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Short communication

Combination of dilute acid and ionic liquid pretreatments of sugarcane bagasse for glucose by enzymatic hydrolysis

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ABSTRACT

Loss of hemicellulose and inability to effectively decrystallize cellulose, result in low yield and high cost of sugars derived from biomass. In this work, dilute sulfuric acid pretreatment could easily remove most of hemicellulose as sugars. The sugars were successfully used for 2,3-butanediol production with relative high yield (36.1%). Then, the remained solid residue after acid-pretreatment was further pretreated by ionic liquid (IL) to decrease its crystallinity for subsequent enzymatic saccharification. The combination of dilute acid- and IL-pretreatments resulted in significant higher glucose yield (95.5%) in enzymatic saccharification, which was more effective than using dilute acid- or IL-pretreatment alone. This strategy seems a promising route to achieve high yield of sugars from both hemicellulose and cellulose for biorefinery.

1. Introduction

Lignocellulose is a promising renewable feedstock for the production of fermentable sugars and biofuels [1,2]. Pretreatment is a critical step for the utilization of biomass. Dilute sulfuric acid-pretreatment has been shown as a leading and most promising process that is currently under commercial development [3]. In dilute acid, hemicellulose and amorphous cellulose are easily and nearly completely hydrolyzed to fermentable sugars accessible to microorganisms for biofuels production [4,5]. But, the remained solid residue (mainly contained crystalline cellulose) is more difficult to be hydrolyzed, and harsh conditions (*e.g.*, high acid concentrations or temperatures) are required for it [6]. However, severe conditions accelerate the secondary decomposition of sugars and the formation of toxic by-products for fermentation, such as 5-hydroxymethylfurfural (5-HMF) and furfural [7,8]. After pretreatment with ionic liquids (ILs), the crystallinity of cellulose decreased remarkably and its structure becomes essentially amorphous and porous for efficient hydrolysis [9,10]. In the previous work, the initial enzymatic hydrolysis rates were approximately 50-fold higher for the regenerated cellulose pretreated by IL [AMIM]Cl (1-allyl-3-methylimidazolium chloride) as compared to untreated cellulose with an enzyme concentration of 50–115 filter paper units (FPU)/g glucan [9]. The IL-pretreatment of lignocellulose also can greatly increase its saccharification rate and the fermentable sugar yield [11]. In addition, IL-pretreatment is considered as an environmentally-friendly alternative to conventional pretreatment methods because IL is recyclable [12].

Studies on different pretreatments of sugarcane bagasse have been widely reported, such as physical- [13], ultrasonic- [14], steam explosion- [15], acid- [16], IL- [9–11,17], alkaline- and urea-pretreatments [18]. Limited studies have been reported on the comparison of dilute acid-, alkali- and IL-pretreatments on sugarcane bagasse [19]. Pretreatment of sugarcane bagasse by acid-catalysed process in aqueous IL solutions has also been studied [20]. Unsatisfactorily, loss of a considerable proportion of hemicellulose and inability to effectively decrystallize cellulose, result in low yield and high cost of sugars derived from biomass [21]. But, little was reported on the combination of dilute acid- and IL-pretreatments on lignocelluloses for their efficient conversion to sugars.

The purpose of this work is to get high glucose yield from sugarcane bagasse by enzymatic hydrolysis, while other C_5 sugars are also recovered and utilized. Sugarcane bagasse is firstly pretreated by dilute sulfuric acid to remove hemicellulose as sugars by hydrolysis, and the fermentability of the hydrolysates is evaluated by the production of 2,3-butanediol with *Klebsiella oxytoca*. The remained cellulose in the solid residue is further pretreated by IL [AMIM]Cl to destroy its crystallinity for glucose by subsequent enzymatic saccharification.

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Table 1
Chemical components and structural characteristics of sugarcane bagasse after different pretreatments.

