Structural and Behavior Changes of Herbaceous and Hardwood Biomass during Steam Explosion Pretreatment and Enzymatic Hydrolysis

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Steam explosion (SE) was used to pretreat rice straw (RS) and *Caragana korshinskii* Kom. (CKK). Enzymatic hydrolysis of pretreated RS and CKK, and a pure cellulose (bacterial cellulose, BC) were performed and compared. The characteristics and changes of different substrates during pretreatment and enzymatic hydrolysis were observed. After SE pretreatment, the hemicellulose content of RS and CKK was decreased from 21.7% to 4.9% and 17.9% to 6.2%, respectively. The cellulose percent of RS and CKK was increased to 45.1% and 38.4%, and the enzymatic hydrolysis conversion of cellulose was increased 1.1 times and 0.9 times, respectively. Although the composition and enzymatic yield of different substrates varied, their enzymatic hydrolysis rates showed a similar declining trend during enzymatic hydrolysis. The adsorption capacities of substrates on the enzyme were increased by pretreatment. The enzymatic hydrolysis efficiency was closely related to the enzyme adsorption capacities of substrates at the initial stage (1 h). The BC and SE pretreated RS first adsorbed and subsequently desorbed the enzyme, while the untreated RS and CKK had weak adsorption capacity and formed irreversible adsorption with the enzyme. The changes in crystallinity after enzymatic hydrolysis suggested that there was no direct correlation between the crystallinity and digestibility of cellulose.

Keywords: Steam explosion; Biomass heterogeneity; Enzymatic hydrolysis; Cellulose crystallinity

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INTRODUCTION

Bioconversion of lignocellulosic biomass to chemicals and fuels has been attracting attention due to the current concerns over food shortages, the energy crisis, and environmental pollutions (Ragauskas *et al.* 2006; Li *et al.* 2018). Lignocellulosic biomass is an abundant and renewable resource, which is mainly composed of cellulose, hemicellulose, and lignin. It can be obtained from energy crops, forest residues, agricultural residues, waste paper, and other materials (Morikawa *et al.* 2014; Weerasai *et al.* 2014). The processes involved in the bioconversion of lignocellulosic biomass are pretreatment and enzymatic hydrolysis of cellulose and hemicelluloses into fermentable sugars (Ungurean *et al.* 2014; Bernal-Lugo *et al.* 2019; Jiang *et al.* 2019). Pretreatment and enzymatic hydrolysis are complicated processes. The structural features of lignocellulosic

biomass include chemical composition, microstructure, and crystallinity, which are known to affect pretreatment and digestibility of cellulose to enzymatic hydrolysis (Ioelovich and Morag 2011; Meng and Ragauskas 2014; Jiang *et al.* 2016; Li *et al.* 2017; Lai *et al.* 2018). However, the mechanism of the enzymatic breakdown of lignocellulosic biomass is still not completely clear.

The diversity of biomass types (termed as "biomass heterogeneity") leads to differences in digestibility among these feedstocks. The heterogeneity is caused by the different chemical compositions, cell wall microstructures, and crystallinity of cellulose, which results in different efficiencies of enzymatic hydrolysis (Mood *et al.* 2013; Chen and Liu 2015; Huang *et al.* 2018). The compact surrounded structure formed by hemicellulose and lignin makes the lignocellulosic biomass much more difficult to hydrolyze (Jurado *et al.* 2009). Therefore, it is necessary to pretreat these feedstocks to improve the enzymatic digestibility of cellulose by altering or removing the structure of lignin or hemicellulose. Steam explosion (SE) pretreatment is a low-cost and effective method to improve the enzymatic hydrolysis of hardwood and herbaceous lignocellulose (Singh *et al.* 2015). No additional chemical reagent is required for steam explosion pretreatment, and it can alter the chemical compositions and physical structures of lignocellulose, which enhances the enzymatic hydrolysis efficiency (Kumar *et al.* 2009). However, the influence of biomass heterogeneity during the pretreatment and enzymatic hydrolysis needs further clarification. For example, the cellulose crystallinity generally hinders enzymatic hydrolysis (Mittal *et al.* 2011). Nevertheless, the enzymatic hydrolysis of bacterial cellulose (BC) with high crystallinity (91%) is easier than that of rice straw (RS, crystallinity 60%) and *Caragana korshinskii* Kom. (CKK, crystallinity 66%). The adsorption capacity of different substrates for the enzyme is also diverse due to their unique structures and compositions. The relationship between the enzyme adsorption and the enzymatic hydrolysis yield is still not very clear. Thus, the investigation of this study will help us to shed light on the enzymatic hydrolysis process of lignocellulosic biomass.

In this experiment, the physical and chemical characteristics of different cellulose substrates and their structural changes during pretreatment and the enzymatic hydrolysis process were compared. The BC, RS, and CKK were selected as the representatives of microbial cellulose, herbaceous biomass, and woody biomass, respectively. BC is synthesized by microorganisms (such as *Gluconacetobacter*, *Achromobacter*, *Aerobacter*, and *Agrobacterium*) with high purity and high crystallinity (Krishnamachari *et al.* 2011). RS is an abundant agricultural waste that has been researched as a possible alternative to fossil fuels (Chen *et al.* 2011; Weerasai *et al.* 2014). CKK is a perennial woody species that is mainly distributed in the arid and semi-arid regions for conserving soil and water resources. The shrubs of CKK must be harvested every 3 years to make them flourish (Wang *et al.* 2012; Zhong *et al.* 2014). Woody biomass is a recalcitrant feedstock and requires more severe pretreatment than herbaceous lignocellulose (Zhu and Pan 2010; Zhao et al. 2012). The SE pretreatment of RS and CKK as well as enzymatic hydrolysis of various types of substrates was performed in this study. The chemical composition of the BC, RS, CKK, and SE pretreated RS and SE pretreated CKK were measured. The glucose and xylose yield from enzymatic hydrolysis of different cellulose substrates were analyzed, and the free enzymes in their hydrolytic supernatant were determined. Scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), and X-ray diffraction (XRD) were used to characterize the microtopography, chemical structure, and cellulose

crystalline structure of untreated and SE pretreated substrates before and after enzymatic hydrolysis.

