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Enzymatic saccharification of high pressure assist-alkali pretreated cotton stalk and structural characterization

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ABSTRACT

Cotton stalk is a potential biomass for bioethanol production, while the conversion of direct saccharification or biotransformation of cotton stalk is extremely low due to the recalcitrant nature of lignocellulose. To enhance the enzymatic conversion of cotton stalks, the enzymatic saccharification parameters of high pressure assist-alkali pretreatment (HPAP) cotton stalk were optimized in the present study. Results indicated that a maximum reducing sugar yield of 54.7 g/100 g dry biomass cellulose was achieved at a substrate concentration of 2%, 100 rpm agitation, 0.6 g/g enzyme loading, 40 °C hydrolysis temperature, 50 h saccharification time, and pH 5.0. Scanning electron microscopy, X-ray diffraction, and Fourier transform infrared spectroscopy were used to identify structural changes in native, pretreated biomass and hydrolyzed residues. Structural analysis revealed large part of amorphous cellulose and partial crystalline cellulose in the HPAP cotton stalk were hydrolyzed during enzymatic treatment. HPAP cotton stalk can be used as a potential feed stock for bioethanol production.

1. Introduction

Lignocellulosic biomass, including agricultural residues such as cotton stalk, corn stover, wheat straw, rice straw and soybean residue, is an inexpensive and abundant resource that is considered a major potential and renewable energy source. These materials contain over 65% cellulose and hemicelluloses and can be hydrolyzed into monomeric sugars, such as glucose and xylose, which can be further converted into bioethanol and other industrial products (John, Nampoothiri, & Pandey, 2007; Singh, Tuteja, Singh, & Bishnoi, 2011). A promising technology involves conversion of this abundant and renewable biomass into ethanol through an enzyme-based process (Hasunuma et al., 2013). Over the last few decades, conversion of lignocellulosic materials into biofuels, such as methane, bioethanol, and biodiesel, has gained worldwide attention (Biswas, Teller, & Ahring, 2015; Biswas, Uellendahl, & Ahring, 2015; Hahn-Hägerdal, Galbe, Gorwa-Grauslund, Lidén, & Zacchi, 2006; Lin & Tanaka, 2006; Mood et al., 2013; Singh et al., 2011). As a significant advantage, lignocellulosic biomass does not contribute to the net rise in CO_2 level in the atmosphere and, consequently, to the greenhouse effect (Lin & Tanaka, 2006; Mood et al., 2013; Sun & Cheng, 2002).

Prior to biofuel production, lignocellulosic biomass must be saccharified into glucose or other fermentable sugars. Acidic and enzymatic saccharification are two well-known saccharification processes. Enzymatic saccharification provides better yields without side-product generation; thus, this process is believed to be the more promising technology of the two procedures (El-Zawawy, Ibrahim, Abdel-Fattah, Soliman, & Mahmoud, 2011). However, enzymatic saccharification can be influenced by the structural features of the substrate, including its cellulose crystallinity, degree of cellulose polymerization, structural composition, and available surface area, enzyme activity, and reaction conditions (Behera, Arora, Nandhagopal, & Kumar, 2014; Mood et al., 2013). Therefore, prior to enzymatic saccharification, pretreatment of lignocellulosic biomass is usually employed. Pretreatment processes alter the structure and composition of the lignocellulosic biomass and render the lignocellulosic feedstock more susceptible to enzyme activity (Behera et al., 2014; Sun & Cheng, 2002).

Cotton stalk, one of the most abundant renewable cellulose resources in China, consists of 32–46% cellulose, 20–28% hemicelluloses and 15–26% lignin. A recent report indicated that the amount of cotton stalk residues generated annually exceeds 40 million tons in China (Du et al., 2013). The high cellulose content of cotton stalk makes it an attractive feedstock for bioethanol production

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(Kaur, Oberoi, Bhargav, Sharma-Shivappa, & Dhaliwal, 2012). However, direct saccharification or biotransformation of cotton stalk is extremely difficult because of the close connection of cellulose and hemicellulose with lignin in the plant cell wall. Effective conversion of lignocellulosic biomass to bioethanol or other value-added products involves three major steps, namely, pretreatment, enzymatic saccharification, and fermentation. Pretreatment is an important step in lignocellulosic ethanol production (Behera et al., 2014). Among the pretreatment methods currently employed, alkali treatment appears to be the most effective for breaking ester bonds between lignin, hemicellulose, and cellulose while limiting degradation of hemicellulose polymers. Thus, this method is suitable for pretreatment of agricultural residues and herbaceous crops (El-Zawawy et al., 2011; Mood et al., 2013). Alkaline pretreatment was recently successfully used to utilize cotton stalks for generating value-added products (Binod et al., 2012; Du et al., 2013; Kaur et al., 2012; Vani et al., 2012).