Bagasse samples	Components (wt%)				CrI (%)	BET surface area (m^2/g)
	Glucan	Xylan	Lignin	Ash		
Raw bagasse	41.1 ± 0.9	22.0 ± 0.5	33.5 ± 1.0	5.7 ± 0.8	51.6	5.8
Acid-pretreatment	58.6 ± 0.9	2.0 ± 0.2	28.5 ± 0.8	5.3 ± 0.4	69.8	5.5
IL-pretreatment	41.2 ± 1.0	20.8 ± 1.4	31.1 ± 0.07	5.4 ± 0.1	39.4	8.8
Acid and IL pretreatments	58.5 ± 0.5	0.9 ± 0.3	27.2 ± 0.2	5.7 ± 0.2	34.5	9.8

2. Materials and methods

2.1. Materials

Sugarcane bagasse was obtained from Dehong in Yunnan, China. The bagasse contained 0.16% sucrose and 0.20% glucose analyzed its aqueous solution after washed with hot-water by high performance liquid chromatography (HPLC). It was air-dried, milled and passed through a 20-mesh sieve and then dried in an oven at 105 °C for 4 h. The elemental composition was analyzed by an Organic Elemental Analyzer (Vario EL III, Hanau, Germany) and the content of carbon accounted for 49.4% in raw material. The chemical components were determined according to the National Renewable Energy Laboratory (NREL) procedure [22] (Table 1). Sulfuric acid (95%) was purchased from Chuandong Chemical (Group) Co. Ltd., Chongqing. [AMIM]Cl (99.5% purity) was purchased from Lanzhou Institute of Chemical Physics (Lanzhou, China). The IL was directly used for pretreatment without drying because no weight loss was found after it was freeze-dried for 12 h. The *pH* of its aqueous solution is about 3.7–4.0 at weight ratio of water/[AMIM]Cl of 16–32.

2.2. Dilute acid-pretreatment

A 350-mL high-pressure autoclave (FCFD05-30, Yantai Jianbang Chemical Mechanical Co. Ltd., Shandong) was used for the acid-pretreatment. Sugarcane bagasse (20.0 g) and dilute sulfuric acid (300 mL; 0.25–1.5 wt%) were loaded and pretreated at 140–160 °C for 0.25–1 h. Reducing-sugars concentration was measured by 3,5-dinitrosalicylic acid (DNS) method [23]. The carbon amount in the aqueous phase was measured by a Total Organic Carbon (TOC) Analyzer (TOC-5000A, Shimadzu, Tokyo). Monosaccharides were measured by HPLC (LC-20A, Shimadzu) fitted with a refractive index (RI) detector and Aminex HPX-87P column (Bio-Rad, Hercules, CA) at 80 °C with distilled water as mobile phase at a flow rate of 0.4 mL/min. The yields (wt%) of xylose, reducing-sugars and water-soluble products were calculated as follows:

$$\text{Xylose yield (wt\%)} = \frac{\text{mass of xylose in the acid hydrolysate (g)}}{\text{xylan mass of biomass (g)}} \times 0.88 \times 100\% \quad (1)$$

$$\text{Reducing - sugars yield (wt\%)} = \frac{\text{mass of reducing sugars in the acid hydrolysate}}{\text{mass of biomass}} \times 100\% \quad (2)$$

$$\text{Water - soluble products yield (wt\%)} = \frac{\text{carbon mass of water soluble organic compounds}}{\text{carbon mass of biomass}} \times 100\% \quad (3)$$

After acid-pretreatment, the solid residue was filtrated, washed with distilled water to remove residual sulfuric acid, and dried in an oven at 70 °C for 24 h. Then, the dry solid was milled passed through an 80-mesh sieve and dried again at 105 °C for 4 h before subsequent IL-pretreatment or enzymatic saccharification. The hydrolysates were neutralized with calcium hydroxide to *pH* 7.0, concentrated under vacuum at 60–70 °C to achieve approximately 100 g/L sugar-concentration and detoxificated with activated charcoal. Similar to the previous work [6], the strain used for 2,3-butanediol production was *K. oxytoca* (CICC 22912) from China Center of Industrial Culture Collection (Beijing), which had a broad substrate spectrum. Fermentation experiments were conducted in a 3-L controlled bioreactor (BioTron, South Korea) with 1 L working volume for 42 h at 37 °C and 200 rpm stirring with 1 L/min airflow. The value of *pH* was maintained at 6.5 automatically by adding 6 M KOH. Dry cell weight (DCW) was determined by measuring the absorbance of broth at 600 nm (OD_{600}) using an ultraviolet (UV)-visible spectrophotometer (UV1800, Shimadzu) and calculated by the calibrated equation: $DCW = 0.4491 \times OD_{600} + 0.0388$ ($R^2 = 0.984$). The concentration of 2,3-butanediol was determined by the same HPLC fitted with a RI detector and Aminex HPX-87H column at 60 °C with 0.005 M H_2SO_4 as mobile phase at a flow rate of 0.6 mL/min. The yield (wt%) of 2,3-butanediol was expressed as follows:

