RSC Advances

PAPER

View Article Online
View Journal | View Issue

Cite this: *RSC Adv.*, 2016, **6**, 99455

Combined pretreatment of lignocellulosic biomass by solid base (calcined Na_2SiO_3) and ionic liquid for enhanced enzymatic saccharification

Xiyan Sun,*[abc] Xitong Sun[d] and Fan Zhang[ab]

Received 2nd September 2016
Accepted 12th October 2016

DOI: 10.1039/c6ra22055j

www.rsc.org/advances

A novel method combining a solid base catalyst (Na_2SiO_3) and a cheaper ionic liquid (1-butyl-3-methylimidazolium, [BMIm]Cl), was proposed and used for the pretreatment of lignocellulosic biomass such as spruce, willow and soybean straw. The addition of calcined Na_2SiO_3 in [BMIm]Cl pretreatment significantly destroyed the recalcitrant cell wall architecture, removed lignin and hemicellulose, decreased cellulosic crystallinity, and strongly broke lignocellulosic morphology, which enhanced the biomass accessibility for enzymatic hydrolysis. The combined pretreatment seemed more suitable for willow and soybean straw than spruce. According to single factor experiments, the maximum enzymatic hydrolysis yield and glucose yield of willow were 98.6% and 39.5 g/100 g biomass, respectively, 2.6-fold of single pretreatment with [BMIm]Cl.

1 Introduction

Lignocellulosic biomass, including softwood, hardwood, herbaceous and agricultural crops waste, is considered as a promising renewable feedstock to meet the fuel and chemical shortages of today and tomorrow.[1,2] The above materials are mainly composed of three types of polymers: cellulose, hemicellulose and lignin.[3] Among them, cellulose, as the most abundant component in lignocellulosic biomass, is a linear homopolymer of glucose and contains numerous inter- and intramolecular hydrogen bonds, which results in the resistance to cellulose dissolving in water and traditional organic solvents. Hemicellulose is one of branched short-chain polymer of xylose and other sugars around cellulose fibers. Lignin is a highly cross-linked aromatic polymer surrounding cellulose and hemicellulose, and is recognized as a major barrier for the enzymatic conversion of biomass.[4] Therefore, pretreatment process is considered as a critical step for increasing the porosity of cellulose and breaking or removing lignin, with the purpose of improving the utilization of biomass.

Nowadays, many pretreatment methods have been developed to improve the degradation of lignocellulosic materials, including physical- (grinding, milling, extrusion and microwave), chemical- (acid, alkali, organosolv and ionic liquid), physico-chemical- (steam explosion, CO_2 explosion and ammonia fiber explosion) and biological pretreatments.[5] Among them, pretreatment by ionic liquids received much attention as it facilitates the release of fermentable sugars from biomass.[6] Ionic liquids (ILs) such as 1-butyl-3-methylimidazolium chloride ([BMIm]Cl) or 1-ethyl-3-methylimidazolium acetate ([EMIm]OAc) are regarded as green solvents to pretreat lignocellulosic biomass within short period because of the excellent properties such as low vapor, high chemical and thermal stability below the temperature of 140 °C,[7] non-flammability and great solvent power, compared with traditional molecular solvents.[8,9] The dissolution of lignocellulosic biomass in ILs improves enzymatic saccharfication through two possible ways: it breaks down the crystalline lattice of cellulose and it removes lignin.[10]

The major drawback of ILs pretreatment is the high cost consumption.[11] Up to now, [EMIm]OAc is found as the most effective ILs for biomass pretreatment, but it's price is up to $50/kg, which occupies half of the cost during the conversion of biomass to ethanol and is hard to be commercialized.[12] Meanwhile, [BMIm]Cl costs only 1/60 of [EMIm]OAc, although it shows less effective in lignocellulose pretreatment.[12] Therefore, developing a general method to improve the performance of cheaper ILs has significant practical meaning. Recently, several methods of combining acids/bases (HCl, H_2SO_4, ammonia and NaOH) with ILs were employed for the pretreatment or fractionation of the lignocellulosic biomass, and exhibited a good performance for the enhancement of the sugar yield.[13–16] A combination of dilute H_2SO_4 and [AMIm]Cl was used for the pretreatment of bagasse.[13] Most of hemicellulose was first removed by dilute H_2SO_4, and a significant higher glucose yield

[a]*Biomass Group, Key Laboratory of Tropical Plant Resources and Sustainable Use, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Kunming, Yunnan 650223, P. R. China. E-mail: sunxiyan168@gmail.com; Fax: +86-871-65137468; Tel: +86-871-65137468*

[b]*University of Chinese Academy of Sciences, 19A Yuquan Road, Beijing 100049, P. R. China*

[c]*Department of Chemistry and Biochemistry, University of California-Los Angeles, Los Angeles, California 90095, USA*

[d]*Key Laboratory of Biobased Materials, Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, Qingdao, Shandong 266101, P. R. China*

of 95.5% was achieved. Geng *et al.* combined NaOH extraction and [BMIm]Cl for the pretreatment of corn stover, with glucose yield of 96% achieved after 5 h hydrolysis.[16] However, these two combined pretreatment was performed in separated steps, while residual acid or alkali in the biomass after pretreatment should be neutralized before the next step. Homogeneous acid or base catalysts could not be reused, which further increased the treatment cost. So it is necessary to replace homogeneous catalyst with a heterogeneous catalyst for the pretreatment process.

In our previous work, calcined sodium silicate (Na_2SiO_3) was studied as a heterogeneous catalyst to synthesize biodiesel, with the advantages of (1) safety: non-volatile, non-corrosive, non-toxic and chemically stable,[17] (2) lower cost: the lower average price of $150/ton (costs only 1/3 of the extensively studied alkali of NaOH), (3) easily available: preparation using an easy calcination technology,[18] (4) strong basic activity: the total basicity is 15-fold higher than that of CaO (total basicity: 0.290 mmol of CO_2/g) which exhibits a strong basic strength,[18–21] and (5) reusability: the remaining catalyst can be recovered by simple centrifugation from reactants and products after reaction and used to catalyze the next batch of reaction.[22] The lost basicity of Na_2SiO_3 after several runs can be easily recovered by calcining spent Na_2SiO_3 with NaOH at 400 °C.[22] This made calcined Na_2SiO_3 a good choice to solve the shortcomings of traditional alkali pretreatment. According to our best knowledge, the combination of solid base catalyst and ILs has not been reported for the biomass pretreatment before. Therefore, a novel method was proposed in this study, which combined solid base catalyst (Na_2SiO_3) with cheaper ionic liquid ([BMIm]Cl) to pretreat lignocellulosic biomass (spruce, willow and soybean straw) at mild conditions. Reaction conditions were investigated to obtain high enzymatic hydrolysis and glucose yield. The correlation between the structural or chemical characteristics of pretreated biomass and saccharification results was discussed to explore the mechanism of this combined pretreatment.

2 Materials and methods

2.1 Materials

Spruce, willow and soybean straw were regarded as typical softwood, hardwood and crop residue biomass, respectively. They were obtained from nearby farms in Zhengzhou, Henan province in China, and were used as raw materials for lignocellulosic pretreatment and enzymatic hydrolysis. All three biomass was milled to 200–300 mesh and then dried in an oven (WFO-710, EYELA, Toky Rikakikai Co, Ltd, Tokyo, Japan) at 105 °C for 24 h. Sodium silicate nonahydrate ($Na_2SiO_3 \cdot 9H_2O$, 19.3–22.8 wt% Na_2O, weight ratio of $Na_2O/SiO_2 = 1.03 \pm 0.03$) was supplied by Xilong Chemical Co. Ltd. (Shantou, China). Na_2SiO_3 powder was calcined at 400 °C for 2 h, ball-milled to 200–300 mesh (SHQM-0.4L, Chunlong Petroleum Instrument Co., Ltd, Lianyungang, Jiangsu, China), and then dried at 105 °C for 24 h before use. [BMIm]Cl, which was heated at 90 °C for 30 min before use, was obtained from Shanghai Chengjie Chemical Co. Ltd. (Shanghai, China). Celluclast 1.5L® (from *Trichoderma reesei* ATCC 26921, 89.4 FPU mL^{-1}), Novozyme 188 (from *Aspergillus niger*, 777 CBU mL^{-1}), and standard sugars of glucose, xylose, arabinose, mannose and galactose (purity > 99%) were purchased from Sigma-Aldrich (Shanghai, China). The cellulase activity (FPU, CBU) was determined by the method proposed by Adney and Baker.[23] Deionized water with electrical conductivity of 18.2 MΩ cm was obtained by a water purification system (Milli-Q Academic, Merck Millipore, Darmstadt, Germany). All other chemicals were analytical grade and purchased from Xilong Chemical Co. Ltd. (Shantou, China).

