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ORIGINAL PAPER

Oxygen-assisted ethanol organosolv pretreatment of sugarcane bagasse for efficient removal of hemicellulose and lignin

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Abstract Hemicellulose and lignin act as physical barriers impeding the hydrolysis of lignocellulosic biomass by cellulases. To develop a process which could simultaneously remove most hemicellulose and lignin in biomass, in this study, oxygen-assisted ethanol organosolv pretreatment (O_2-EOP) of sugarcane bagasse (SCB) was carried out. The effects of temperature, time, oxygen pressure and ethanol concentration on the pH of the hydrolysate, the solubilization and the chemical composition of SCB were investigated. Compared with autocatalytic EOP and acid-catalyzed EOP, O_2-EOP could remove most xylan and lignin from SCB at much milder conditions. At optimized conditions (40/60 ethanol/water (v/v), 1.5 MPa O_2, 160 °C and 80 min), 82.9% xylan and 83.3% lignin were removed. Glucan content in the residue reached 86.3%, much higher than results obtained by autocatalytic EOP and acid-catalyzed EOP. In subsequent enzymatic hydrolysis (1% solid loading, 4.96 mg protein/g cellulose, 72 h), O_2-EOP pretreated SCB produced 3.97 and 1.90 times more glucose than untreated material and N_2-EOP pretreated SCB, respectively. O_2-EOP also produced abundant organic acids, total amounts of formic acid and acetic acid in the hemicellulose hydrolysate reached 5.42 g/L. In conclusion, O_2-assisted EOP was an effective process for the production of pulp with high cellulose purity and good accessibility to cellulases.

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Introduction

Lignocellulosic biomass is one of the most promising alternatives to fossil resources for the production of carbon-based chemicals and transportation fuels. Lignocellulose mainly consists of hemicellulose (23–32%, a biopolymer of pentose), cellulose (38–50%, a biopolymer of glucose), and lignin (15–25%, a heterogeneous biopolymer of phenylpropane). These constituents are strongly intermeshed and bonded to each other (Sun et al. 2016). Thus, pretreatment is necessary to improve the accessibility of cellulose to cellulases for hydrolysis.

Among the numerous pretreatment methods, organosolv pretreatment has attracted much attention because it facilitates the removal of lignin and/or hemicellulose from biomass and greatly improves accessibility of cellulose (Zhang et al. 2016). After organosolv pretreatment, lignocellulosic biomass is usually fractionated into two components: (1) the liquor containing hydrolysate of hemicellulose and dissolved lignin, and (2) cellulose-enriched pulp with high accessibility to cellulases (Zhao et al. 2009). Many organic solvents have been tested for the pretreatment of biomass including methanol, ethanol, propanol, 1-butanol, 1-pentanol, tetrahydrofurfuryl alcohol, tetrahydrofuran, 2-methyltetrahydrofuran and so on (Arato et al. 2005; Gandolfi et al. 2014; Ho et al. 2009; Nguyen et al. 2015; Teramura et al. 2016; vom Stein et al. 2011). Ethanol is inexpensive, non-toxic and easy-to-be-recovered due to its low boiling point. Moreover, it can be derived from biomass by fermentation of glucose. Ethanol-based organosolv pretreatment (EOP) has been widely studied for different lignocelluloses (Arato et al. 2005; Mesa et al. 2010; Schwiderski et al. 2014; Teramoto et al. 2009; Wildschut et al. 2013). However, using catalyst is still a key problem to be overcome for organosolv pretreatment based on low-boiling-point organic solvents such as ethanol.

EOP improves the accessibility of lignocellulosic biomass to cellulases mainly by removing lignin and hemicellulose. EOP without exogenous catalyst addition, called autocatalytic process, is usually carried out at high temperature (185–210 °C) (Duff and Murray 1996) while the explosive and combustive nature of ethanol at high pressure limit its application. Moreover, this process can only solubilize part of hemicellulose and lignin (< 50%) and cellulose content in the pretreated sugarcane bagasse usually doesn't exceed 70%. Acid-catalyzed EOP can remove most of hemicellulose (> 80%) and part of lignin (> 50%). However, the high severity of this process may cause severe degradation of cellulose. Adding base accelerate the solubilization of lignin but could not effectively remove hemicellulose and thus in the subsequent enzymatic hydrolysis hemicellulase is usually needed to obtain high yield of sugars. Besides, adding acid or base also brings new problems such as equipment corrosion and high cost due to catalyst recovery (Agbor et al. 2011). Molecular oxygen (O_2) could significantly accelerate lignin degradation in alkaline medium by inducing the fragmentation of the aromatic ring of lignin and side chain scission (Yang et al. 2003) and thus facilitate lignin removal during alkaline pretreatment of LCB. Pretreatment of SCB in 12 bar O_2 at 195 °C, 15 min and alkaline pH could solubilize 93–94% of hemicellulose and 40–50% of lignin (Martin et al. 2007). In addition, O_2 was also found to accelerate lignin removal in organosolv pulping process (Evtuguin et al. 1999). However, to the best of our knowledge, oxygen-assisted ethanol process has not yet been used for the pretreatment of lignocellulosic biomass. In this study, to develop a process which could remove most lignin and hemicellulose in lignocellulosic biomass but doesn't cause severe degradation of cellulose, EOP of sugarcane bagasse in O_2 atmosphere was systematically studied. The effect of temperature, time, O_2 pressure and ethanol/water ratio on residue recovery, pH of the hydrolysate and chemical composition of SCB were investigated. Enzymatic accessibility of pretreated bagasse was evaluated.