$$\text{2, 3-Butanediol yield (wt\%)} = \frac{\text{mass of 2, 3-butanediol (g)}}{\text{mass of consumed reducing sugars (g)}} \times 100\% \quad (4)$$

2.3. IL-pretreatment

Biomass suspension in IL was prepared by adding 0.15-g (5 wt%) biomass in a 100-mL flask containing 3-g IL. Then, without removing air, the flask was sealed with a cork and placed in oil bath at 110 °C with stirring at 200 rpm for 1 h. After pretreatment, deionized water at 90 °C (15-mL) was added to the mixture to precipitate biomass with vigorous shaking for 10 s. The regenerated biomass was then transferred into a 100-mL beaker with deionized water at 75 °C, washed with 50-mL water (75 °C) thoroughly for 10 times to remove residual IL. The biomass was dried for 24 h with an EYELA 1200 freeze dryer (Rikakikai Co., Ltd., Tokyo), and dried in an oven at 105 °C for 4 h before subsequent enzymatic saccharification.

2.4. Structural characteristics

Specific surface area of samples was determined by Bruner Emmett and Teller (BET) method (Tristar II 3020, Micromeritics Instrument Co., Ltd., Northcross, GA). Samples were degassed at 100 °C for 3 h and nitrogen with relative pressure of 0.05–0.985 was applied in the analyses. Biomass samples were measured by X-ray diffraction (XRD) in a Rigaku TTR III X-ray diffractmeter (Tokyo) at 40 kV and 200 mA. Cu radiation ($\lambda = 1.54$ Å) was scanned over diffraction angle ($2\theta^\circ$) of 5–50° with a step of 0.01°. Its crystallinity index (CrI) was determined by the equation [24]:

$$\text{CrI (\%)} = \frac{I_{002} - I_{am}}{I_{002}} \times 100\% \quad (5)$$

where I_{002} was the highest peak intensity at an angle of diffraction of $2\theta = 22.5°$, I_{am} was the peak for the amorphous cellulose at $2\theta = 18°$.

2.5. Enzymatic saccharification

Enzymatic saccharification (0.1 g biomass) was carried out in a 50-mL Erlenmeyer flask containing 9.8-mL sodium citrate (50 mM, *pH* 4.8) reaction buffer and 100-μL 2% sodium azide at 50 °C with shaking at 90 rpm [25]. The samples were hydrolyzed with the cellulase mixture (60 FPU/g biomass) that consisted of Celluclast 1.5L® (from *Trichoderma reesei* ATCC 26921, ≥700 U/g) and Novozyme 188 (from *Aspergillus niger*) (Sigma, Shanghai) at a weight ratio of 4:1. The mixture activity (FPU) was determined by the method proposed by Andey and Baker [26].

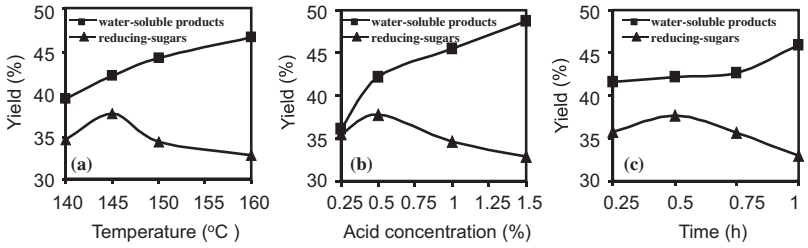

Fig. 1. Effects of reaction variables on hydrolysis yields of bagasse with dilute acid: (a) reaction temperature (reaction time 0.5 h, acid concentration 0.5%); (b) acid concentration (reaction time 0.5 h, reaction temperature 145 °C); (c) reaction time (reaction temperature 145 °C, acid concentration 0.5%).