2.2 Biomass dissolution and regeneration

For a typical pretreatment reaction, 0.25 g of biomass powder was added into 5.0 g of [BMIm]Cl in a 25 mL glass-stoppered test tube equipped with a magnetic bar, which was then fully mixed by whirlpool mixer. The mixed reactant was heated to 120 °C in oil bath under stirring (200 rpm) and remained for 10 min. A setting dosage of calcined Na_2SiO_3 powder (1, 3, 5, 7 or 10 g/100 g IL) was then dispersed into the above biomass solution, and treated at 120 °C for a certain time (0.5, 1.0, 1.5, 2.0, 2.5 or 3.0 h).

The pretreated biomass was regenerated by adding 20 mL deionized water at 90 °C and vigorously shaking for 10 s on whirlpool mixer. The precipitated biomass was transferred into a beaker with 50 mL fresh deionized water at 70 °C, and washed thoroughly with deionized water to remove the residual solvent. The regenerated biomass was freeze-dried for 24 h (Eyela 1200 freeze dryer; Tokyo Rikakikai Co, Ltd, Tokyo, Japan) for the following enzymatic hydrolysis. The Na_2SiO_3 and [BMIm]Cl in residual solvent were recovered by distillation at reduced pressure. The recovery yield of regenerated biomass was calculated as follows:

$$\text{Recovery yield}_{\text{biomass}}\ (\%) = \frac{\text{mass of recovered biomass (g)}}{0.25\ \text{g}} \times 100\% \quad (1)$$

2.3 Enzymatic hydrolysis

In a typical run, enzymatic hydrolysis of pretreated and untreated biomass was conducted in a 50 mL Erlenmeyer flask containing 10 mL sodium citrate (50 mM, pH 4.8) reaction buffer, with a substrate concentration of 0.32% (w/v) and cellulase dosage of 0.05 FPU mg^{-1} biomass. The cellulase mixture consisted of Celluclast 1.5L® and Novozyme 188 at a volume ratio of 1 : 1, and the activity of this cellulase mixture was determined according to the National Renewable Energy Lab method.[23] The samples were incubated for 3, 6, 12, 24, 48 and 72 h at 50 °C with shaking at 100 rpm. Tetracycline (400 μg) and cycloheximide (300 μg) were added to prevent bacterial growth during digestion. After enzymatic hydrolysis, 150 μL of the supernatant was transferred from the product mixture to a 1.5 mL Eppendorf centrifuge tube, and centrifugated at 12 000 rpm for 10 min. The concentration of glucose in samples was measured in triplicate using a biosensor analyzer (SBA-40D, Shandong Key Laboratory of Biosensor, Jinan, China) with immobilized glucose-oxidase membranes. Each sample was

diluted to the concentration of 0–100 mg dL^{-1} before analysis. The hydrolysis yield, glucose yield and glucose release rate were determined using the following equations:[24]

$$\text{Hydrolysis yield (\%)} = \frac{\text{mass of glucose in hydrolysis reaction (g)} \times 0.9}{\text{mass of cellulose in regenerated biomass (g)}} \times 100\% \quad (2)$$

$$\text{Glucose yield (g/100 g)} = \frac{\text{mass of glucose in hydrolysis reaction (g)}}{\text{mass of raw biomass (g)}} \times 100 \quad (3)$$

$$\text{Glucose release rate (\%)} = \frac{\text{mass of glucose in hydrolysis reaction (g)}}{\text{mass of cellulose in raw biomass (g)} \times \frac{1}{0.9}} \times 100\% \quad (4)$$

2.4 Characterization of untreated and pretreated lignocellulose

2.4.1 Chemical composition. Chemical composition of biomass was characterized according to the National Renewable Energy Laboratory (NREL) procedure.[25] In brief, 0.3000 g of dried biomass was treated with 3 mL of 72% H_2SO_4 for 1 h at 30 °C. Next, the mixture was diluted with 84 mL of deionized water and further hydrolyzed at 121 °C for 1 h in an autoclave. The hydrolysate was separated from solid residue by filtering, and the sugar content in the neutralized filtrate was determined by high performance liquid chromatography (HPLC-20A, Shimadzu, Japan) equipped with Aminex Hi-Plex Pb column (Bio-Rad, USA). HPLC analysis was performed at 70 °C using deionized water as mobile phase and a flow rate of 0.5 mL min^{-1}. The content of acid soluble lignin in the filtrate was analyzed by an ultraviolet (UV)-visible spectrophotometer (UV 1800, Shimadzu, Japan).[25] The residue after acid hydrolysis was collected for the measurement of the content of ash and acid insoluble lignin.[25] The recovery yields of cellulose, hemicellulose and lignin after [BMIm]Cl or [BMIm]Cl + Na_2SiO_3 pretreatment were determined as follows:

$$\text{Recovery yield}_{\text{components}} \text{ (\%)} = \frac{\text{mass of component after regeneration}}{\text{mass of component in raw biomass}} \times 100\% \quad (5)$$

2.4.2 Morphology. The morphology of untreated and pretreated biomass samples was characterized by a scanning electron microscopy (SEM, Hitachi s-3400N, Japan) at ×10 000 magnification and accelerating voltage of 30 kV. Before imaging, the samples were first sputter-coated with gold to make the samples conductive and avoid possible degradation of photo quality.

2.4.3 Crystallinity. The crystallinity index (CrI) of samples was analyzed by X-ray diffraction (XRD, Rigaku TTR III, Japan) at 40 kV and 200 mA. Cu radiation ($\lambda = 1.54$ Å) was used to scan over diffraction angle ($2\theta^\circ$) of 5–45° with a step of 0.05°. CrI was calculated by the following equation:[26]

$$\text{CrI (\%)} = \frac{I_{002} - I_{\text{am}}}{I_{002}} \times 100\% \quad (6)$$

where I_{002} was the highest peak intensity at diffraction angle of 22.5°, and I_{am} was the peak at 18° for amorphous cellulose.

2.4.4 Specific surface area. The specific surface area (SSA) of samples was measured on Tristar II 3020 (Micromeritics Instrument Co. Ltd, Northcross, GA, USA) using the Brunauer–Emmett–Teller (BET) method.[27] The analysis was performed using nitrogen as adsorbate within a relative pressure range of 0.05–0.985, while samples were degassed at 100 °C for 3 h before analysis.

2.4.5 Thermogravimetric analysis. Thermogravimetric analysis (TGA) of untreated and pretreated biomass was conducted on thermogravimetric and differential scanning calorimetry synthetic analyzer (Model STA449F3, NETZSCH, Germany). Sample (20 mg) was heated from 38 to 800 °C at a constant heating rate of 10 °C min^{-1} under nitrogen atmosphere.

2.5 Kinetics of enzymatic hydrolysis

The kinetics of enzymatic hydrolysis was simulated using an empirical equation according to the previous literature, which was given as follow:[28]

$$X = \frac{1}{k} \times \ln(1 + k \times v_0 \times \tau) \quad (7)$$

where X (%) was hydrolysis yield, k is the rate retardation constant that was also related to the change of hydrolysis rate, v_0 (%/h) was the initial hydrolysis rate, and τ (h) was the hydrolysis time. Based on this equation, the experimental data of hydrolysis yields and times were used to determine the values of k and v_0 *via* nonlinear curve fitting with Origin software.