Experimental

Materials

Anhydrous ethanol (≥ 99.7%) was purchased from Xilong Chemical Factory Co., Ltd. (Shantou, Guangdong). Anhydrous oxygen (≥ 99.5%) was provided by

Kunming Guangruida Co., Ltd. (Yunnan). Standard agents including acetic acid, formic acid, levulinic acid, arabinose, glucose, xylose, furfural and 5-hydroxymethylfurfural (HMF) (> 99%) were bought from Aladdin Factory Co., Ltd. (Shanghai). Microcrystalline cellulose Avicel PH101 was purchased from Sigma-Aldrich (Shanghai).

Methods

Pretreatment of bagasse

Bagasse was provided by Yunnan Kangfeng Sugar Co., Ltd. (Baoshan). It was milled in a pulverizer (9FC-15, Xudong machinery manufacturing Co., Ltd., Leshan, Sichuan) equipped with a sieve with diameter of 2 mm and air-dried at 105 °C in oven (WFO-710, EYELA, Tokyo Rikakikai Co., Ltd.) until constant weight. Pretreatment was carried out in a 30 mL high-pressure autoclave (YZPR-50, Yanzheng Shanghai Experimental Instrument Co., Ltd.). Bagasse (1.0 g, oven-dried basis), liquid solution (20 mL) and a magnetic stir bar (agitation speed of 600 rpm) were added into the vessel. For different runs of experiments, volumetric ratio of ethanol to water were set as 70:30, 60:40, 50:50, 40:60 and 30:70, respectively. The tightly sealed autoclave was initially charged with 0–2.0 MPa O_2. N_2 was used to bring the total pressure to 2.0 MPa if the pressure of O_2 is less than 2.0 MPa. For experiments performed in pure N_2 atmosphere, the vessel was purged with N_2 for 1 min and charged with 2.0 MPa N_2. The reactor was electrically heated by the heating jacket to a desired temperature (130–170 °C) in 12–25 min and kept for 40–120 min. After pretreatment, the vessel was quenched by tap water to 40 °C within 5 min. The vessel was then depressurized and opened. The pH of the obtained mixture was measured by pH meter (UB-10, Ultrabasic, Denver Instrument Co., Ltd.) directly. And then the mixture was decanted from the vessel and filtered though a 0.22 μm membrane. The obtained residue was rinsed with 10 mL anhydrous ethanol and 30 mL deionized water twice. After freeze-drying (PDU-1200, EYELA, Tokyo Rikakikai Co., Ltd.) for 48 h, the solid residue was weighted and used for composition analysis and enzymatic hydrolysis. Residue recovery was defined as follows:

$$\text{Residue recovery}\,(\%) = (\text{dry weight of residue})/(\text{dry weight of raw bagasse}) \times 100 \quad (1)$$

Analysis of sugars and degradation products in the hydrolysate

After the pretreatment conducted at optimized conditions (temperature 160 °C, time 80 min, pressure of 1.5 MPa O_2, ethanol/water (v/v) 40/60), about 2 mL sample was drawn from the mixture by a syringe and filtered (0.22 μm membrane). Then, concentrations of sugars (i.e., xylose, arabinose and glucose) and degradation products (i.e., acetic acid, formic acid, levulinic acid, HMF and furfural) were analyzed by HPLC (LC-20A, Shimadzu) equipped with an automatic sampler, ultraviolet (UV) and refractive index (RI) detectors, and Bio-Rad Aminex® HPX-87H column. The mobile phase was 5 mM H_2SO_4 at flow rate of 0.6 mL min^{-1}, and temperature for both oven and the detectors was set at 40 °C. Furfural and HMF were determined by UV detector at 284 nm and other products (sugars and organic acids) by RI detector, respectively.

Chemical components of raw and pretreated bagasse

Chemical components of raw bagasse were determined according to the National Renewable Energy Laboratory (NREL) procedure (Sluiter et al. 2008). Firstly, raw bagasse was extracted in Soxhlet extractor with refluxed water for 24 h and then with refluxed ethanol for another 24 h. The components dissolved into water and ethanol was denoted as extractives. The extracted bagasse was then hydrolyzed by 72% sulfuric acid at 30 °C for 60 min and subsequently by 4% sulfuric acid at 121 °C for 60 min. After neutralization with calcium carbonate, concentrations of sugars in the hydrolysate were measured by HPLC equipped with Agilent Hi-Plex Pb column. The mobile phase was deionized water at flow rate of 0.5 mL/min. The temperature of oven and RI detector was 70 and 55 °C, respectively. Standard calibration curves for these products were constructed with five points (0.1, 0.5, 1.0, 1.5 and 2.0 mg/mL, $R^2 > 0.999$). The amount of acid-soluble lignin in the hydrolysate was measured

by UV–Vis spectrophotometer (UV-1800, Shimadzu, Kyoto) at 240 nm. The residue after hydrolysis was dried at 105 °C, weighted and then oxidized in a muffle furnace (4-10, Yongguangming Instrument Factory, Beijing) at 575 °C for 24 h, the weight loss of residue was denoted as acid-insoluble lignin. The ash content of raw bagasse was measured by directly oxidizing the sample in the muffle furnace at 575 °C for 24 h. The analysis was performed twice in parallel. Similarly, the composition of the pretreated bagasse was also analyzed without extraction. After being hydrolyzed by sulfuric acid, the weight of the residue was denoted as acid insoluble lignin while further oxidation procedure was omitted. Other procedures were the same as those for raw bagasse analysis.

