM-6360LA, JEOL, Japan) that was operated at 15 kV. Fourier transform infrared (FTIR) was to verify the change of the chemical structure of SBH and SBS before and after pretreatment. The spectra were analyzed using a FTIR spectrometer (Nicolet, USA). The sample spectra were obtained using 32 scans over the range of 500–4000 cm^{-1}. Samples were ground and mixed with the spectroscopic grade KBr then pressed in a standard device to produce diameter pellets (Qing et al., 2016). The porosity of the samples was determined by N_2 adsorption/desorption isotherms at 77 K using a Brenauer-Emmett-Teller (BET) surface area analyzer (ASAP 2010 M). Before the measurement, all samples were dried at