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Research paper

Hemicellulose and lignin removal to improve the enzymatic digestibility and ethanol production

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ABSTRACT

Fourteen herbaceous energy crops were selected to investigate their recalcitrance to cellulosic enzyme. The results about enzymatic hydrolysis of sorghum and *Pennisetum* hybrids indicated that both hemicellulose and lignin content had a negative influence on enzymatic digestibility (ED), especially for the latter.

Furthermore, liquid hot water (LHW) and aqueous ammonia (AA) methods were applied to enhance the ED of sugarcane bagasse (SCB). A high ED of 90% was obtained for sample with 50% removal of xylan and lignin in the AA process, which was 20% higher than that of LHW due to the significant removal of lignin hindering and exposing of fibers. However, the total sugars recovery of LHW were about 10% higher than that of AA resulted to the high yield of hemicellulose-derived sugars.

Moreover, SCB hemicellulose and lignin were removed with the help of LHW and following AA process. The ethanol concentration reached to 30.6 g/L after a fed-batch enzymatic hydrolysis following simultaneous saccharification and fermentation process with a high substrates loadings of 20% (w/v), low enzyme loading of 20 FPU/g glucan, and low fermentation time of 24 h.

1. Introduction

Biorefinery of lignocellulosic biomass to fuels can enhance the energy security and reduce the GHG emissions [1]. However, the complex cell wall structure built by cellulose, hemicellulose and lignin protects native lignocellulosic biomass from the attack of enzymes and microorganisms [2]. This recalcitrance to sugar release is a major limitation for cost-effective bio-finery process. Understanding and overcoming biomass recalcitrance are central research themes of the U.S. Department of Energy BioEnergy Science Center (BESC), and the researchers hope to use a single microbe or microbial consortium to deconstruct lignocellulose and convert it directly to product. However, many literatures indicated that pretreatment using catalysts, acids, bases, ionic liquids, water, heat, milling, microorganism or other means to breakdown the complex cell wall structure of cellulose-hemicellulose-lignin is the inevitable course for the improvement of fermentable sugars release at present [3,4]. An effective pretreatment can reduce the downstream pressure by making cellulose more accessible to the enzymes and minimize the formation of degradation products that inhibit the growth of fermentative microorganisms [5].

Comparing with the chemical methods, liquid hot water (LHW) pretreatment with no chemical addition and little erosion on equipment is becoming attractive. And it has a good performance of hemicellulose dissolution and low inhibitors formation for the following enzymatic hydrolysis and fermentation [6–8]. Wyman proposed a partial flow LHW process for corn stover after optimizing the operating strategy and reaction conditions [9], and we designed the two-step LHW approach for sorghum bagasse [10] and wood chips [11] by changing the reaction temperature and flow rate. All of them gave a good total sugar recovery and enzymatic digestibility (ED) of cellulose. However, less than 20% lignin were removed after LHW pretreatment, which usually resulted to a high loading of enzyme and low concentration of substrates loading.

Alkaline pretreatment using sodium hydroxide (NaOH) [12] and aqueous ammonia (AA) has a good performance of lignin dissolution. Particularly, AA can be recyclable due to its high volatility,

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non-polluting and non-corrosive [13]. A pretreatment method combined LHW and AA was proposed in our previous work [14,15] and other literatures [16,17]. However, how the LHW, AA or their combined pretreatment overcome biomass recalcitrance and enhance cellulose accessibility is still unclear.

In this study, fourteen herbaceous energy crops were selected to investigate their recalcitrance to cellulosic enzyme. In particular, the contribution of chemical composition and structures changes during LHW and AA process on the enhancement of cellulose accessibility for sugarcane bagasse (SCB) were evaluated systematically. Then a simultaneous saccharification and fermentation (SSF) process was developed for LHW-AA pretreated SCB. The related results would provide insight into understanding of LHW and AA pretreatment mechanism and developing efficient cellulosic ethanol biorefinery process.

2. Materials and methods

2.1. Materials

Eleven energy sorghums were kindly supported by China Agricultural University. Two *Pennisetum* hybrid a, b were harvested in May, 2015 from Zengcheng of Guangzhou City, China. Sugarcane bagasse (SCB) was provided by Guangxi FengHao Group Co. Ltd. (Pingxiang, China). Their chemical composition (on a dry weight basis) had been reported in our previous works [18–20]. The cellulase (234 FPU/g) was purchased from Imperial Jade Biotechnology Co., Ltd. China.

2.2. Pretreatment

LHW and AA are treated in Batch stirred tank reactor, the details of experimental apparatus and operation steps were described in previous works [15,20].

