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Comparison of two-stage acid-alkali and alkali-acid pretreatments on enzymatic saccharification ability of the sweet sorghum fiber and their physicochemical characterizations

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HIGHLIGHTS

- A two-stage pretreatment process was studied for lignocelluloses pretreatment.
- The two-stage process improved the saccharification efficiency of sweet sorghum fiber.
- 0.23 $g \cdot g^{-1}$ of glucose yield was achieved under the optimized condition of alkali-acid sequence.

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ABSTRACT

Two-stage acid/alkali pretreatment was used to enhance the saccharification efficiency of sweet sorghum fiber. The physicochemical characterizations of the pretreated fibers were evaluated by SEM, FTIR and XRD. The acid and alkali sequence in the two-stage pretreatment process was compared, and their dosage was optimized. The results indicated that the two-stage pretreatment showed better saccharification performance when compared with conventional single stage pretreatment. And compared with the acid-alkali sequence, the alkali-acid sequence achieved higher glucose yield (0.23 $g \cdot g^{-1}$) under the optimized conditions, which was 1.64 and 1.21 times higher than that of the single stage and the acid-alkali pretreatments, respectively. Overall, the two-stage pretreatment process is a promising approach to achieve high fermentable glucose conversion rate of cellulosic material.

1. Introduction

Conversion of sugars in abundant cellulosic materials into valuable chemicals has drawn great interest of scientists in recent decades as it provides a potential route to respond to the global environmental change and unsustainable fossil fuel supplies (Guilliams et al., 2016). However, physico-chemical structural and compositional factors in lignocellulosic biomass materials hinder the biodegradation of the complex cellulose and hemicellulose components into monomeric sugars, which is recognized as the key barrier for further usage of the biomass materials (Behera et al., 2014). To obtain the cheap sugars from lignocellulosic materials, effective pretreatment is encouraged (Qin et al., 2013).

Among different types of pretreatment methods, chemical pretreatment was proved as a promising one, and alkali or acid pretreatments were investigated in biorefinery processes (Cai et al., 2016a). By applying alkali pretreatment, the ester and ether bonds in lignin carbohydrate complexes were broken while the internal surface area of biomass materials increased (Mou et al., 2013). By the acid catalysis, the hemicelluloses fraction in lignocellulosic materials could be hydrolyzed into monosaccharides, supplying sufficient cellulose accessible surface to cellulase. It was proved that the acid pretreatment was more important than the alkali pretreatment in the procedure of enzymatic hydrolysis of the cellulosic materials (Leu and Zhu, 2012; Zhang et al., 2013). As the removal of hemicellulose and lignin from lignocellulosic materials is important for the effective enzymatic saccharification of cellulose fraction, it thus calls for the improvement of the pretreatment process that could remove both lignin and hemicelluloses (Kim et al., 2011; Azelee et al., 2014). Nevertheless, most of the single stage acid and alkali processes could only partially remove the lignin or hemicelluloses fractions, leading to relatively low sugar yields and relatively severe pretreatment conditions (Zu et al., 2014).

In previous studies, by integrating the acid and alkali pretreatment process based on two-stage strategy, the successive

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delignification was effective improved by the removal of both lignin and hemicelluloses fractions in the raw biomass material (Sakdaronnarong et al., 2014; Kim et al., 2011; Sun et al., 2015a). Hence, the surface area and porosity of cellulosic fibers effectively increased and the efficiency of enzymatic hydrolysis was improved (Kim et al., 2012). These processes could be divided into two sequences: acid pretreatment followed by alkali pretreatment, and alkali pretreatment followed by acid pretreatment. Although numerous literatures have been done based on different acid and alkali for the pretreatment of biomass materials and production of fermentable sugars, limited researches compared the two sequences based on physicochemical evaluations.

In the current work, sweet sorghum fiber, the photoperiod-sensitive lignocellulosic biomass, was used as the raw material for two-stage pretreatment based on acid-alkali or alkali-acid sequences for the first time. The physicochemical behaviors of the raw material and the pretreated fibers were tested by surface morphology, fourier transform infrared spectroscopy and crystallinity analysis. Then, enzymatic hydrolysis was further practiced to saccharify the two-stage pretreated fibers.

2. Methods

2.1. Raw material

Sweet sorghum stalk was kindly provided by Chinese Academy of Agricultural Sciences and was harvested in October in 2015 on an experimental plot at Shunyi District of Beijing, China. After juice extraction, the solid residues were washed and collected, followed by dried out at 105 °C. Then, the lignocellulosic fiber was milled and screened to 60 meshes and stored at −20 °C before use. The chemical composition of the sweet sorghum fiber was determined by the standardized methods of National Renewable Energy Laboratory (Sluiter et al., 2008). The chemical compositions of raw material were: 38.2 ± 4.2% cellulose, 22.8 ± 2.7% hemicelluloses, 21.3 ± 1.6% lignin, 3.5 ± 0.4% ash and 14.2 ± 2.5% extractives.