Raw bagasse consisted of 6.87 ± 0.26% extractives, 42.25 ± 0.55% glucan, 22.32 ± 0.08% xylan, 2.24 ± 0.17% arabinan, 20.48 ± 0.05% lignin and 2.41 ± 0.21% ash. Xylan removal and lignin removal were defined as follows:

$$\begin{aligned}&\text{Xylan removal}\,(\%)\\&\quad=100-((\text{weight of xylan in solid residue})/\\&\qquad(\text{weight of xylan in raw bagasse})\times 100)\end{aligned}\tag{2}$$

$$\begin{aligned}&\text{Lignin removal}\,(\%)\\&\quad=100-(\text{weight of lignin in solid residue})/\\&\qquad(\text{weight of lignin in raw bagasse})\times 100\end{aligned}\tag{3}$$

SEM and FTIR analysis of samples

The pretreated residue obtained under the optimized conditions was characterized by FTIR (Fourier transform infrared spectroscopy) and SEM (scanning electron microscopy), and compared with raw bagasse. FTIR measurements were performed on Nicolet *is10* spectrometer (Madison, WI) with a resolution of 4 cm^{-1} at 500–4000 cm^{-1} using standard KBr disk method. SEM (ZEISS EVO LS10, Cambridge, UK) was operated at 10 keV with 1000× magnifications (Li et al. 2016).

Enzymatic hydrolysis of samples

Commercial Cellulase (Cellic CTec2) was kindly provided by Novozymes (Bagsvaerd, Denmark).This cellulase preparation possesses high β-glucosidase activity and lytic polysaccharides monoxygenase (LPMO) and these new components increase the performance of the cellulase preparation (Cannella and Jorgensen 2014). The activity of the enzyme solution was 238.4 ± 2.6 FPU/mL, measured by the method published by Ghose (Ghose 1987). The concentration of protein in the solution was determined by the bicinchoninic acid assay (BCA) (Smith et al. 1985) and was 283.2 mg/mL. Accessibility of raw and pretreated bagasse to cellulase were evaluated based on the NREL TP-5100-63351 protocol (Resch et al. 2015). The mass/volume of all materials were amplified by 50/7 times to enhance accuracy of the results. Solid sample (100, 400 or 800 mg) with 300 μL of citrate buffer (1.0 M, pH 5.0), 40 μL of 5% sodium azide and a given amount of enzymes were added into a 25 mL ground-glass stoppered flask. Cellulase loadings were 4.96, 9.93 and 14.9 mg protein per gram of bagasse, corresponding to 4.225, 8.450 and 12.675 FPU/g of bagasse respectively. Deionized water was added to control the total volume of hydrolysis mixtures to 10 mL. The mixtures in flasks were incubated at 50 °C, agitated at 150 rpm in a thermostat incubator (SPH-100B, Shanghai Shiping laboratory equipment Co., Ltd.). At 72 h, 300 μL of hydrolysate was drawn out of flasks by a 1 mL pipette. These samples were bathed in boiling water for 10 min to stop enzymatic hydrolysis. After that, they were diluted and centrifuged (3–30 K, Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany) at 10,000 rpm (9391 rcf) for 5 min. The supernatant was analyzed by HPLC to determine glucose and xylose amount. Oven temperature was 60 °C and temperature of RI detector was 50 °C. Other conditions were all the same as described above. Glucan digestibility (wt%) was defined as follows:

$$\begin{aligned}&\text{Glucan digestibility}\,(\text{wt}\%)\\&\quad=0.90\times(\text{glucose weight in hydrolysate})/\\&\qquad(\text{glucan weight in residue})\times 100\end{aligned}\tag{4}$$

$$\begin{aligned}&\text{Xylan digestibility}\,(\text{wt}\%)\\&\quad=0.88\times(\text{xylose weight in hydrolysate})/\\&\qquad(\text{xylan weight in residue})\times 100\end{aligned}\tag{5}$$

Results and discussion

Hemicellulose and lignin primarily act as physical barriers to prevent cellulose in the lignocellulosic biomass from being attacked by cellulase and thus their contents were highly related to the enzymatic accessibility of the substrate (Ohgren et al. 2007). Therefore, we investigated the effects of temperature, reaction time, O_2 pressure and ethanol/water ratio on the solubilization and removal of xylan and lignin.

Pretreatment of bagasse

Effect of temperature

The pretreatment was carried out at temperatures of 130–170 °C. Reaction temperature significantly influenced the solubilization of bagasse and pH of the hydrolysate (In Fig. 1a). When treated at 130 °C, only 19.0% of bagasse was solubilized, and pH of the hydrolysate was 4.0. Increasing reaction temperature accelerated the solubilization of bagasse and decreased the pH of the hydrolysate. At 160 °C, residue recovery decreased from 81.0% at 130 °C to 45.0% while pH of the hydrolysate decreased to 2.9. The decline of pH indicated that high temperature facilitated the formation of organic acids. Organic acids could in turn accelerate the solubilization of bagasse. Analysis of the composition of pretreated residue showed that at 130 °C only 10.1% of xylan and 8.2% of lignin was removed from bagasse and glucan content slightly increased from 42.2 to 51.4% (Fig. 1b). High temperature improved removal of xylan and lignin. At 160 °C, 87.6% of xylan and 87.4% of lignin was removed and cellulose content in pretreated residue was enhanced to 87.6%, suggesting that 93.2% of glucan was maintained in the pulp. Further increasing reaction temperature to 170 °C did not improve the removal of xylan and lignin significantly. Hence the optimal temperature was set at 160 °C with 87.4% lignin removal, 87.6% xylan removal and 87.6% glucan in the residue.