3 Results and discussion

3.1 Na_2SiO_3-associated [BMIm]Cl pretreatment of lignocellulosic biomass

A novel Na_2SiO_3-associated [BMIm]Cl pretreatment method ([BMIm]Cl + Na_2SiO_3) was proposed in this study. Spruce (softwood), willow (hardwood) and soybean straw (crop residue) were selected to evaluate the efficiency of this method. The recovery and chemical composition of biomass after [BMIm]Cl or [BMIm]Cl + Na_2SiO_3 pretreatment were investigated, with the results summarized in Table 1. Compared with [BMIm]Cl pretreatment, the recovery yield of spruce, willow and soybean straw after [BMIm]Cl + Na_2SiO_3 pretreatment were decreased by 17.8%, 17.5% and 23.9%, respectively. After [BMIm]Cl + Na_2SiO_3 pretreatment, most of cellulose was remained in the pretreated materials, while certain amounts of hemicellulose and lignin were destroyed and removed. From Table 1, it could be seen that the recovery of lignin in lignocellulose decreased by only 12.7–20.0% with [BMIm]Cl pretreatment. However, after [BMIm]Cl + Na_2SiO_3 pretreatment, lignin recovery decreased to 55.4–68.3%, although the cellulose part seemed less influenced. It implied that the addition of Na_2SiO_3 as a solid base benefited the removal of lignin and hemicellulose in the raw

Table 1 The chemical compositions and recovery yields of untreated, [BMIm]Cl-pretreated and [BMIm]Cl + Na_2SiO_3-pretreated biomass

Biomass species	Pretreatment methods[a]	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Recovery yield (%)			
					Regenerated biomass	Cellulose	Hemicellulose	Lignin
Spruce (softwood)	Untreated	39.3 ± 0.1	24.0 ± 1.5	30.7 ± 0.5	—	—	—	—
	[BMIm]Cl	41.9 ± 0.7	23.1 ± 0.9	30.3 ± 0.5	88.2 ± 1.9	94.1	85.0	87.3
	[BMIm]Cl + Na_2SiO_3	49.5 ± 0.8	24.1 ± 0.3	28.8 ± 0.1	72.5 ± 0.2	91.3	72.9	68.1
Willow (hardwood)	Untreated	37.5 ± 0.3	21.0 ± 1.6	26.8 ± 0.5	—	—	—	—
	[BMIm]Cl	39.1 ± 0.5	20.4 ± 0.1	23.9 ± 1.9	92.4 ± 0.4	96.3	89.5	82.5
	[BMIm]Cl + Na_2SiO_3	47.7 ± 0.1	17.2 ± 0.4	24.0 ± 1.8	76.2 ± 0.1	96.8	62.4	68.3
Soybean straw (crop residue)	Untreated	36.9 ± 0.1	25.0 ± 0.1	17.5 ± 0.2	—	—	—	—
	[BMIm]Cl	42.8 ± 2.8	25.2 ± 0.7	16.8 ± 0.2	83.3 ± 1.1	96.5	84.0	80.0
	[BMIm]Cl + Na_2SiO_3	52.3 ± 2.0	22.0 ± 0.1	15.4 ± 0.6	63.4 ± 2.6	89.7	55.6	55.4

[a] Other pretreatment conditions: biomass dosage, 0.25 g; [BMIm]Cl dosage, 5.0 g; Na_2SiO_3 dosage, 0 or 5.0 g/100 g IL; temperature, 120 °C; and time, 1 h.

lignocellulose. The content and chemical structure of lignin were reported to have great adverse influence on the competitive adsorption of hydrolysis substrate on enzymes,[29] while the removal of lignin and hemicellulose benefited the exposure of cellulose core of cell-wall microfibrils.[30] Therefore, it might improve the cellulose accessibility for enzymatic hydrolysis.[31]

The pretreated spruce, willow and soybean straw were then enzymatic hydrolyzed with the cellulase mixture (Celluclast 1.5L®: Novozyme 188 = 1 : 1, v/v) at 0.05 FPU mg^{-1} biomass. The results were shown in Fig. 1. The hydrolysis yields of untreated spruce, willow and soybean straw at 72 h were only 15.7%, 11.6% and 26.1%, respectively. In contrast, the hydrolysis yields of [BMIm]Cl-pretreated spruce, willow and soybean straw at 72 h were increased to 62.2%, 31.5% and 46.6%, respectively, which was in agreement with previous works on ionic liquid pretreatment.[32–35] When the combined pretreatment using [BMIm]Cl and Na_2SiO_3 was conducted, interesting results were observed. The hydrolysis yield of [BMIm]Cl + Na_2SiO_3-pretreated willow and soybean straw were 54.5% and 57.7%, which were improved to 1.7- and 1.2-fold compared with [BMIm]Cl-pretreated biomass, indicating that the removal of lignin and hemicellulose by Na_2SiO_3 benefited the enzymatic hydrolysis of cellulose. However, the hydrolysis yield of [BMIm]Cl + Na_2SiO_3-pretreated spruce at 72 h was slightly decreased to 59.3%. This result might have several interpretations: (1) it was assumed that spruce, as a typical kind of low density softwood, showed better accessibility to ionic liquids and cellulase than high density hardwood.[36] Therefore, [BMIm]Cl pretreatment was sufficient to destroy the construction of spruce. (2) Alkali pretreatment might bring both advantageous and disadvantageous effects on the enzymatic accessibility of biomass at the same time, according to the reaction severity. The proper conditions for the pretreatment of hardwood and herbaceous plant might become unfavorable for the pretreatment of softwood. (3) Lignin content was the key factor negatively correlated with enzymatic digestibility, and was significantly reduced during [BMIm]Cl + Na_2SiO_3-pretreatment of spruce. However, considering the complex structure of lignocellulosic biomass, comprehensive and interactive effects from many factors including the polymerization degree of cellulose, and cellulosic crystallinity, lignin chemical structure, lignin-carbohydrate complex, porosity, particle size, the integrity of cell wall architecture and others also naturally exist on the enzymatic digestibility of lignocellulose. Lignin content was a key but not the sole factor.[37–39] Therefore, it could be concluded that the combined pretreatment using [BMIm]Cl and Na_2SiO_3 was more efficient for willow and soybean straw than spruce.

The kinetics of enzymatic hydrolysis was simulated with an empirical equation reported in the literature.[40] As shown in Table 2, [BMIm]Cl pretreatment increased the initial hydrolysis rate v_0 of woody biomass (willow and spruce) by 50.6% and 1.7%, respectively, but decreased the v_0 of crop residue (soybean straw) by 12.4% compared to untreated biomass. In contrast, [BMIm]Cl + Na_2SiO_3 pretreatment decreased the v_0 of woody biomass (willow and spruce) by 38.7% and 24%, respectively, but increased the v_0 of crop residue (soybean straw) by 49.5% compared to [BMIm]Cl-pretreated biomass. In general, the initial hydrolysis rate (v_0) of cellulosic materials was determined by multiple influencing factors such as the concentration of the most easily degradable polysaccharides and the inhibition effects from impurities in hydrolysis substrates, while the increase or decrease in initial rate of biomass after pretreatment must result from the complex contribution of these factors. However, parameter k expressed the overall effects of various rateretarding factors during the whole hydrolysis process, such as enzyme inhibition, the orientation of cellulose, the formation of byproducts during hydrolysis and so on.[40] In Table 2, the rate retardation constant k of all three types of biomass decreased dramatically after pretreatments with [BMIm]Cl and [BMIm]Cl + Na_2SiO_3. After [BMIm]Cl pretreatment, k value of spruce, willow and soybean straw decreased by 82.8%, 73.3% and 57.1%, respectively compared to untreated biomass. For the [BMIm]Cl + Na_2SiO_3 pretreatment, k value of spruce, willow and soybean straw decreased by 20%, 58.3% and 16.7%, respectively compared to [BMIm]Cl-pretreated biomass. It indicated that the pretreatment enhanced the enzymatic hydrolysis of biomass mainly by alleviating multiple rateretarding factors during hydrolysis, and slowing down the decline in hydrolysis rate,