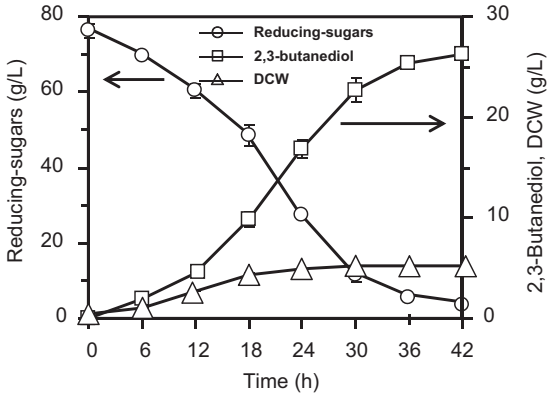

Fig. 2. Production of 2,3-butanediol with *Klebsiella oxytoca* from the hydrolysates of sugarcane bagasse pretreated with dilute sulfuric acid.

Glucose yield (wt%) was defined as:

$$\text{Glucose yield (wt\%)} = \frac{\text{mass of glucose in enzymatic hydrolysate (g)}}{\text{glucan mass of biomass after different pretreatments (g)}} \times 0.9 \times 100\% \quad (6)$$

3. Results and discussion

3.1. Hemicelluose removal by acid-pretreatment

Effects of reaction variables including temperature, sulfuric acid concentration and reaction time on hydrolysis yields of bagasse were studied (Fig. 1). Based on our previous work on dilute acid hydrolysis of *Jatropha* hulls [6], reaction conditions were selected as: temperature of 140–160 °C, sulfuric acid concentration of 0.25–1.5% and reaction time of 0.25–1 h. All the three variables had great influences on sugar yield as showed in Fig. 1. In this study, each variable was optimized one by one under given conditions. As temperature increased from 140 to 160 °C (Fig. 1a), the yield of water soluble products rose gradually because of the hydrolysis of bagasse to sugars. But, after the yield of reducing-sugars reached the maximum of 37.7% at 145 °C, it decreased gradually due to the secondary decomposition of sugars at higher temperatures (>145 °C). Similar trends occurred as sulfuric acid concentration and reaction time increased (Fig. 1b and c). The degraded toxic products for fermentation such as 5-HMF (*e.g.*, 0.3 g/L) and furfural (*e.g.*, 0.5 g/L) were formed. Therefore, the pretreatment conditions were selected as: temperature 145 °C, acid concentration 0.5 wt% and reaction time 0.5 h. Under these conditions, the maximum reducing-sugars yield was 37.7% (containing 4.1 g/L glucose, 14.6 g/L xylose, 1.4 g/L arabinose, 0.6 g/L mannose, 0.7 g/L galactose). The xylose yield was 87.6% and glucose yield was 13.3%. Raw bagasse contains 22.0 wt% xylan. After the pretreatment, the remained solid residue had only 2.0% xylan (Table 1). As the main component of hemicellulose in bagasse, xylan was almost removed by hydrolysis to xylose after the acid-pretreatment. To evaluate the fermentability, the hydrolysates with the initial 76.3 g/L reducing-sugars were used for 2,3-butanediol production. After 42 h, 5.2 g/L DCW and 26.2 g/L 2,3-butanediol were obtained, giving a productivity of 0.6 g/(L h) and a yield of 36.1% (equivalent to 72.2% of the theoretical value) (Fig. 2). In past decades, the utilization of low cost lignocelluloses as raw materials for 2,3-butanediol production to improve the economics of the process received considerable attention. Wood acid hydrolysates were used for 2,3-butanediol production, obtaining 13.3 g/L 2,3-butanediol with a yield of 29% and a productivity of 0.28 g/(L h) [27]. In our previous work [6], after washed by neutral detergent, *Jatropha* hulls were hydrolyzed and used for 2,3-butanediol fermentation, and 25.91 g/L diol was achieved with a yield of 35.6%, giving a diol productivity of 0.42 g/(L h). For the fermentation of IL-pretreated *Jatropha* hulls hydrolysate, products contained 24.13 g/L diol, with a yield of 33.29% and a productivity of 0.40 g/(L h) [28]. Corncob acid hydrolysates, detoxificated by boiling, overliming and activated chaocoal adsorption, were also used as feedstocks for fermentation. After 60 h of fed-batch fermentation, a maximal 35.7 g/L 2,3-butanediol was obtained, giving a productivity of 0.59 g/(L h) and a highest diol yield of 50% reported so far [5]. Thus, the hemicellulose hydrolysate of sugarcane bagasse in this study was an attractive raw material for 2,3-butanediol production.