Effect of reaction time

Reaction time varied from 40 to 120 min. Other conditions were: 1.0 g bagasse, 20 mL 50/50 (v/v) ethanol/water solution, 1.5 MPa O_2, 160 °C, magnetic stirring rate of 600 rpm. Reaction time didn't have an impact on the pH of the hydrolysate significantly, which was in the range of 3.3–2.9 (Fig. 2a), indicating that the formation of organic acids and their subsequent degradation to carbon dioxide are in equilibrium. When reaction time was 40 min, 40.3% of bagasse was solubilized. Removal of xylan and lignin was 57.4 and 62.0% respectively and glucan content was 67.8% (Fig. 2b). Extending reaction time to 80 min, residue recovery was reduced to 47.3%, removal of xylan and lignin were improved to 77.5 and 84.2%, respectively. Glucan content increased to 83.8%, suggesting that 93.8% glucan remained in the residue. Prolonging reaction time further had little effect on the solubilization of bagasse. At 100 and 120 min, residue recovery was 46.0 and 45.0% respectively. Hence reaction time of 80 min was the

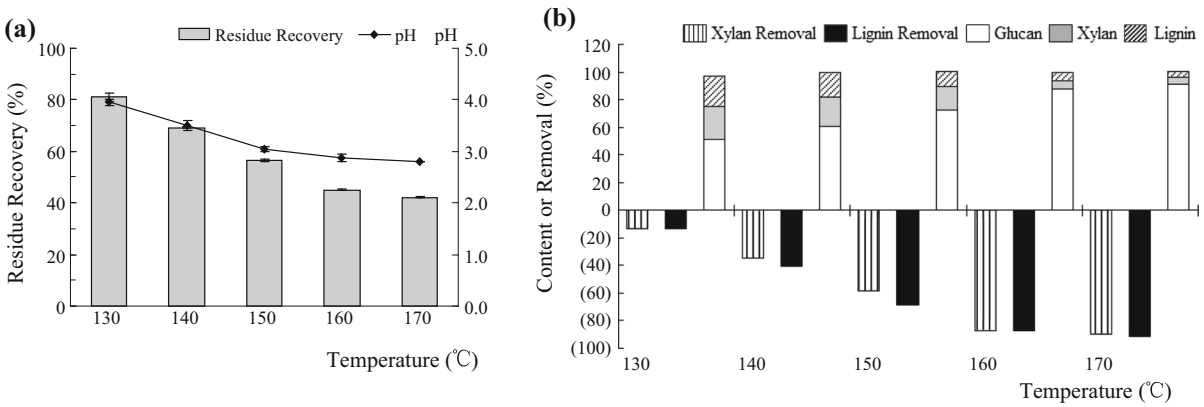

Fig. 1 Effect of temperature on residue recovery and pH of the hydrolysate (**a**) and the chemical composition of SCB (**b**) (50/50 ethanol/water (v/v), 1.5 MPa O_2, 120 min, 130–170 °C

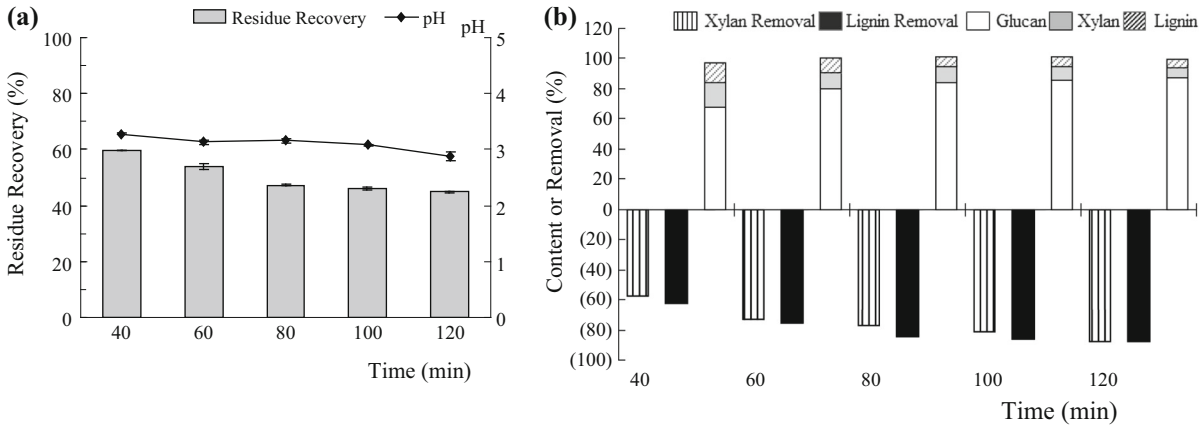

Fig. 2 Effect of time on residue recovery and pH of the hydrolysate (**a**) and the chemical composition of pretreated SCB (**b**) (50/50 ethanol/water (v/v), 1.5 MPa O2, 40–120 min, 160 °C)

best choice with 84.2% lignin removal, 77.5% xylan removal and 83.8% glucan in the residue.