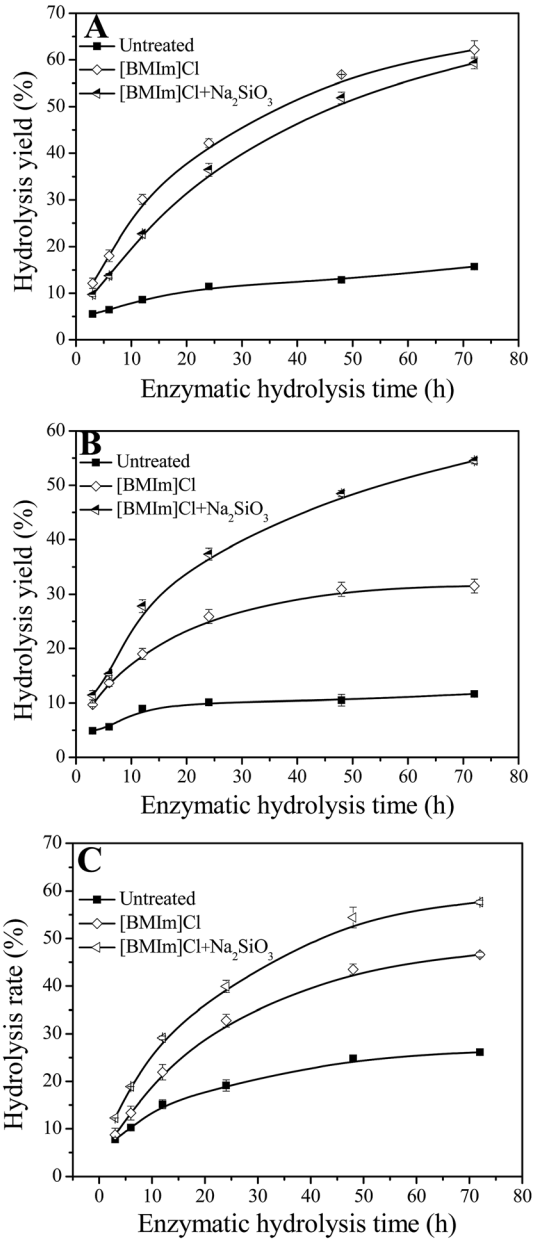

Fig. 1 Enzymatic hydrolysis of untreated, [BMIm]Cl-pretreated and [BMIm]Cl + Na_2SiO_3-pretreated spruce (A), willow (B) and soybean straw (C) (pretreatment conditions: biomass dosage, 0.25 g; [BMIm]Cl dosage, 5.0 g; Na_2SiO_3 dosage, 0 or 5.0 g/100 g IL; temperature, 120 °C; and time, 1 h; enzymatic hydrolysis conditions: biomass dosage, 0.32% (w/v); cellulase mixture, 0.05 FPU mg^{-1} biomass; temperature, 50 °C; and time, 72 h).

which was accordance with previous biological pretreatment with the selective white-rot fungi (*Echinodontium taxodii* 2538) on enzymatic hydrolysis of two native woods: Chinese willow (hardwood) and China-fir (softwood) reported by H. Yu *et al.*[40]

3.2 Characterization of regenerated biomass

As discussed in Section 3.1, the improvement of lignocellulose accessibility by pretreatment had strong correlation with the altered chemical composition or physical structure of biomass.

Table 2 Kinetics parameters of enzymatic hydrolysis of untreated, [BMIm]Cl-pretreated and [BMIm]Cl + Na_2SiO_3-pretreated biomass

Pretreatment methods[a]	Initial hydrolysis rate v_0 (%/h)	Rate retardation constant k	R^2
Spruce (softwood)			
Untreated	3.48 ± 0.85	0.29 ± 0.03	0.97
[BMIm]Cl	5.24 ± 0.44	0.05 ± 0.003	0.99
[BMIm]Cl + Na_2SiO_3	3.21 ± 0.24	0.04 ± 0.002	0.99
Willow (hardwood)			
Untreated	6.39 ± 3.18	0.45 ± 0.07	0.92
[BMIm]Cl	6.50 ± 1.24	0.12 ± 0.01	0.98
[BMIm]Cl + Na_2SiO_3	4.94 ± 0.53	0.05 ± 0.004	0.99
Soybean straw (crop residue)			
Untreated	4.36 ± 0.50	0.14 ± 0.008	0.99
[BMIm]Cl	3.82 ± 0.45	0.06 ± 0.005	0.99
[BMIm]Cl + Na_2SiO_3	5.71 ± 0.60	0.05 ± 0.003	0.99

[a] Other pretreatment conditions: biomass dosage, 0.25 g; [BMIm]Cl dosage, 5.0 g; Na_2SiO_3 dosage, 0 or 5.0 g/100 g IL; temperature, 120 °C; and time, 1 h.

To get a better understanding of [BMIm]Cl + Na_2SiO_3 pretreatment, the morphology, cellulosic crystallinity, specific surface area and thermal degradation feature of all three biomass before and after pretreatment were characterized by SEM, XRD, BET and TGA.

Fig. 2 showed the SEM images of spruce, willow and soybean straw. Untreated lignocellulose exhibited an intact or unbroken cell wall architecture, namely invisible cellulose microfibrils bundled or enveloped by a coating layer, which mainly contained lignin and hemicellulose (Fig. 2, A/B/C-untreated). After [BMIm]Cl pretreatment, the coating layer was significantly removed or delocalized, with more cellulose microfibrils exposed to the outside, which resulted in a relatively rough and broken surface (Fig. 2, A/B/C-[BMIm]Cl). However, after [BMIm]Cl + Na_2SiO_3 pretreatment, it seemed that the surface architecture of biomass materials was destroyed to a higher extent, and resulted in a highly rough and irregular morphology with porous texture (Fig. 2, A/B/C-[BMIm]Cl + Na_2SiO_3). This result demonstrated that ionic liquid and Na_2SiO_3 played an important role in destroying the coating layer structure of lignocellulosic biomass, by removing lignin and hemicellulose.[41,42] It improved the cellulosic accessibility for enzymatic hydrolysis.

Fig. 3 showed the X-ray diffraction patterns and BET results of untreated, [BMIm]Cl-pretreated and [BMIm]Cl + Na_2SiO_3-pretreated biomass. In the XRD pattern of untreated material, two typical peaks were observed at angles of around 15 and 22.5°, corresponding to (101) and (002) planes of cellulose I.[43] In the XRD pattern of [BMIm]Cl-pretreated willow and soybean straw, the intensities of these two peaks were significantly reduced, which demonstrated the destroy of crystalline structure of cellulose. But for [BMIm]Cl-pretreated spruce, the peaks at around 15 and 22.5° disappeared, and a strong peak at 21° was observed, which was attributed to the feature of cellulose II. The transition from cellulose I to cellulose II increased the

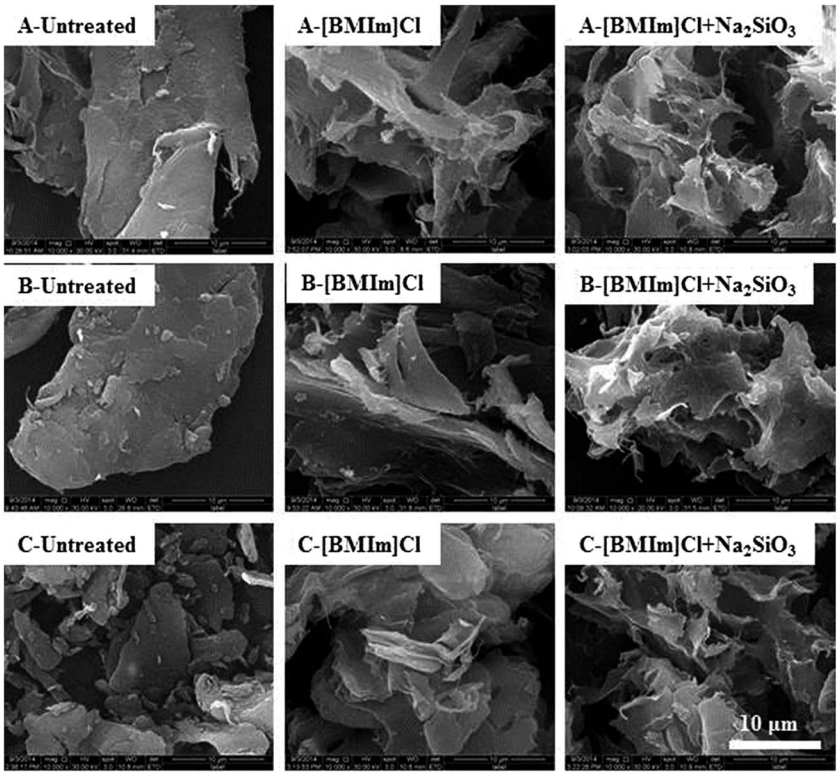

Fig. 2 SEM images of untreated, [BMIm]Cl-pretreated and [BMIm]Cl + Na_2SiO_3-pretreated spruce (A), willow (B) and soybean straw (C) at 10 000× magnification.

spacing between the stacked sheets of cellulose molecules,[44] which resulted in a lower ordered structure and less recalcitrant to enzymatic digestibility.[45] In the XRD pattern of [BMIm]Cl + Na_2SiO_3-pretreated biomass, the crystalline structure of cellulose in the three biomass all transformed from cellulose I to cellulose II.

