Full Length Article

Comparative study of two different alkali-mechanical pretreatments of corn stover for bioethanol production

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ABSTRACT

Biomass pretreatment is still a major challenge for improving bioethanol production. The performance of two alkaline-mechanical pretreatments, alkaline twin-screw extrusion (ATSE) pretreatment where both alkaline treatment and refining occur simultaneously and alkaline pretreatment followed by PFI refining (APR), was compared in pretreating corn stover for fermentable sugar production at the same pretreatment conditions: 99 °C for 1 h with alkali to biomass ratio of 0.06 g NaOH/g oven-dry corn stover and a 1:2 biomass-to-liquid ratio. The enzymatic hydrolysis efficiency of glucan and total sugar yield of ATSE-treated samples was 87.47% and 78.75% compared to 64.80% and 54.83% of APR-treated samples (alkaline pretreatment with the supplement of 10000 revolutions refining) with a cellulase loading of 20 FPU/g for 48 h. X-ray diffractometer (XRD), and scanning electron microscopy (SEM) explained why ATSE pretreatment obtained the satisfied total sugar yield with low temperature and high biomass/liquid ratio and also demonstrated ATSE pretreatment was an effective method for improving enzymatic hydrolysis rate of corn stover.

1. Introduction

With the depletion of fossil energy, the exploration and utilization of feasible pathways for the conversion of abundant and renewable biomass to biofuels is highly desirable. In comparison with fossil energy, the usage of biofuels not only reduces greenhouse gas emissions but also enhances rural economy and national energy security [1–5]. Due to the properties of abundant availability and low-cost, corn stover has been regarded as one of the most important raw materials to produce biofuels in some countries. As is well known, enzymatic saccharification is an environmentally friendly process to produce fermentable sugars, but enzymatic conversion efficiency is limited by the natural recalcitrance of complex plant structure. In natural plants, cellulose is protected and sheathed by lignin and hemicellulose, and lignin can also be covalently linked to carbohydrates, which make cellulose difficult to access by cellulase enzymes [6,7]. Therefore a pretreatment process of lignocellulosic materials is essential to alter the structure of cellulosic biomass to make cellulose more accessible to the enzymes that would convert the carbohydrate polymers into fermentable sugars [8].

A large number of pretreatment approaches such as physical (comminution, grinding and milling), chemical (alkali, acid, oxidizing agents, and organic solvents), physic-chemical (steam explosion, ammonia fiber explosion, SPORL (Sulfite Pretreatment to Overcome Recalcitrance of Lignocellulose)), biological, or a combination of these pretreatment techniques have been investigated to improve the accessibility of enzymes to cellulosic fibers [9,10]. Generally, the physical pretreatments are not economically feasible due to the high energy consumption [11]. Acid pretreatment can remove hemicellulose and expose cellulose for enzymatic digestion, while it has to deal with the equipment corrosion problems, environment problems and higher concentration of inhibitors in hydrolysate such as furfural and 5-hydroxymenthyl furfural (HMF) [12]. On the contrary, alkaline processes have less sugar degradation, furan derivative formation is avoided and many of the caustic salts can be recovered. Due to the mild pretreatment conditions (low loading of NaOH and low temperature), small amount of enzyme inhibitors would be produced, which were verified by our previous work [13]. In addition, alkali pretreatments require lower temperature and produce relatively lower pressure [6,14,15]. Recovery of sodium hydroxide from black liquor is a mature technology in the pulp and paper industry, whereas the recovery of sodium hydroxide from a dilute alkaline solution remains a challenge to be addressed [16]. Four reaction agents including NaOH, KOH, Ca(OH)$_2$ and NH$_4$OH, had been tested by many researchers during alkaline pretreatments [17–20]. Among these four agents, NaOH is one of the most

effective alkaline reagents and has been widely used to treat a variety of lignocellulose feedstocks [21–25]. Silverstein et al. investigated the effectiveness of dilute NaOH pretreatment by analyzing the cellulose conversion and lignin removal, and reported that 60.8% cellulose conversion and 65.63% delignification were achieved after pretreatment with 2% NaOH at 121 °C for 90 min [26]. MacDonald et al. studied NaOH pretreatment of corn stover and reported that almost all the lignin in corn stover was dissolved and 80% of the potential glucose in raw biomass was recovered after pretreating using 2% alkali solution at 150 °C for 15 min [23]. It was also reported that, using 0.75% NaOH to treat Coastal Bermuda grass at 121 °C for 15 min, 86% lignin was removed, 71% total reducing sugar yields was obtained, and the overall conversion efficiencies for glucan and xylan were 90.43% and 65.11%, respectively [19].

Integration of chemical pretreatment and mechanical disruption would be a good method to overcome the above problems. Extruder, as a readily scalable equipment, has been widely used for different lignocellulosic biomass, resulting in subsequently high enzymatic hydrolysis rates. Our previous work employed the method of alkaline twin-screw extrusion (ATSE) to pretreat corn stover for fermentable sugar production with a biomass/liquid ratio of 1/2 (wt) and NaOH loading of 0.06 g/g biomass at a temperature of 99 °C without heating equipment. The conversions of glucan and xylan could reach up to 83% and 89%, respectively. In addition, 71% lignin removal was achieved [27]. However, the functions of physicochemical disruption and single alkali reaction were not fully differentiated. To further understand the fundamentals of ATSE pretreatment for improving enzymatic hydrolysis conversion of corn stover with low temperature, high biomass/liquid ratio and low dosage of alkali, we systematically compared ATSE pretreatment with alkaline pretreatment followed by PFI refining (APR) in this study. ATSE and APR are both physicochemical pretreatment methods, but the former is a continuous process. From the separated pretreatment method (APR), we could know what kind of functions can chemical and mechanical pretreatment bring during the process, respectively. The chemical changes of the samples were investigated before and after the ATSE and alkaline pretreatment (AP). The enzymatic digestibility of the raw materials and the pretreated substrates were evaluated. In addition, the morphology changes were examined using scanning electron microscope. According to the comparison between the continuous physicochemical pretreatment and separate physicochemical pretreatment, ATSE method can be considered as a promising pretreatment method due to the efficient conversion of lignocellulosic biomass to fermentable sugars. But none of the cellulosic ethanol from extrusion pretreatment technology has been commercialized to date. In our work, ATSE pretreatment was conducted with a pilot-scale equipment, while the APR was conducted with cooking reactor and PFI refiner which are mature and simulated production library equipment. Thus, we believe that the two different scale pretreatment processes are comparable. Moreover, the data would provide a reference for further optimizing the ATSE pretreatment parameters and contribute to the understanding of the pretreatment mechanism.