Effect of O_2

The pretreatments were performed in 0–2.0 MPa O_2. Other conditions were: 1.0 g bagasse, 20 mL 50/50 (v/v) ethanol/water solution, 160 °C, 80 min and magnetic stirring rate of 600 rpm. After being treated in pure N_2 atmosphere, as shown in Fig. 3a, the pH of the hydrolysate was 4.8. Hemicellulose contained in bagasse consisted of acetyl group. Under hydrothermal conditions, hydrolysis of acetyl group produced acetic acids, which caused descending of the *pH* of the hydrolysate. Treatment in O_2 atmosphere led to lower *pH* of the hydrolysate. When the pressure of O_2 was 0.5, 1.0, 1.5 and 2.0 MPa, the *pH* of the hydrolysate decreased to 3.6, 3.3, 3.1 and 3.2, respectively. Under hydrothermal conditions, hemicellulose, cellulose and lignin contained in lignocellulosic biomass could be degraded and partially oxidized by O_2 to organic acids. Demesa et al. (2015) studied the hydrothermal oxidation of lignin in alkaline solution at 175–225 °C in 1.0 MPa O_2. They found that the main products were formic acid, acetic acid, succinic acid, oxalic acid and glutaconic acid. Li et al. (2014) found that hydrothermal oxidation of polysaccharides, monosaccharides, furfural, levulinic acid and humins at 190 °C in 2 MPa O_2 led to the formation of formic acid and acetic acid.

Overall performance of pretreatment in pure N_2 was poor, only 23% of bagasse was solubilized. Positive effect of O_2 on the chemical composition of

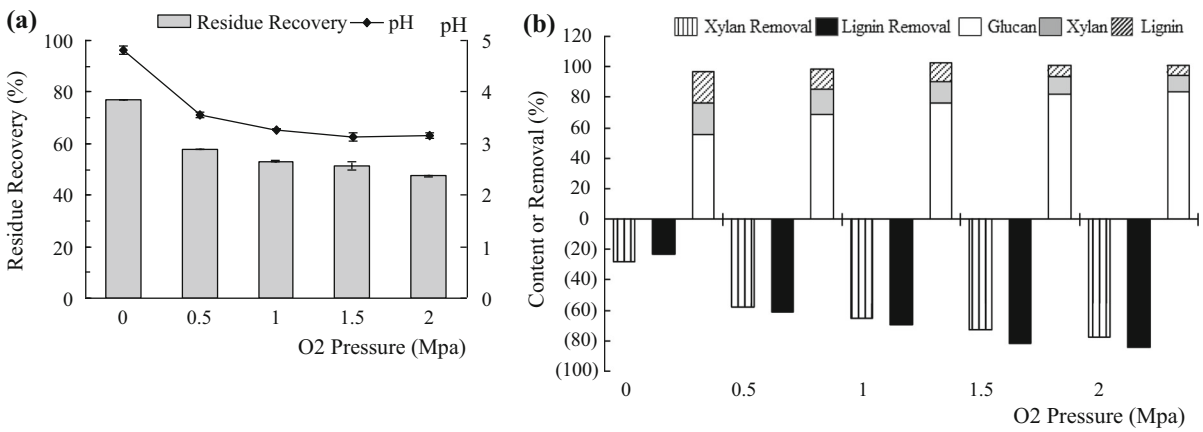

Fig. 3 Effect of O_2 pressure on residue recovery and pH of the hydrolysate (**a**) and the chemical composition of pretreated SCB (**b**) (50/50 ethanol/water (v/v), 0–2.0 MPa O_2, 80 min, 160 °C)

pretreated bagasse was shown in Fig. 3b. Removal of xylan and lignin were 28.2 and 23.0% respectively. Glucan content was enhanced from 42.2 to 55.2%. In inert gas atmosphere or without exogenous gas, organosolv pretreatment of bagasse usually needed severe conditions. Wei et al. (2017) studied auto-catalyzed organosolv pretreatment of bagasse. After treated at 195 °C for 30 min in 40/60 ethanol/H_2O (v/v), only 49.8% of hemicellulose and 66.2% of lignin was removed while glucan content was enhanced from 43.7 to 66.2%. Area et al. (2009) also studied auto-catalyzed EOP of bagasse. After treated at 175 °C for 240 min in 50% (v/v) aqueous ethanol, residue recovery was 48.2 and 85.2% of lignin was removed. Even with the assistance of catalyst, organosolv pretreatment of bagasse needed harsh conditions. Agnihotri et al. (2015) investigated EOP of bagasse catalyzed by formic acid. 75% of lignin was removed after treatment at 175 °C for 90 min in 50% (v/v) aqueous ethanol (pH of the solution was adjusted to 3.5 by formic acid).

O_2 atmosphere dramatically improved the removal of xylan and lignin from bagasse. After treatment in 0.5 MPa O_2, 57.5% of xylan and 61.4% of lignin were removed respectively and glucan content increased to 68.7%, compared with 55.2% in pure N_2. With the existence of O_2, bagasse was partially oxidized to organic acids, which could accelerate xylan and lignin removal. Moreover, hydroxyl radical formed in O_2 atmosphere could also attack the aromatic ring and aryl ether bond contained in lignin and accelerate its removal (Ricq et al. 2000). Increasing O_2 pressure to 1.5 MPa significantly promoted removal of xylan and lignin to 72.9 and 81.5% respectively. Meanwhile cellulose content in pretreated pulp was enhanced to 82.1%. When O_2 pressure was increased to 2.0 MPa, cellulose content in the pulp only slightly enhanced to 83.8% (Fig. 3b). Thus, O_2 pressure should be 1.5 MPa with 81.5% lignin removal, 72.9% xylan removal and 82.1% glucan in the residue.