The CrI value and specific surface of untreated and pretreated biomass were also calculated or determined, with the results listed in Fig. 3. Comparing with untreated samples, the CrI of [BMIm]Cl-pretreated spruce, willow and soybean straw decreased from 46.7%, 40.1% and 41.6–29.1%, 34.3% and 28.2%, respectively, with specific surface hardly changed. The addition of Na_2SiO_3 in [BMIm]Cl-assisted biomass pretreatment further decreased the CrI value to 24.9% and 23.3% for willow and soybean straw, but it seemed less efficiently for the pretreatment of spruce, which could be a possible reason for the lower enzymatic hydrolysis yield of [BMIm]Cl + Na_2SiO_3-pretreated spruce than [BMIm]Cl-pretreated spruce. Meanwhile, the addition of Na_2SiO_3 slightly increased the specific surface of willow and soybean straw, but was less pronounced for the increase of porosity for spruce.

The thermal degradation analysis of pretreated biomass was also performed by TGA, with the results listed in Fig. 4. In Fig. 4, the left vertical axis was the weight change of analysis sample as the function of heating temperature, while the right vertical axis showed the 1st derivative of weight loss in the left vertical axis. The differential thermal gravity (DTG) curve of unpretreated and pretreated biomass showed a strong peak at 230–370 °C with a shoulder at 200–270 °C, which was attributed to the characteristics of the pyrolysis of cellulose and hemicellulose, respectively.[46] The temperature range for the mass loss of lignin part was relatively broaden. It started at the temperature lower than 250 °C, and extended to the temperature of >600 °C.[46] As shown in Fig. 4, although the [BMIm]Cl and [BMIm]Cl + Na_2SiO_3 pretreatment provided different influences on the thermal degradation behaviors of different biomass materials, some common rules were also observed. Firstly, the mass loss of [BMIm]Cl- and [BMIm]Cl + Na_2SiO_3-pretreated biomass at 200–270 °C decreased, attributed to the removal of some easily hydrolyzed components such as hemicellulose, according to the analysis from Perez-Pimienta *et al.*[47] Secondly, the temperature for the main DTG peak of [BMIm]Cl- and [BMIm]Cl + Na_2SiO_3-pretreated biomass at 350–370 °C seemed migrating to higher temperature, which illustrated the improvement of thermal stability of cellulose part as the removal of hemicellulose and lignin.

3.3 Influencing factors of enzymatic hydrolysis

In order to determine the performance of combined pretreatment with both [BMIm]Cl and Na_2SiO_3, the influencing factors of pretreatment and enzymatic hydrolysis, including Na_2SiO_3 dosage, pretreatment time, enzyme loading and enzymatic hydrolysis time, were also studied by single factor experiments, and the willow served as unmanageable biomass. The results were presented in Fig. 5–8.

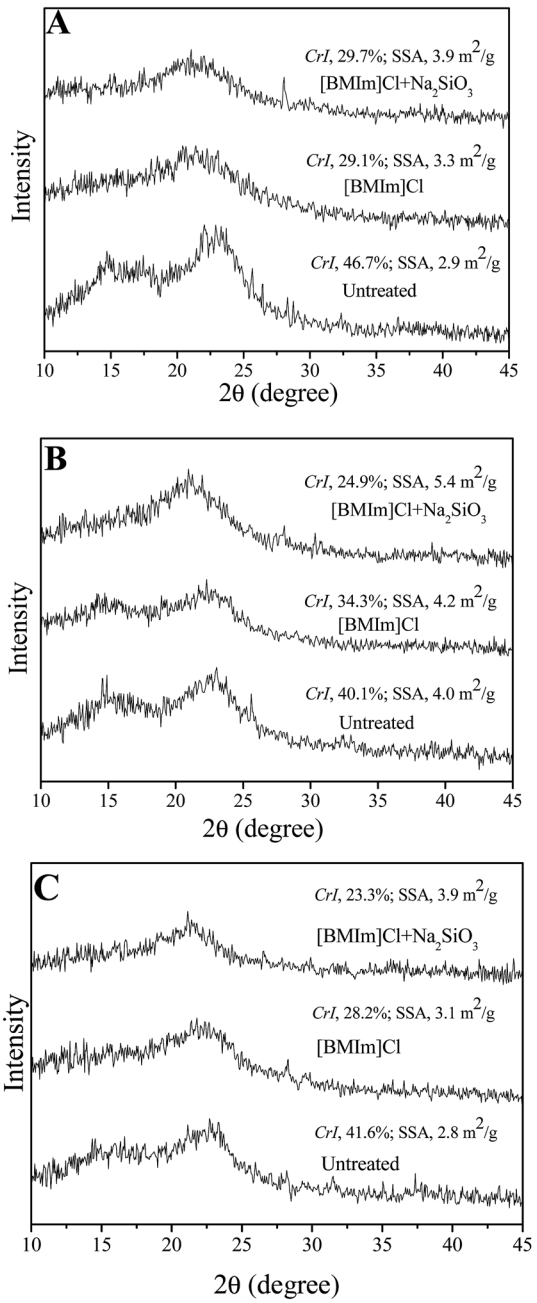

Fig. 3 X-ray diffraction patterns, crystallinity index (CrI) and specific surface area (SSA) of untreated, [BMIm]Cl-pretreated and [BMIm]Cl + Na_2SiO_3-pretreated spruce (A), willow (B) and soybean straw (C).

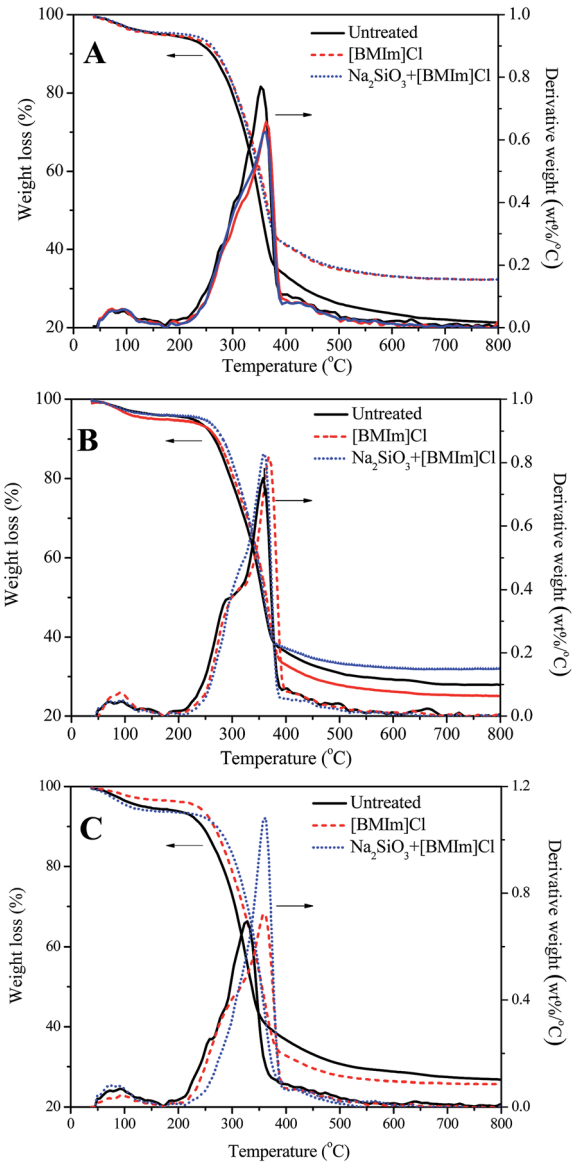

Fig. 4 TG curves of untreated, [BMIm]Cl-pretreated and [BMIm]Cl + Na_2SiO_3-pretreated spruce (A), willow (B) and soybean straw (C).

As shown in Fig. 5, willow was pretreated using [BMIm]Cl + Na_2SiO_3 system with Na_2SiO_3 dosage of 1, 3, 5, 7 and 10 (g/100 g IL), and 5.0 g of [BMIm]Cl at 120 °C for 1 h, and the regenerated solid was then enzymatic hydrolyzed. The hydrolysis yield was firstly increased and then decreased as Na_2SiO_3 dosage increased from 1 to 10 g/100 g IL, with the highest hydrolysis yield of 56.4% at Na_2SiO_3 dosage of 7 g/100 g IL. The decrease of hydrolysis yield at high Na_2SiO_3 dosage might be due to the difficulty in mixing the liquid reactants with high concentration of catalyst under magnetic stirring, while the decline in product yield with high ratio of solid catalyst to liquid reactant was also observed by Zhang *et al.*[19]

As shown in Fig. 6, the [BMIm]Cl + Na_2SiO_3 system was performed with different pretreatment time of 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 h. High hydrolysis yield of over 65% was achieved after pretreatment time of 0.5 h, while the increase of pretreatment time from 0.5 to 2.5 h gradually but significantly increased the hydrolysis of pretreated willow.