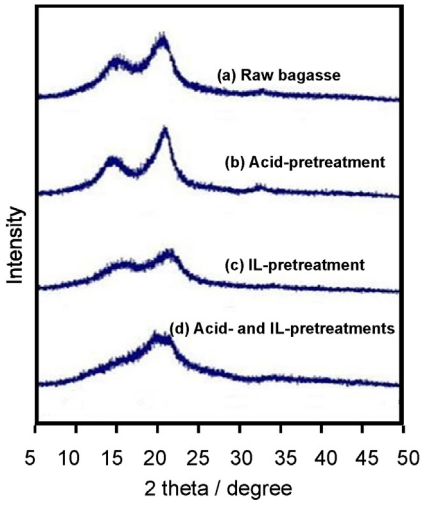

Fig. 3. XRD analyses of sugarcane bagasse with different pretreatments.

3.2. Chemical components and structural characteristics after pretreatments

Chemical components of sugarcane bagasse after different pretreatments were analyzed and summarized in Table 1. After IL-pretreatment of bagasse, the content of glucan changed little (41.2% *vs.* 41.1%) but xylan slighly decreased (20.8% *vs.* 22.0%). After both acid- and IL-pretreatments, glucan increased to 58.5% due to the removal of xylan (only 0.9%) in the acid-pretreatment step (only 2.0%). At the same time, lignin decreased from 33.5% to 27.2%. After acid-pretreatment, CrI increased from 51.6% to 69.8% and BET surface area decreased slightly from 5.8 to 5.5 m^2/g. The increase in CrI after dilute acid-pretreatment was consistent with the results reported previously [19,21]. In the case of dilute acid-pretreatment, the amorphous components broke down more but this pretreatment process was unable to break the inter- and intra-chain hydrogen-bonding in cellulose fibrils, and crystalline cellulose broke down less. More crystalline cellulose content means higher crystallinity and lower surface area of the acid-pretreated biomass. After IL-pretreatment, the intensity of crystalline peaks at 22.7° and 34.5° decreased remarkably or even disappeared with a flat and broad peak remained at around 20.7° (Fig. 3c). The possible reason was due to the transformation of cellulose I to cellulose II [29]. After both pretreatments with acid and IL, CrI decreased from 51.6% to 34.5% (Fig. 3d) and BET surface area increased from 5.5 to 9.8 m^2/g. Acid-pretreatment was used to remove hemicellulose for producing fermentable sugars. The subsequent IL-pretreatment decreased CrI and increased BET surface area for the efficient hydrolysis of cellulose. The efficiencies of different pretreatment methods were examined by enzymatic saccharification.

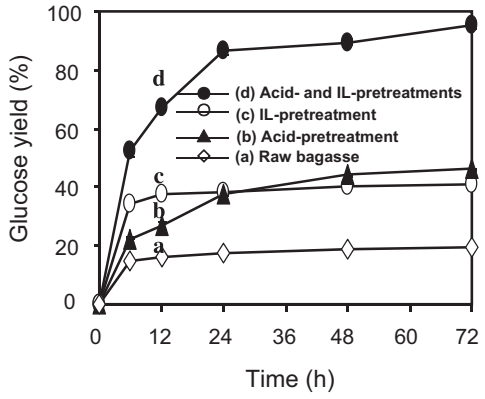

Fig. 4. Glucose yields from sugarcane bagasse with different pretreatments in enzymatic saccharification.