2.3. Enzymatic hydrolysis

Substrate was put into 0.05 M sodium citrate buffer (pH4.8, calculated based on the residual moisture content) with 5% (w/v) or 10% (w/v) (just for LHW-AA treated samples) concentration, cellulose dosage was 40 FPU/g substrate or 20 FPU/g glucan (just for LHW-AA treated samples). 0.1% (100μL/100 mL digestibility system) sodium azide was added to avoid microbial growth. The tests were performed at 50 °C with an agitation speed of 150 rpm on a rotary shaker for 72 h. Samples were taken per 24 h for untreated, LHW and AA treated SCB. Every experiment was duplicated twice.

2.4. SSF

The process of SSF for LHW-AA pretreated SCB was conducted based on our previous works [21]. Fed-batch enzymatic hydrolysis was firstly performed at 50 °C for 72 h before SSF process. 30 g substrates were loaded into the reactor with 300 mL 0.05 M sodium acetic buffer, then 15 g substrates were fed to the reactor at 24 h and 48 h, separately, which made the total loadings of substrates achieve to 20% (w/v). Simultaneously, cellulase were added with 20 FPU/g glucan. Then the temperature was adjusted to 30 °C, *Saccharomyces. cerevisiae* Y2034 (purchased from the National Center for Agricultural Utilization Research) and nutrients (5 g/L yeast extract, 5 g/L peptone, 5 g/L KH_2PO_4, 0.2 g/L $(NH_4)_2SO_4$ and 0.4 g/L $MgSO_4{\cdot}7H_2O$) were added into the hydrolysate slurry without sterilization. 72-h fermentation was carried out at the speed of 100 rpm. The time when the yeast was inoculated was marked as 0 h. Samples were taken and analyzed for the ethanol yield and sugars consumption.

2.5. Characteristic analysis of biomass

The internal pore distribution and specific surface area of SCB and pretreated residues were analyzed by Automated Surface Area and Pore Size Analyzer (SI-MP-10/Pore Master 33, Quantachrome, USA). Crystallinity index of unpretreated and pretreated samples was measured by XRD using a X′ Pert Pro MPD generator (PW3040/60, Philips, Holand). The dried samples were scanned in 2^{θ} range from 5° to 80° using Cu radiation generated at 40 kV and 40 mA.

2.6. Measurement of products

The sugars and ethanol contents were respectively measured with the high performance liquid chromatography and gas chromatography following the previous method [21]. The yield of total xylose was calculated as the ratio of the total xylose in the liquid fraction per 100 g of potential xylose in the raw material.

3. Results and discussion

3.1. Biomass recalcitrance from chemical composition

Two *Pennisetum* hybrids with same hybridization parents were selected to evaluate their recalcitrance to cellulosic enzyme. Fig. 1 indicated that they have similar anatomic structure after stained by malachite green and sarranine. Moreover, the differences of content of cellulose, hemicellulose and lignin, CrI and surface characteristics were lower than 10%, and they were more than 15% for their arabinose/xylose ratio (Ara/Xyl), acetyl group, DP of cellulose and lignin [18]. Specially, cellulose DPn and DPw of *P.*a were 1.45 and 1.35 times the amount of the *P.*b, respectively. In contrast, *P.*b had a bigger DPn and DPw value of lignin compared with that of *P.*a. Despite this, there were no significant differences in term of 72 h ED of *P.*a and *P.*b, they were 39.2% and 38.5% respectively under the enzyme loading of 20 FPU/g, and 60.9%, 57.3% under 40 FPU/g.

Eleven energy sorghums with different chemical composition were digested with cellulosic enzyme to further investigate the effect of lignin and xylan content on glucose release. With the in-depth statistical analysis, there was a negative correlation both for the lignin and xylan level to ED as shown in Fig. 2a. It has been reported that getting rid of the shields from lignin and hemicellulose can enhance the accessibility of cellulose to enzyme [14]. However, R^2 values of the correlation equations of lignin level to ED (0.79) was higher than that of xylan (0.47). We speculate that lignin inhibits the access of enzyme to cellulose not only by a steric hindrance but the non-productive adsorption of cellulase. The high ED of 57.9% was obtained for the sorghum with low lignin content of 15.3%, but ED was only 47.8% although with the low xylan level of 17.9%.

With the objective of getting a more comprehensive understanding of contribution of lignin content on ED, energy sorghums were pretreated at 160 and 180 °C for 60 min. Fig. 2b presents that, independent of the removal of lignin, the ED is lower than 50% resulted from the low xylan removal with range of 14.4%–57.7% under the condition of LHWP-160 °C. Moreover, ED appears to bear no relation to lignin level in spite of the 100% xylan removal at 180 °C. All of these information indicates that lignin level is an important restraining factor in the cellulosic digestibility, but is not the unique factor. Other primary substrate related factors includes CrI, surface area, and so on [22].

3.2. LHW and AA pretreatments

In order to deep understand the influence of hemicellulose and lignin on the enzymatic hydrolysis. SCB was pretreated with