Cellulase mixture with a volume ratio of Celluclast 1.5L to Novozyme 188 of 1 : 1 was used for the hydrolysis of [BMIm]Cl + Na_2SiO_3-pretreated willow, while different enzyme usages of 0.05, 0.15, 0.3, 0.6, 1.2 and 2.4 FPU mg^{-1} biomass were tested. As shown in Fig. 7, hydrolysis yield increased quickly from 0 to 98.7% as the enzyme loading increased from 0 to 0.3 FPU mg^{-1} biomass. The hydrolysis yield remained constant with further

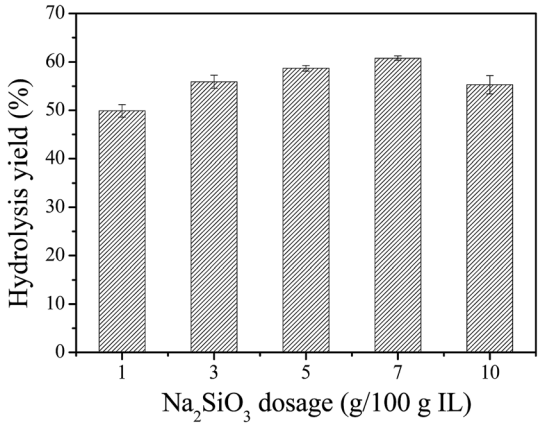

Fig. 5 Enzymatic hydrolysis of regenerated willow after [BMIm]Cl + Na_2SiO_3 pretreatment with different Na_2SiO_3 dosage (pretreatment conditions: biomass dosage, 0.25 g; [BMIm]Cl dosage, 5.0 g; Na_2SiO_3 dosage, 1.0–10.0 g/100 g IL; temperature, 120 °C and time, 1 h; enzymatic hydrolysis conditions: biomass dosage, 0.32% (w/v); cellulase mixture, 0.05 FPU mg^{-1} biomass; temperature, 50 °C; and time, 72 h).

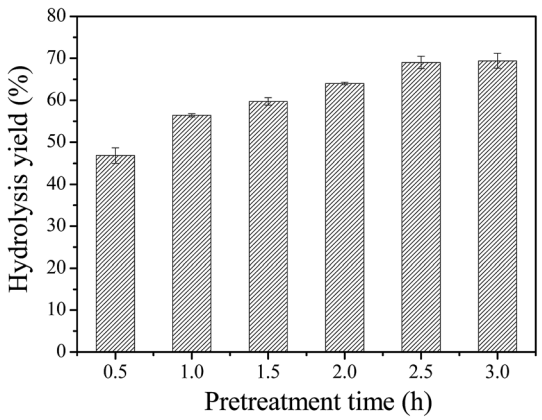

Fig. 6 Enzymatic hydrolysis of regenerated willow after [BMIm]Cl + Na_2SiO_3 pretreatment for different pretreatment time (pretreatment conditions: biomass dosage, 0.25 g; [BMIm]Cl dosage, 5.0 g; Na_2SiO_3 dosage, 7.0 g/100 g IL and temperature, 120 °C; enzymatic hydrolysis conditions: biomass dosage, 0.32% (w/v); cellulase mixture, 0.05 FPU mg^{-1} biomass; temperature, 50 °C; and time, 72 h).

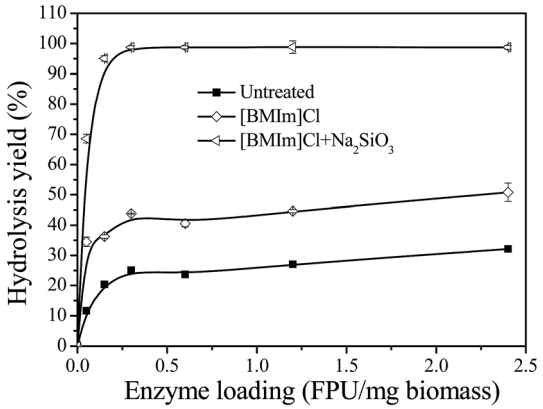

Fig. 7 Effect of the enzyme loading on enzymatic hydrolysis of untreated and regenerated willow (pretreatment conditions: biomass dosage, 0.25 g; [BMIm]Cl dosage, 5.0 g; Na_2SiO_3 dosage, 0 or 7.0 g/100 g IL; temperature, 120 °C and time, 2.5 h; enzymatic hydrolysis conditions: biomass dosage, 0.32% (w/v); temperature, 50 °C; and time, 72 h).

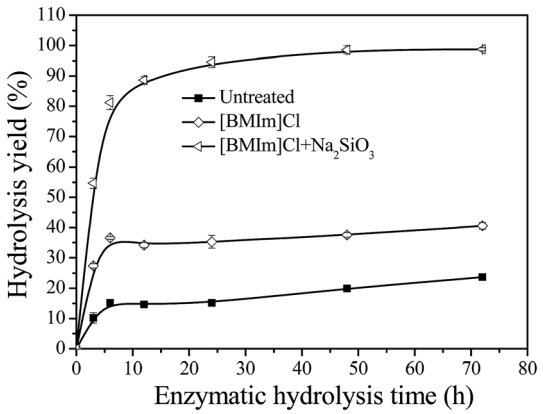

Fig. 8 Effect of enzymatic hydrolysis time on the hydrolysis yield of untreated and regenerated willow (pretreatment conditions: biomass dosage, 0.25 g; [BMIm]Cl dosage, 5.0 g; Na_2SiO_3 dosage, 0 or 7.0 g/100 g IL; temperature, 120 °C; and time, 2.5 h; enzymatic hydrolysis conditions: biomass dosage, 0.32% (w/v); enzyme loading, 0.3 FPU mg^{-1} biomass; and temperature, 50 °C).

increase of enzyme loading, indicating that an equilibrium was reached between enzyme and cellulose.

Therefore, considering the balance between hydrolysis rate, equipment cost and energy consumption, Na_2SiO_3 dosage of 7 g/100 g IL, pretreatment time of 2.5 h, enzyme loading of 0.3 FPU mg^{-1} biomass were suggested as the reaction conditions for the pretreatment and enzymatic hydrolysis of willow. Under these preferable conditions than Fig. 1B, enzymatic hydrolysis of willow was performed at different hydrolysis time, with the results listed in Fig. 8. It was demonstrated that an equilibrium of enzymatic hydrolysis were quickly reached within 6 h for untreated and [BMIm]Cl-pretreated willow, with the final hydrolysis yield of 20.0 and 37.5%, respectively. However, for [BMIm]Cl + Na_2SiO_3-pretreated willow, enzymatic hydrolysis equilibrium was achieved after 48 h, providing a much higher hydrolysis yield of up to 98.6%, which was over 5 and 2.6 times of untreated and [BMIm]Cl-pretreated willow. The maximum glucose yield of [BMIm]Cl + Na_2SiO_3-pretreated willow was 39.5 g glucose per 100 g as-received biomass after 48 h hydrolysis (glucose release rate = 94.8%), which proved that the combined pretreatment by [BMIm]Cl and Na_2SiO_3 was a promising method for the transformation of willow to fermentable sugars.

3.4 Comparison of different pretreatments of willow with literatures

Compared with different pretreatments of willow in other reports, the combined method of [BMIm]Cl and Na_2SiO_3 exhibited higher pretreatment ability of lignocellulosic biomass

Table 3 Comparison of different pretreatments of willow between literature reports and this study