2. Materials and methods

2.1. Materials

Corn stover used in this study was obtained from the city of Qingdao, China. Before pretreatment, the air-dried corn stover was cut into 2–5 cm pieces in length and stored in sealed plastic bag at room temperature for pretreatment. The key chemical composition including polysaccharides and lignin contents of corn stover was analyzed before pretreatment. All results are based on the oven dry weight. The enzymes used for enzymatic hydrolysis were Celluclast 1.5L (cellulase, enzyme activity 192 FPU/mL), Novozyme 188 (β-glucosidase, enzyme activity 741 IU/mL) and xylanase (enzyme activity 236 U/g) provided by Sigma-Aldrich China Inc. The enzymes activities were determined

according to the method reported by Ghose [28]. All chemicals and enzymes were used as received.

2.2. Pretreatment of corn stover

ATSE pretreatment. A pilot-scale extruder was used in the pretreatment process with the capacity of 200 kg oven dry corn stover per hour and the experiment was conducted according to the previously conditions as described: alkali to biomass ratio was 0.06 g NaOH/g oven-dry corn stover, biomass/liquid ratio of 1/2, heat preservation time of 1 h at a temperature of 99 °C [27].

APR pretreatment. APR pretreatment mean alkaline pretreatment plus PFI refining pretreatment. The alkaline pretreatment (AP) was carried out in a cooking reactor (Model PL1-00, Xianyang TEST Equipment Co., Ltd., Xianyang, China) containing 4 bombs with batch capacity of oven dry corn stover of 50 g per bomb, and the pretreatment conditions were same as the ATSE pretreatment. After pretreatment, the solid substrate was beaten by a PFI refiner (mode PL 11-00, Xianyang Test Equipment Co., Ltd., Xianyang, China). Beating conditions were as follows: pulp consistency, 10%; beating gap, 0.3 mm; and the PFI refining revolution, 1500 r, 3000 r, 4500 r, 6000 r, 8000 r, or 10,000 r. The refined pulp was collected and stored in a refrigerator.

2.3. Enzymatic hydrolysis

Enzymatic hydrolysis of the substrates was conducted on a 0.4 g (dry basis) in a serum bottle substrate consistency of 2% (W/V) at 50 °C using an incubator shaker at 95 rpm for 48 h. Sodium citrate buffer (pH = 4.8) was added to maintain the pH at 4.8 and 0.02% sodium azide was used in the mixture to inhibit the microbial infections. An enzyme cocktail of cellulase (20 PFU/g substrate), β-glucosidase (10 IU/g substrate) and xylanase (5 U/g substrate) was used for enzymatic hydrolysis.

2.4. Analytical hydrolysis

Components of corn stover such as cellulose, hemicellulose, and lignin were determined according to the procedures described by National Renewable Energy Laboratory (NREL) [29]. The sugar compositions of the acid and enzymatic hydrolysate were determined by high performance liquid chromatography (HPLC) (Model 1200, Agilent, USA). The HPLC system was equipped with a Bio-Rad Aminex HPX-87H column (300 × 7.8 mm) and refractive index detector. The analytical column was operated at 55 °C with sulfuric acid (0.05 M) as the mobile phase (0.5 mL/min). The effectiveness of pretreatments was evaluated by the solid yield (Y_{solid}), enzymatic hydrolysis efficiency of glucan or xylan ($E_{glucan/xyla}$), delignification rate ($R_{delignification}$), and sugar yields. The calculation equations were listed as follows:

$$Y_{solid}(\%) = (M_{pretreated\ corn\ stover}/M_{original\ corn\ stover}) \times 100 \quad (1)$$

$$E_{glucan}(\%) = (M_{glucose\ in\ hydrolyzate} \times 0.9/M_{glucan\ in\ pretreated\ corn\ stover}) \times 100 \quad (2)$$

$$E_{xylan}(\%) = (M_{xylose\ in\ hydrolyzate} \times 0.88/M_{xylan\ in\ pretreated\ corn\ stover}) \times 100 \quad (3)$$

$$R_{delignification}(\%) = 1 - (Y_{solid} \times C_{lignin\ of\ pretreated\ corn\ stover})/C_{lignin\ of\ raw\ corn\ stover} \times 100 \quad (4)$$

where, M was the oven dry weight of corresponding substance (g), C was the percent of the corresponding component content (wt%), Y_{solid} was the percentage of solid yield, E_{glucan} was the enzymatic hydrolysis efficiency of glucan, E_{xylan} was the enzymatic hydrolysis efficiency of xylan, and $R_{delignification}$ was the delignification rate.

The total sugar yield (%) was expressed as the percentage of glucan and xylan in enzymatic hydrolysate divided by the glucan and xylan in