Numerous research studies demonstrate that the efficiency of enzymatic saccharification of pretreated lignocellulosic biomass depends on several process factors, such as substrate concentration, enzyme loading, reaction time, pH, reaction temperature, surfactant addition, and so on (Mood et al., 2013; Qi, Chen, Shen, Su, & Wan, 2009; Singh & Bishnoi, 2012; Sun & Cheng, 2002). Therefore, optimization of the saccharification process is one of the most important stages in the development of an efficient and cost-effective saccharification strategy. The conventional optimization technique is to deal with one-factor at a time. However, this optimization approach is time-consuming and also does not reveal the interactions between independent variables. Response surface methodology (RSM) is an effective optimization tool in which various factors and their interactions affecting the response can be identified with fewer experimental trials (Myers & Montgomery, 2002). RSM has been successfully used to model and optimize various steps of lignocellulosic biomass bioprocessing, such as pretreatment, cellulase production, enzymatic hydrolysis, fermentation, or simultaneous saccharification and fermentation (Avci, Saha, Dien, Kennedy, & Cotta, 2013; Ferreira, Duarte, Ribeiro, Queiroz, & Domingues, 2009; Hari Krishna & Chowdary, 2000; Kim, Oh, Shin, Eom, & Kim, 2008). In addition, in order to get the data for RSM, Plackett–Burman design (PBD) is normally used to arrange the experiments from which the variables having significant influence on the dependent variable are selected, together with the optimum value of each variable. And then, Central composite design (CCD) is used to arrange the experiments in which only most important variables and their optimum values are selected according to the results from PBD. Finally, the data obtained from CCD is subjected to RSM to get the optimum conditions for getting the highest value of dependent variable under the consideration of interaction between all selected variables in CCD.

In this study, hydrolysis of high pressure assist-alkali pretreatment (HPAP) cotton stalk using commercial cellulase was investigated in detail using RSM. Factors affecting enzymatic hydrolysis were sequentially optimized by PBD and CCD. Saccharification residues were assessed by scanning electron microscopy (SEM), X-ray diffraction (XRD), and Fourier transform infrared spectroscopy (FT-IR). The aim of the present study is to optimize the saccharification conditions of cotton stalks to improve their utilization efficiency and to reveal structure difference of native, pretreated biomass, and hydrolyzed residue.

2. Material and methods

2.1. Pretreatment of lignocellulosic biomass

Cotton stalks provided by the Institute of Cotton Research of CAAS were air-dried to moisture of 8–9%, cut into 1–2 cm lengths, and milled to approximately 0.7 mm particle size using a sawtooth mill. Ground cotton stalk was pretreated with NaOH at a concentration of 3.0% (w/v), pressure of 130 kPa (125 °C), and liquid/solid ratio of 20:1 for 40 min (Du et al., 2013). The mixtures were placed in a closed conical flask and treated with alkali. Pretreatment was performed using a commercial autoclave (ES-315, Tomy Kogyo Co., Ltd., Japan). After pretreatment, the contents were filtered through a Büchner funnel lined with Whatman filter paper, and residues were collected and repeatedly washed with distilled water to pH 7. The neutralized alkali-treated cotton stalk powders were dried at 50 °C to constant weight and used as the substrate for saccharification experiments and compositional analysis.

2.2. Enzymatic saccharification of cotton stalk

Enzymatic saccharification of untreated or treated cotton stalks was carried out using commercial cellulase (activity, 60 ± 3.1 FPU/g) from Shanghai Boao Biotech. Corp., China. The conditions of enzymatic saccharification were carried out according to the design plan provided in Tables 1 and 2. After hydrolysis, the samples were centrifuged at $3000 \times g$ for 10 min to remove unhydrolyzed residues. The reducing sugar content of the supernatant was determined using the 3,5-dinitrosalicylic acid (DNS) method (Miller, 1959). Reported values were corrected for the sugar contribution from enzyme mixture as found in the blanks. Results are expressed as grams of glucose per 100 g of dry biomass cellulose.

2.3. Experimental design and data analysis for hydrolysis

2.3.1. Plackett–Burman design

PBD is an efficient mathematical approach used to determine the most significant variables in any study; it offers fast screening, mathematically computes the significance of a large number of factors in one experiment, which is time saving, and gives the effect of change in more than one factor in a single experiment (Qi et al., 2009). Six independent variables, namely, substrate concentration (g/L), enzyme loading (g/g), hydrolysis temperature (°C), hydrolysis time (h), agitation (rpm), and pH, were investigated by PBD in the present study. Each variable was set at a high level (+1) and a low level (−1). The experimental design is provided in Table 1. Each experiment was repeated twice with the mean considered as the response.

2.3.2. Central composite design

On the basis of the results of the PBD experiment, significant factors, including hydrolysis temperature, enzyme loading, pH, and hydrolysis time, were selected as major variables for further study. The levels of these major variables were selected according to the results obtained from PBD, taking the required experimental conditions and literature into consideration. The coded and uncoded values of these major variables are provided in Table 2. Substrate concentration, buffer solution, and agitation were set at 2% (w/v), sodium acetate buffer, and 100 rpm, respectively.

CCD was used to investigate the significance of enzyme loading, hydrolysis temperature, pH, and hydrolysis time on the saccharification process. A five-level, four-factor factorial CCD was designed by Design-Expert 8.0.6 software (Stat-Ease Inc.). The experimental design plan is shown in Table 2, with four variables at five coded levels (−2, −1, 0, +1, +2) resulting in 30 combinations. A total of 30 experiments, including 16 for factorial design, 8 for axial points, and 6 repetitions at the central point, were performed. All experiments were carried out in duplicate and mean values were presented. Results were analyzed using Design Expert 8.06. A polynomial quadratic equation was used to evaluate the