		Pretreatment performance				Enzymatic hydrolysis test		
Pretreatment methods	Pretreatment conditions	Cellulose recovery	Delignification ratio	CrI of cellulose[b]	Hydrolysis conditions	Hydrolysis yield	Glucose release rate	Refs.
Sulfolane	T = 170 °C, time = 1.5 h, sulfolane : willow = 5 : 1 w/w	96.2%	84.7%	Increased by 23.7%	Cellulase (lot no. K1220003) = 20 FPU, β-glucosidase (lot no. BCBH2676 V) = 20 CBU, xylanase (X2753-10G) = 1.5 FXU g^{-1} pretreated materials, T = 50 °C, time = 72 h	80.3%.	77.2%	44
Steam	H_2SO_4 impregnation (0.5%, w/w), T = 200 °C, time = 8 min	~100%	26%	n.a.[a]	Celluclast 1.5L = 65 FPU, β-glucosidase (Novozym 188) = 376 IU g^{-1} substrate, T = 40 °C, time = 96 h	~93.7%	93.7%	48
Catalytic organosolv fractionation	T = 190 °C, time = 3 h, 0.01 mol L^{-1} H_2SO_4 as catalyst	99.6%	5.7%	n.a.	Accellerase 1500 = 33 FPU g^{-1} substrate, T = 50 °C, time = 72 h	87.3%	87.0%	49
White-rot fungi	T = 25 °C, time = 120 days	73.3%	45.6%	n.a.	Cellulase = 20 FPU g^{-1} substrate, T = 50 °C, time = 120 h	33%	n.a.	40
Alkaline	T = 50 °C, time = 12 h, NaOH dosage = 5 g/100 g biomass	n.a.	n.a.	n.a.	Celluclast® dosage = 50 FPU g^{-1} biomass, T = 50 °C, time = 24 h	~82%	n.a.	50
Ionic liquid–water mixtures	[BMIm][HSO_4] : water = 80 : 20 v/v, T = 120 °C, time = 22 h	40.2%	92.8%	n.a.	Celluclast = 60 FPU, β-glucosidase (Novozym 188) = 64 pNPGU g^{-1} substrate, T = 50 °C, time = 96 h	n.a.	>80%	33
[BMIm]Cl + Na_2SiO_3	T = 120 °C, time = 2.5 h, willow: [BMIm]Cl = 0.25 g/5 g, Na_2SiO_3 dosage = 7.0 g/100 g IL	96.4%	56.6%	Decreased by 37.9%	Celluclast 1.5L® + Novozyme 188 (1 : 1, v/v), cellulase dosage = 0.3 FPU mg^{-1} substrate, T = 50 °C, time = 48 h	98.6%	94.8%	This study

[a] Word "n.a." meant not analyzed. [b] Compared to untreated biomass.

(Table 3). The [BMIm]Cl + Na_2SiO_3 pretreatment effectively removed hemicellulose and lignin at lower temperature and shorter time for a more powerful dissolution of lignocellulose, while considerable recovery of cellulose was achieved with decreased after regeneration (Table 3). The pretreated materials gave better results in enzymatic hydrolysis than sulfolane,[44] steam,[48] catalytic organosolv fractionation,[49] ionic liquid[33] and white-rot fungi[40] pretreatment at shorter hydrolysis time. NaOH pretreatment reported by Wilkinson *et al.*[50] was completed at milder temperature (50 °C), but long reaction time (12 h) and the removal of hazardous substances might increase the overall production cost and the environment pollution.

4 Conclusions

In this study, a novel pretreatment method, combining solid base catalyst with cheaper ionic liquid ([BMIm]Cl + Na_2SiO_3), was proposed and used for the pretreatment of different lignocellulosic biomass, including spruce (softwood), willow (hardwood) and soybean straw (crop residue). The [BMIm]Cl + Na_2SiO_3 pretreatment was more suitable for willow and soybean straw than spruce. The combination of Na_2SiO_3 and [BMIm]Cl effectively destroyed the structure of biomass through the removal of lignin and hemicellulose, and significantly decreased cellulosic crystallinity, which resulted in an enhanced biomass accessibility and the improvement of enzymatic hydrolysis. The maximum enzymatic hydrolysis yield and glucose yield for willow reached 98.6% and 39.5 g/100 g willow (Na_2SiO_3 dosage, 7 g/100 g IL; pretreatment time, 2.5 h; enzyme loading, 0.3 FPU mg^{-1} biomass and enzymatic hydrolysis time, 48 h), respectively, which was 2.6-fold of those with single [BMIm]Cl. Therefore, combined [BMIm]Cl + Na_2SiO_3 method had a potential application in the pretreatment of hardwoods and herbaceous plants for the effective production of value-added sugars.

Acknowledgements

The authors wish to acknowledge the financial support from Chinese Academy of Sciences (CAS, China) [CAS 135 program (XTB-T02) and equipment R&D grant (No. YZ201260)], the Yunnan Provincial Government (Baiming Haiwai Gaocengci Rencai Jihua), and the Natural Science Foundation of China (No. 31400518). We also thank Prof. Zhen Fang (Department of Engineering, Nanjing Agricultural University), Dr Xiaofei Tian (Department of Bioscience and Bioengineering, South China University of Technology) and Dr Jia Luo (Key Laboratory of Tropical Plant Resources and Sustainable Use, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences) for their kind assistance and advices on this study.

References

1 X. R. Liu, X. C. Wang, S. X. Yao, Y. J. Jiang, J. Guan and X. D. Mu, Recent advances in the production of polyols from lignocellulosic biomass and biomass-derived compounds, *RSC Adv.*, 2014, **4**, 49501–49520.

2 J. N. Putro, F. E. Soetaredjo, S. Y. Lin, Y. H. Ju and S. Ismadji, Pretreatment and conversion of lignocellulose biomass into valuable chemicals, *RSC Adv.*, 2016, **6**, 46834–46852.

3 S. P. Magalhaes da Silva, A. M. da Costa Lopes, L. B. Roseiro and R. Bogel-Łukasik, Novel pre-treatment and fractionation method for lignocellulosic biomass using ionic liquids, *RSC Adv.*, 2013, **3**, 16040–16050.

4 L. T. P. Trink, Y. J. Lee, J. W. Lee and H. J. Lee, Characterization of ionic liquid pretreatment and the bioconversion of pretreated mixed softwood biomass, *Biomass Bioenergy*, 2015, **81**, 1–8.

5 S. H. Mood, A. H. Golfeshan, M. Tabatabaei, G. S. Jouzani, G. H. Najafi, M. Gholami and M. Ardjmand, Lignocellulosic biomass to bioethanol, a comprehensive review with a focus on pretreatment, *Renewable Sustainable Energy Rev.*, 2013, **27**, 77–93.

6 M. E. Zakrzewska, E. Bogel-Lukasik and R. Bogel-Lukasik, Solubility of carbohydrates in ionic liquids, *Energy Fuels*, 2010, **24**, 737–745.

7 Y. Cao and T. Mu, Comprehensive investigation on the thermal stability of 66 ionic liquids by thermogravimetric analysis, *Ind. Eng. Chem. Res.*, 2014, **53**, 8651–8664.

8 E. R. E. Hassan, F. Mutelet, J. C. Moise and N. Brosse, Pretreatment of miscanthus using 1,3-dimethylimidazolium methyl phosphonate (DMIMMPh) ionic liquid for glucose recovery and ethanol production, *RSC Adv.*, 2015, **5**, 61455–61464.

9 J. Holm and U. Lassi, *Ionic liquids in the pretreatment of lignocellulosic biomass*, INTECH Open Access Publisher, 2011.

10 L. Sun, C. L. Li, Z. J. Xue, B. A. Simmons and S. Singh, Unveiling high-resolution, tissue specific dynamic changes in corn stover during ionic liquid pretreatment, *RSC Adv.*, 2013, **3**, 2017–2027.

11 S. Q. Xia, G. A. Baker, H. Lia, S. Ravula and H. Zhao, Aqueous ionic liquids and deep eutectic solvents for cellulosic biomass pretreatment and saccharification, *RSC Adv.*, 2014, **4**, 10586–10596.

12 D. Groff, A. George, N. Sun, N. Sathitsuksanoh, G. Bokinsky, B. A. Simmons, B. M. Holmes and J. D. Keasling, Acid enhanced ionic liquid pretreatment of biomass, *Green Chem.*, 2013, **15**, 1264–1267.

13 L. Q. Jiang, Z. Fang, X. K. Li, J. Luo and S. P. Fan, Combination of dilute acid and ionic liquid pretreatments of sugarcane bagasse for glucose by enzymatic hydrolysis, *Process Biochem.*, 2013, **48**, 1942–1946.

14 D. Groff, A. George, N. Sun, N. Sathitsuksanoh, G. Bokinsky, B. A. Simmons, B. M. Holmes and J. D. Keasling, Acid enhanced ionic liquid pretreatment of biomass, *Green Chem.*, 2013, **15**, 1264–1267.

15 T. A. D. Nguyen, K. R. Kim, S. J. Han, H. Y. Cho, J. W. Kim, S. M. Park, J. C. Park and S. J. Sim, Pretreatment of rice straw with ammonia and ionic liquid for lignocellulose conversion to fermentable sugars, *Bioresour. Technol.*, 2010, **101**, 7432–7438.