Effect of ethanol/water ratio

Ethanol/water (v/v) ratio varied from 70/30 to 0. Other conditions were: 1.0 g bagasse (1.5 g bagasse for wet oxidation pretreatment without ethanol), 20 mL aqueous ethanol solution, 1.5 MPa O_2, 160 °C, 80 min and magnetic stirring rate of 600 rpm. Ethanol/water ratio significantly influenced the pH of the hydrolysate. When ethanol/water (v/v) decreased from 70/30 to 30/70, the pH of the hydrolysate decreased from 3.7 to 2.7 (Fig. 4a). Ethanol and water had different auto-protolysis constants (Ks), Ks of ethanol and water were 19.1 and 14.0 respectively. In addition, the dielectric constant (ε) of water was 80.2, much higher than that of ethanol (25.0). So, even the same amount of acid showed different apparent pH in solutions with varied ethanol/water ratio. Increasing ethanol/water ratio led to higher pH (Bates et al. 1963). Moreover, ethanol/water ratio influenced the degradation of bagasse. High ethanol/water ratio decelerated the degradation of polysaccharides and lignin to monomers, and thus decelerated subsequent oxidation of monomers to organic acids. When ethanol/water was 70/30, residue recovery was 62.7%. Declining ethanol/water ratio accelerated the solubilization of bagasse. Residue recovery was reduced to 46.6% when ethanol/water was 40/60.

The chemical composition of pretreated pulp was also strongly related to ethanol/water ratio. Removal of xylan and lignin were respectively 43.7 and 56.1% when ethanol/water was 70/30. Decreasing ethanol/water ratio accelerated the solubilization of xylan and lignin. 82.9% of xylan and 83.3% of lignin were removed when ethanol/water was 40/60. Meanwhile, glucan content in the residue reached 86.3%, suggesting that 95.1% of cellulose was remained. Further decreasing ethanol/water ratio to 30/70 had little impact on the removal of xylan and lignin (Fig. 4b). However, without the assistance of ethanol, wet oxidation pretreatment could not efficiently remove lignin from SCB. Although xylan was entirely removed, the pretreated residue still consisted of 32.7% lignin, suggesting that only 11.6% lignin was removed. Therefore, the best conditions for the pretreatment are: temperature 160 °C, time 80 min, 1.5 MPa O_2 and ethanol/water (v/v) 40/60 with 46.6% residue recovery, 83.3% lignin removal, 82.9% xylan removal and 86.3% glucan in the residue. The sample pretreated at optimized conditions was characterized by FT-IR and SEM and was subjected to subsequent enzymatic hydrolysis.

Acid-catalyzed EOP of SCB was investigated by Wei et al. (2017). SCB was treated in 60/40 ethanol/water (v/v) at 190 °C for 60 min, with 5 wt% acetic acid used as catalyst. Glucan content was enhanced from 39.4 to 67.6%. Autocatalytic EOP of SCB was also studied. After treated with 40/60 ethanol/water (v/

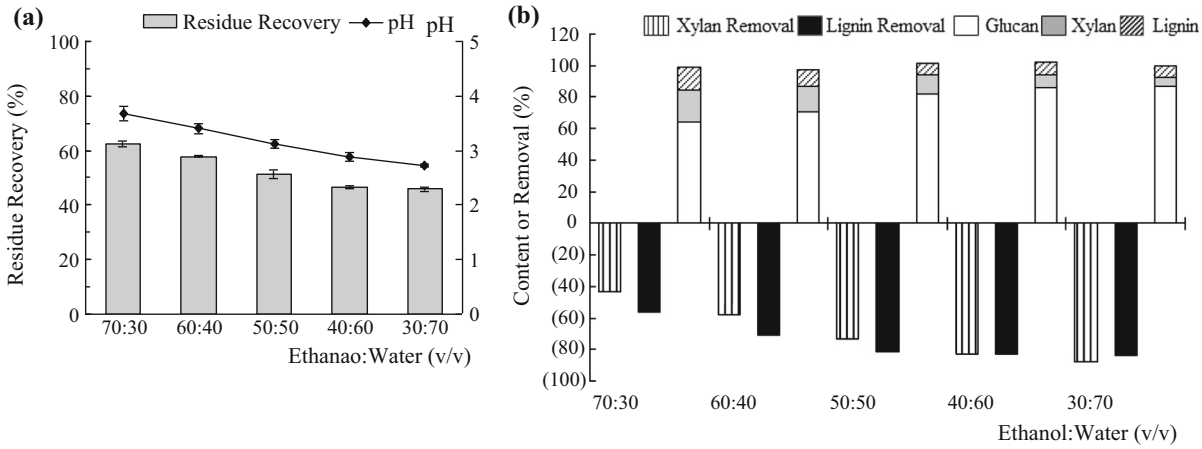

Fig. 4 Effect of ethanol/water ratio on residue recovery and pH of the hydrolysate (**a**) and the chemical composition of pretreated SCB (**b**) (ethanol/water (v/v) 70/30-0, 1.5 MPa O_2, 80 min, 160 °C)

v) at 195 °C for 30 min, glucan content was promoted from 43.7 to 66.2% (Zhang and Wu 2015). Thus, compared with these studies, much higher glucan content in the residue was obtained at milder conditions by O_2-assisted EOP due to higher removal of hemicellulose and lignin.

Characterization of pretreated bagasse

Figure S1 shows the photos and SEM images of untreated and pretreated bagasse. Lignocellulosic biomass treated in dilute acid or organic solvent often shows color of brown or dark brown, due to the change of chromophoric group in lignin on the surface of the sample. However, the color of treated sample in this study was close to white, indicating that O_2-assisted EOP had bleaching effect. The SEM image of treated bagasse indicated that due to high degree of xylan and lignin removal, the structure of cell wall was severely disrupted.

FTIR spectrum of sample pretreated under optimal conditions was presented in Fig. 5 and was compared with those of untreated bagasse and Avicel PH101. All spectra showed characteristic cellulose bands around 1000–1200 cm^{-1}(Cao and Tan 2004). Compared with raw sugar cane bagasse, no new peaks on the FTIR spectra were observed, indicating that no new functional groups were formed during the pretreatment process. The spectrum of pretreated sugar cane bagasse was similar to that of Avicel PH 101. Compared with untreated bagasse, main changes of the FTIR spectra of pretreated sugar cane bagasse were as follows.