2.2. Two-stage pretreatment process

Sweet sorghum fiber was pretreated by two-stage integrated processes. 0.5–2% (v/v) of H_2SO_4 and 0.5–2% (w/v) of NaOH were investigated in the acid-alkali sequence and the alkali-acid sequence, respectively. In the first-stage of pretreatment, the raw material was treated by a certain concentration of acid/alkali aqueous in a 500 ml bioreactor with agitation rate of 200 rpm. The solid to liquid ratio was 1: 10 while the reaction temperature was maintained at 120 °C for 30 min. After the first stage of pretreatment, the bioreactor was cooled down with circulating water and the solid remains was collected by filtration. And it was washed by deionized water until reached the native pH. For the second-stage of pretreatment, the opposite alkali/acid aqueous was mixed with the solid fraction obtained from the first-stage. The other reaction conditions in the second-stage pretreatment were similar with the first-stage pretreatment. After pretreatment, the solid was also washed by deionized water until reached the native pH.

2.3. Enzymatic saccharification

All the pretreated fibers were attempted to produce glucose by enzymatic saccharification. The enzymatic saccharifications were performed in a 200 ml conical flasks with 15% (w/v) solid loading and kept at 50 °C and 200 rpm. According to previous study (Cai et al., 2016b), 0.01 M KH_2PO_4/H_3PO_4 buffer (pH 4.7) was used, and cellulase dosage was 15 FPU/g cellulose. The cellulase (60 ± 5 FPU) was purchased from KDN Co. Ltd., (Qingdao, China). After 72 h enzymatic saccharification, the hydrolysates were centrifuged and the liquid fraction was collected to analyze the fermentable sugars concentration. In consideration of accuracy and reliability, all the above experiments were performed in duplicate.

2.4. Analysis methods

The content of monosaccharides was determined by high performance liquid chromatograph (Shimadzu LC-10A, Japan) equipped with a HPX-87H column (Bio-Rad Labs, USA) according to the method of Cai et al. (2016c). Scanning electron microscope (SU-1510, Hitachi Hightechnologies Co., Japan) images were performed at 5kv. And fourier transform infrared was performed using a Nicolet 8700 spectrophotometer in the range from 400 to 4000 cm^{-1} at 4 cm^{-1} resolutions with 128 scans. X-ray diffraction was measured using ADVANE X'Pertdiffractometer (RigakuCo., Tokyo, Japan) with Cu Kα radiation (λ = 1.54 nm) and operated at 30 kV. The scattering angle was ranged from 10° to 30°, and the speed was 0.1 s per step. The Crystallinity index (CrI) was determined by the following equation (Jiang et al., 2015):

$$CrI = \frac{I002 - Iam}{I002} \times 100\% \quad (1)$$

where I_{002} represented the highest peak intensity at 2θ = 22.4°, and I_{am} represented the peak at 2θ = 15.5°.

The recovery rate of the pretreated substrates fraction and the cellulose conversion rate were calculated by the following formulas:

$$Recovery\,(\%) = \frac{Wpre}{Wraw} \times 100\% \quad (2)$$

where W_{pre} and W_{raw} were the weight of pretreated substrates and raw materials, respectively.

$$Cellulose\ conversion\ (\%) = \frac{Amount\ of\ gluclose\ in\ hydrolysate \times 0.9}{Amount\ of\ cellulose\ in\ substrates} \times 100\% \quad (3)$$

$$Cellulose\ remaining\ (\%) = \frac{Amount\ of\ cellulose\ in\ pretreated\ material}{Amount\ of\ cellulose\ in\ substrates} \times 100\% \quad (4)$$

$$Hemicellulose\ removal\ (\%) = 1 - \frac{Amount\ of\ Hemicellulose\ in\ pretreated\ material}{Amount\ of\ Hemiellulose\ in\ substrates} \times 100\% \quad (5)$$

$$Delignification\ (\%) = 1 - \frac{Amount\ of\ lignin\ in\ pretreated\ material}{Amount\ of\ lignin\ in\ substrates} \times 100\% \quad (6)$$

$$Cellulose\ yeild\ (\%) = \frac{Amount\ of\ cellulose\ in\ hydrolysate}{amount\ of\ raw\ material} \times 100\% \quad (7)$$

3. Results and discussion

3.1. Compositional analysis

To compare the influence of the pretreated methods on the composition of biomass, the compositions of the pretreated fibers after the first and the second stages of pretreatment were evalu-