3.3. Enzymatic saccharification

In the enzymatic saccharification, the sugarcane bagasse after acid and IL pretreatments exhibited significantly higher cellulose digestibility (Fig. 4d), glucose yield reaching 95.5% as compared with 46.1% for acid-pretreated, 40.8% for IL-pretreated samples, and 19.3% for raw material after 72 h hydrolysis.

The high glucose yield could be explained as: first, the removal of hemicellulose by acid-pretreatment could increase the enzymatic digestibility of cellulose [30]. Enhancement in glucose yield (46.1%) after acid-pretreatment was verified (19.3% for untreated sugarcane bagasse) (Fig. 4b *vs.* a). Hemicellulose was biomass recalcitrance by covering and protecting the cellulose fibrils from enzymatic deconstruction [3]. Xylan had high affinity to cellulose and could absorb on cellulose surface irreversibly [31]. Xylo-oligomers had inhibition effects on cellulase action and that cellulase bound more strongly to xylan than cellulose [32,33]. In this study, hemicellulose was hydrolyzed to fermentable sugars for efficient 2,3-butanediol production and consequently increased the degree of glucose yield from cellulose. Secondly, after IL-pretreatment, the sharp decrease in CrI and increase in BET surface area would improve cellulose surface accessibility to enzymes for efficient hydrolysis. Enhancement in glucose yield of IL-pretreated bagasse (40.8%) was also observed as compared with untreated material (19.3%) (Fig. 4c *vs.* a). After combined pretreatments, hemicellulose was removed by hydrolysis with acid-pretreatment, and CrI was decreased after further IL-pretreatment, resulting in the highest glucose yield.

In this study, after acid-pretreatment, glucose yield was improved to 46.1% as compared to 19.3% for untreated material, but the yield still resulted in large proportion cellulose lost. Similar result also occurred when using IL-pretreatment alone. In addition, part of hemicellulose was lost during IL-pretreatment. In previous study [34], a combined process of dilute acid and IL treatments on sugarcane bagasse, could partially remove xylose and lignin. Another widely used method, steam explosion was also used in sugarcane bagass pretreatment [15]. The highest recovery of glucose from raw bagasse was 73.7% but part of hemicellulose and cellulose were lost during the pretreatment. To make lignocellulose conversion economically feasible, it was essential that both hemicellulose and cellulose were efficiently utilized in large scale application. Compared to the generally used pretreatments, the application of combined pretreatments of dilute acid and IL appeared to offer several advantages: (i) high yield of sugars derived from hemicellulose was obtained; (ii) the hemicellulose hydrolysate of sugarcane bagasse was efficiently utilized for 2,3-butanediol fermentation; (iii) the removal of hemicellulose and amorphous cellulose could cut down the cost by using less amount of IL and enzymes; (iv) the remained cellulose in the solid residue after the combined pretreatments could nearly completely saccharification; and (v) the final solid residue after enzymatic saccharification likely suggested a promising aspect of lignin study. However, these advantages need to be counterbalanced by the high cost associated with ILs. To overcome this difficulty, it is necessary to develop effective recovery and recycling methods and reduce energy consumption for recycling ILs. Then, new ILs should be designed to improve the capacity of lignocellulose dissolution at lower temperatures within shorter times.

4. Conclusions

In this study, dilute sulfuric acid-pretreatment could remove most of hemicellulose for fermentation and subsequent IL-pretreatment significantly decreased cellulose crystallinity. The pretreatments with both dilute acid and IL resulted in remarkably higher glucose yield (95.5%) as compared with the yields of 19.3% for untreated bagasse, 46.1% for only acid- and 40.8% for IL-pretreated samples in enzymatic saccharification. This strategy seems a promising route to achieve high yield of sugars from both hemicellulose and cellulose for the fermentation to value-added products.

Acknowledgements

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