The band at 1737 cm^{-1}, which was related to the stretching of unconjugated C=O group in hemicellulose (Zhang et al. 2011), was much lower than that in raw bagasse. Hemicellulose in raw bagasse contained uronic acid and acetyl group, leading to the absorption at 1737 cm^{-1}. After pretreatment, 82.9% of hemicellulose was removed and hemicellulose content decreased from 24. 6 to 8.0%. Along with the removal of hemicellulose, these groups were also removed and thus their absorption was reduced.

The bands at 1240, 1516 and 1636 cm^{-1} were attributed to C–O stretching of the guaiacyl ring (Liu et al. 2011), the stretching of the phenyl ring and the stretching of C=O in lignin and bending mode of the absorbed water respectively (Zhang et al. 2011). These groups were all contained in lignin. Absorptions at these wave numbers were all significantly alleviated compared with those in raw bagasse. And this was consistent with lower lignin content in pretreated bagasse (7.2%) than in untreated bagasse (20.5%).

Monosaccharides and degradation products in the hydrolysate

Total concentration of glucose and xylose was 4.97 mg/mL. The hydrolysate was also rich in organic acids, total amount of formic acid and acetic acid reached 5.42 mg/mL. Acetic acid was partly derived from the hydrolysis of acetyl group in hemicellulose.

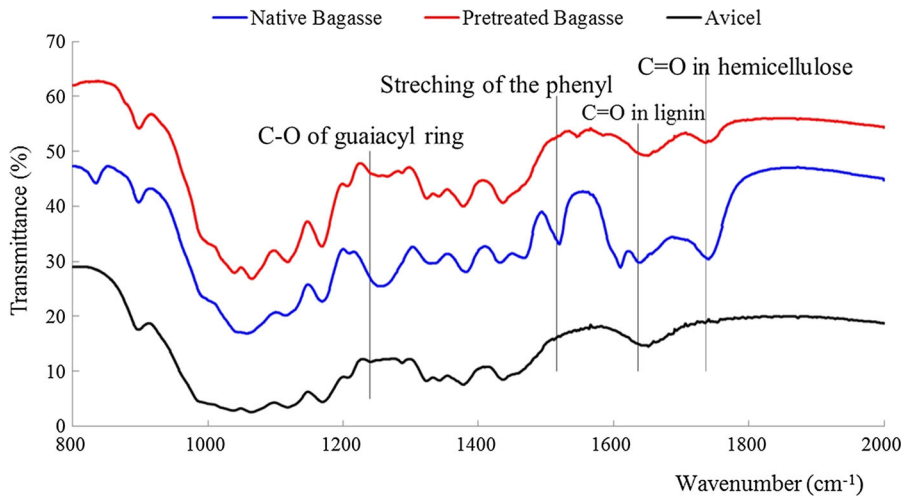

Fig. 5 FT-IR spectra of Avicel PH101 (black), raw (blue) and pretreated (red) bagasse (a: wavenumber at 400–4000 cm^{-1}; b: wavenumber at 800–2000 cm^{-1}). (Color figure online)

Moreover, oxidation of lignin, monosaccharides, furfural and HMF also produced acetic acid and formic acid. Furfural and HMF was not detected in the hydrolysate. These chemicals were rich in double bonds and were prone to be attacked by O_2. The highly acidic hemicellulose hydrolysate could be used for the production of furans without exogenous catalyst (Table 1).

Enzymatic hydrolysis

At low cellulase loading of 4.96 mg protein/g bagasse, we compared the enzymatic hydrolysis of untreated bagasse, O_2-EOP pretreated bagasse, N_2-EOP pretreated bagasse and Avicel PH101. As can be seen from Fig. 5, untreated bagasse was recalcitrant to enzymatic hydrolysis. Glucan digestibility was only 9.62% after 72-h reaction. N_2-EOP slightly improved the accessibility of SCB to cellulases, glucan digestibility slightly increased to 16.5%, in consistent with the limited removal of xylan and lignin by N_2-EOP. In comparison, 47.8% of glucan in O_2-EOP pretreated SCB was converted into glucose, which was 4.97 and 1.90 times of untreated bagasse and N_2-EOP pretreated bagasse. O_2-EOP pretreatment effectively removed 82.9% of xylan and 83.3% of lignin from bagasse, after xylan and lignin were removed, cellulose in bagasse became more accessible to cellulase. Meanwhile, the unproductive absorbance of cellulase by lignin was alleviated. At the same cellulase loading, 40.3% Avicel PH101 was converted into glucose, indicating that pretreated bagasse was more accessible

Table 1 Concentrations of monosaccharides and degradation products in the hydrolysate (temperature 160 °C, time 80 min, 1.5 MPa O_2, ethanol/water (v/v) 40/60)

	Concentration (mg/mL)	Yield (g per one kg bagasse)[a]
Glucose	0.689	11.0
Xylose	4.28	68.5
Formic acid	3.50	56.0
Acetic acid	1.92	30.7
Levulinic acid	< 0.01	–
5-Hydroxylmethylfurfural	< 0.01	–
Furfural	< 0.01	–