16 X. L. Geng and W. A. Henderson, Pretreatment of corn stover by combining ionic liquid dissoluyion with alkali extraction, *Biotechnol. Bioeng.*, 2012, **109**, 84–91.
17 J. Duo, Z. S. Zhang, G. D. Yao, Z. B. Huo and F. M. Jin, Hydrothermal conversion of glucose into lactic acid with sodium silicate as a base catalyst, *Catal. Today*, 2016, **263**, 112–116.
18 F. Guo, Z. G. Peng, J. Y. Dai and Z. L. Xiu, Calcined sodium silicate as solid base catalyst for biodiesel production, *Fuel Process. Technol.*, 2009, **91**, 322–328.
19 F. Zhang, X. H. Wu, M. Yao, Z. Fang and Y. T. Wang, Production of biodiesel and hydrogen from plant oil catalyzed by magnetic carbon-supported nickel and sodium silicate, *Green Chem.*, 2016, **18**, 3302–3314.
20 H. V. Lee, J. C. Juan, N. F. B. Abdullah, M. F. Rabiah Nizah and Y. H. Taufiq-Yap, Heterogeneous base catalysts for edible palm and non-edible Jatropha-based biodiesel production, *Chem. Cent. J.*, 2014, **8**, 30.
21 K. Tanabe, T. Yamaguchi and T. Takeshita, Solid bases and their catalytic activity, *J. Res. Inst. Catal., Hokkaido Univ.*, 1968, **16**, 425–447.
22 F. Guo, N. N. Wei, Z. L. Xiu and Z. Fang, Transesterification mechanism of soybean oil to biodiesel catalyzed by calcined sodium silicate, *Fuel*, 2012, **93**, 468–472.
23 B. Adney and J. Baker, *Measurement of cellulase activities, Laboratory Analytical Procedure*, NREL LAP-006, 1996.
24 M. Selig, N. Weiss and Y. Ji, *Enzymatic Saccharification of Lignocellulosic Biomass, Laboratory Analytical Procedure*, NREL/TP-510–42629, 2008.
25 A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter and D. Templeton, *Determination of Structural Carbohydrates and Lignin in Biomass, Laboratory Analytical Procedure*, NREL LAP-002, 2004.
26 L. Segal, J. J. Creely, A. E. Martin and C. M. Conrad, An empirical method for estimating the degree of crystallinity of native cellulose using the X-ray diffractometer, *Text. Res. J.*, 1959, **29**, 786–794.
27 S. Brunauer, P. H. Emmett and E. Teller, Adsorption of gases in multimolecular layers, *J. Am. Chem. Soc.*, 1938, **60**, 309–319.
28 X. Tian, Z. Fang, D. Jiang and X. Sun, Pretreatment of microcrystalline cellulose in organic electrolyte solutions for enzymatic hydrolysis, *Biotechnol. Biofuels*, 2011, **4**, 53.
29 W. S. Cardoso, F. D. Tardin, G. P. Tavares, P. V. Queiroz, S. S. Mota, M. C. M. Kasuya and J. H. de Queiroz, Use of sorghum straw (Sorghum bicolor) for second generation ethanol production: pretreatment and enzymatic hydrolysis, *Quim. Nova*, 2013, **36**, 623–627.
30 Y. L. Loow, T. Y. Wu, J. M. Jahim, A. W. Mohammad and W. H. Teoh, Typical conversion of lignocellulosic biomass into reducing sugars using dilute acid hydrolysis and alkaline pretreatment, *Cellulose*, 2016, **23**, 1491–1520.
31 G. Bali, X. Meng, J. I. Deneff, Q. N. Sun and A. J. Ragauskas, The effect of alkaline pretreatment methods on cellulose structure and accessibility, *ChemSusChem*, 2015, **8**, 275–279.
32 T. Auxenfans, S. Buchoux, D. Larcher, G. Husson, E. Husson and C. Sarazin, Enzymatic saccharification and structural properties of industrial wood sawdust: recycled ionic liquids pretreatments, *Energy Convers. Manage.*, 2014, **88**, 1094–1103.
33 A. Brandt, M. J. Ray, T. Q. To, D. J. Leak, R. J. Murphy and T. Welton, Ionic liquid pretreatment of lignocellulosic biomass with ionic liquid–water mixtures, *Green Chem.*, 2011, **13**, 2489–2499.
34 F. da Cunha-Pereira, R. Rech, M. A. Z. Ayub, A. P. Dillon and J. Dupont, Liberation of fermentable sugars from soybean hull biomass using ionic liquid 1-butyl-3 methylimidazolium acetate and their bioconversion to ethanol, *Biotechnol. Prog.*, 2016, **32**, 312–320.
35 S. H. Lee, T. V. Doherty, R. J. Linhardt and J. S. Dordick, Ionic liquid-mediated selective extraction of lignin from wood leading to enhanced enzymatic cellulose hydrolysis, *Biotechnol. Bioeng.*, 2009, **102**, 1368–1376.
36 B. Li, J. Asikkala, I. Filpponen and D. S. Argyropoulos, Factors affecting wood dissolution and regeneration of ionic liquids, *Ind. Eng. Chem. Res.*, 2010, **49**, 2477–2484.
37 X. Z. Meng and A. J. Ragauskas, Recent advances in understanding the role of cellulose accessibility in enzymatic hydrolysis of lignocellulosic substrates, *Curr. Opin. Biotechnol.*, 2014, **27**, 150–158.
38 Y. Q. Pu, F. Hu, F. Huang, B. H Davison and A. J. Ragauskas, Assessing the molecular structure basis for biomass recalcitrance during dilute acid and hydrothermal pretreatments, *Biotechnol. Biofuels*, 2013, **6**, 15.
39 Y. N. Zeng, S. Zhao, S. H. Yang and S. Y. Ding, Lignin content is the key factor negatively correlated with enzyme digestibility, *Curr. Opin. Biotechnol.*, 2014, **27**, 38–45.
40 H. Yu, G. Guo, X. Zhang, K. Yan and C. Xu, The effect of biological pretreatment with the selective white-rot fungus *Echinodontium taxodii* on enzymatic hydrolysis of softwoods and hardwoods, *Bioresour. Technol.*, 2009, **100**, 5170–5175.
41 X. Geng and W. A. Henderson, Pretreatment of corn stover by combining ionic liquid dissolution with alkali extraction, *Biotechnol. Bioeng.*, 2012, **109**, 84–91.
42 D. G. MacDonald, N. N. Bakhshi, J. F. Mathews, A. Roychowdhury, P. Bajpai and M. Moo-Young, Alkali treatment of corn stover to improve sugar production by enzymatic hydrolysis, *Biotechnol. Bioeng.*, 1983, **25**, 2067–2076.
43 C. Croitoru and S. F. C. Patachia, Structural properties of cellulose regenerated from its ionic liquid solutions, *Bulletin of Romanian Chemical Engineering Society*, 2014, **1**, 113–129.
44 K. Wang, X. Xie, J. Jiang and J. X. Wang, Sulfolane pretreatment of shrub willow to improve enzymatic saccharification, *Cellulose*, 2016, **23**, 1153–1163.
45 C. Li, B. Knierim, C. Manisseri, R. Arora, H. V. Scheller, M. Auer, K. P. Vogel, B. A. Simmons and S. Singh, Comparison of dilute acid and ionic liquid pretreatment of switchgrass: biomass recalcitrance, delignification and enzymatic saccharification, *Bioresour. Technol.*, 2010, **101**, 4900–4906.

46 H. P. Yang, R. Yan, H. P. Chen, D. H. Lee and C. G. Zheng, Characteristics of hemicellulose, cellulose and lignin pyrolysis, *Fuel*, 2007, **86**, 1781–1788.

47 J. A. Perez-Pimienta, M. G. Lopez-Ortega, J. A. Chavez-Carvayar, P. Varanasi, V. Stavila, G. Cheng, S. Singh and B. A. Simmons, Characterization of agave bagasse as a function of ionic liquid pretreatment, *Biomass Bioenergy*, 2015, **75**, 180–188.

48 P. Sassner, C. G. Martensson, M. Galbe and G. Zacchi, Steam pretreatment of H_2SO_4-impregnated Salix for the production of bioethanol, *Bioresour. Technol.*, 2008, **99**, 137–145.

49 W. J. J. Huijgen, A. T. Smit, J. H. Reith and H. den Uil, Catalytic organosolv fractionation of willow wood and wheat straw as pretreatment for enzymatic cellulose hydrolysis, *J. Chem. Technol. Biotechnol.*, 2011, **86**, 1428–1438.

50 S. Wilkinson, D. Greetham and G. A. Tucker, Evaluation of different lignocellulosic biomass pretreatments by phenotypic microarray-based metabolic analysis of fermenting yeast, *Biofuel Res. J.*, 2016, **3**, 357–365.