[a]The volume of the hemicellulose hydrolysate was 16.0 mL

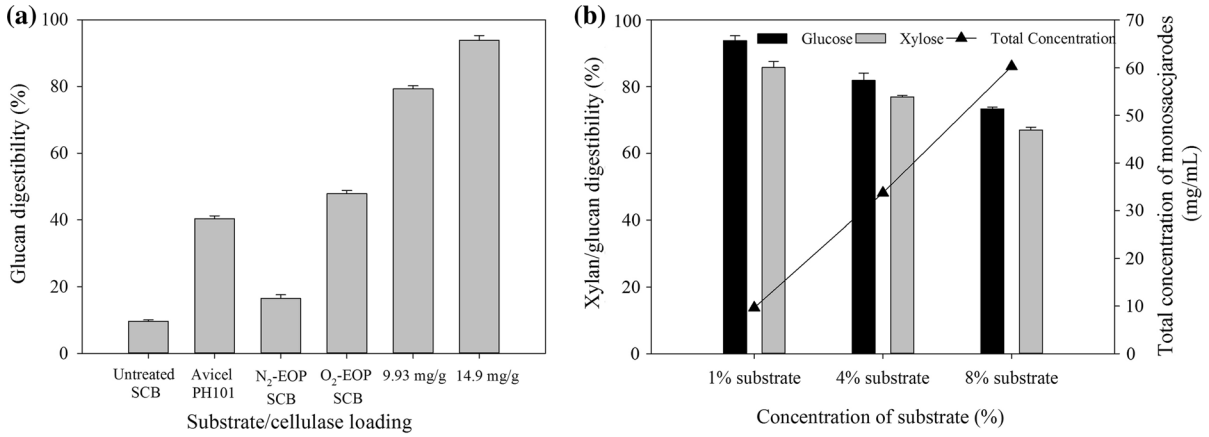

Fig. 6 Enzymatic hydrolysis of samples (**a** 1% solid loading, 4.96–14.9 mg protein/g bagasse, 72 h, 50 °C and rotation rate of 150 rpm; **b** 1–8% solid loading, 14.9 mg protein/g bagasse, 72 h, 50 °C and rotation rate of 150 rpm)

to cellulase than Avicel PH101. Avicel PH101 was prepared by removing amorphous cellulose from α-cellulose using dilute-acid hydrolysis. So it had high degree of crystallinity (about 0.77) and polymerization (> 200) (Gupta and Lee 2009). And this limited its hydrolysis by cellulase. On the other side, Evtuguin et al. (1999) found that when residual lignin was similar, the degree of polymerization of pulp prepared by O_2-organosolv treatment was two times lower than that prepared by sulfate pulping. During O_2-organosolv treatment, both organic acid and free radical caused breaking of glycosidic bond.

Increasing cellulase loading greatly improved the enzymatic hydrolysis. At cellulase loading of 9.93 mg protein/g bagasse, glucan digestibility reached 79.3%. High glucan digestibility of 93.3% were obtained at cellulase loading of 14.9 mg protein/g bagasse. Zhang and Wu (2015) investigated EOP of bagasse by using acetic acid as catalyst. Bagasse was treated in 60/40 (v/v) ethanol/water solution at 190 °C for 1 h. The catalyst was 5% acetic acid. After the treatment, content of xylan and acid-insoluble lignin were decreased from 21.6 and 24.3 to 12.8 and 16.7% respectively. The enzymatic hydrolysis of treated bagasse was evaluated. Cellulase loading was 20 FPU/g of bagasse, solid loading was 2%. After 72-h reaction, glucan digestibility reached 91.4%. Compared with their research, we obtained higher glucan digestibility by much milder pretreatment.

Increasing sugar concentration in the hydrolysate could yield higher concentration of fermentation products such as ethanol, 1-butanol, etc., which is necessary for industrial practice. Hence, we investigated the effect of solid loading on the enzymatic hydrolysis of pretreated bagasse. As shown in Fig. 6b, with increasing solid loading, glucan digestibility decreased. Kristensen et al. (2009) investigated the role of different factors including insufficient mixing, lignin and hemicellulose-derived inhibitors, product inhibition (glucose and cellobiose) and adsorption of cellulases in the decrease of glucan digestibility with increasing solid loading (5–30%). It was found that the inhibition of cellulase adsorption to cellulose was the main factor causing decreased glucose yield. Moreover, Xue et al. (2015) found that oligosaccharides with higher degree of polymerization inhibited the performance of CeTec2 during the hydrolysis of 25% corn stover pretreated by AFEX. However, even when cellulase loading reached 8%, glucan digestibility was more than 70%. Concentration of glucose in the hydrolysate was 8.84, 30.9 and 55.3 mg/mL respectively, when solid loading was 1, 4 and 8%. The residual xylan contained in the pretreated SCB was also hydrolyzed into xylose by xylanases in the enzyme solution and total concentrations of glucose and xylose reached 9.64, 33.7 and 60.3 mg/mL respectively.

Conclusions

O_2-assisted EOP could efficiently remove xylan and lignin from sugarcane bagasse at mild conditions. After treatment in 40/60 (v/v) ethanol/water and

1.5 MPa O_2 at 160 °C for 60 min, 82.9% xylan and 83.3% lignin were removed. Glucan content in the residue reached 86.3%, much higher than results obtained by autocatalytic EOP or acid-assisted EOP. Besides, this process didn't cause severe degradation of cellulose, 95.1% of glucan was remained in the solid residue. The enzymatic accessibility of bagasse was greatly improved. At cellulase loading of 4.96 mg protein/g bagasse, glucan digestibility from the pretreated bagasse was 4.97 times the yield of untreated bagasse and higher than that from Avicel PH101. Glucan digestibility reached 93.3% at 14.9 mg protein/g bagasse and total concentrations of glucose and xylose reached 60.3 g/L when solid loading was enhanced to 8%.

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