The goal of this work is to deepen understanding of the changes of herbaceous and hardwood biomass during the pretreatment, as well as the relationship between enzymatic hydrolysis and adsorption of enzymes. The conclusion of this study could be used as the foundation to further improve and optimize the method for biomass pretreatment and to improve the efficiency of enzymatic hydrolysis.

EXPERIMENTAL

Materials

RS was harvested from Xintian county in Hunan province, China. CKK was obtained from Liangcheng county in Inner Mongolia, China. These feedstocks were air-dried, passed through a screen of 2×2 cm^2, and stored at the sealed plastic bag at room temperature. BC was secreted by *Gluconacebactor xylinus* (CGMCC NO. 2955) and obtained from the laboratory (Zhong *et al.* 2013). Cellulast 1.5L and xylanase were purchased from Sigma (Shanghai, China). *β*-glucosidase was obtained from Youtell Biochemical Co., Ltd (Shanghai, China). All other chemicals were analytical grade and obtained from commercial sources.

Methods

SE pretreatment

The SE pretreatment was performed in QBS-200B steam explosion equipment (Hebi, Henan Province, China). First, 200 g of RS or CKK were loaded into the high-pressure reactor (5 L), and high-pressure steam was imported to reach the desired pressure (2.2 MPa). When the pretreatment time was achieved at 5 min, the ball valve was opened immediately, and the steam was released suddenly to the atmospheric pressure. The pretreated sample was collected and washed until the filtrate pH was 7.0. The BC, pretreated RS and CKK were oven-dried at 40 °C until the weight was constant. The sample was milled by a beater pulverizer and screened through a square mesh 0.85×0.85 mm^2 for further experiments.

Composition analysis

Composition analysis was carried out according to the National Renewable Energy Laboratory (NREL) standard protocol (Sluiter *et al.* 2012). Glucose and xylose were detected by high-performance liquid chromatography (HPLC) using a refractive index detector. The column used was the Aminex HPX-87H (Bio-Rad, Hercules, CA, USA). The temperature of the column was maintained at 65 °C, and 0.6 mL/min of 5 mM H_2SO_4 was used as a mobile phase.

Enzymatic hydrolysis

Enzymatic hydrolysis of samples was conducted in 100 mL Erlenmeyer flasks with 1% glucan loading and a final working weight of 20 g (Resch *et al.* 2015). The enzymes were loaded at 15 FPU/g cellulose of Celluclast 1.5 L, 11 mg protein/g cellulose of *β*-glucosidase, and 8 mg protein/g cellulose of xylanase. The enzymatic hydrolysis was conducted at 50°C, pH 4.8, and 200 rpm for 72 h. The samples were centrifuged (12,000

rpm, 5 min) and filtered with a 0.22 μm membrane filter. The concentration of protein in the hydrolytic supernatant was determined by the Bradford method to estimate the amount of adsorbed enzyme in the solid residual during the enzymatic hydrolysis process (Bradford 1976).

SEM

All samples were sputter-coated with platinum and imaged with a scanning electron microscope (JSM–6380LV, JEOL Ltd, Tokyo, Japan).

XRD

The XRD patterns were obtained using an x-ray diffractometer (D/max–2500, Rigaku Corporation, Tokyo, Japan) in the 2θ range of 10 to 40°. Samples were sieved with an 80-mesh screen and positioned on a quartz sample holder. The scan speed was 4° min^{-1} with a step size of 0.02°. The crystalline index of the samples was calculated based on the peak height method following Eq. 1 (Cao and Tan 2005),

$$CrI\ (\%) = (I_{002} - I_{am}) / I_{002} \times 100\% \quad (1)$$

where I_{002} is the peak height at 2θ = 22.6° of the lattice planes (002) and I_{am} is the peak height at 2θ = 19.0° of the amorphous phases.

FTIR

The samples were mixed with KBr and pressed into a plate using a tableting machine. The IR spectrum was recorded at a range of 4000 to 400 cm^{-1} wavenumbers with 16 scans and a resolution of 4 cm^{-1} (TENSOR 27, Bruker, Karlsruhe, Germany).

RESULTS AND DISCUSSION

Composition Analysis

The chemical composition of BC, untreated and SE pretreated lignocellulose is shown in Table 1.

Table 1. Chemical Composition of BC, Untreated, and SE Pretreated Samples

Samples	Component content (Mean ± SD, %)			
	Cellulose	Hemicellulose	Lignin (AIL)	Ash
BC	>99	0	0	0
Untreated RS	33.3 ± 0.6	21.7 ± 1.6	5.7 ± 1.2	14.1 ± 1.5
SE pretreated RS	45.1 ± 0.6	4.9 ± 3.7	28.2 ± 3.3	13.8 ± 0.7
Untreated CKK	30.6 ± 1.9	17.9 ± 0.6	23.7 ± 0.7	3.3 ± 2.1
SE pretreated CKK	38.4± 2.0	6.2± 4.5	41.5 ± 1.5	2.9 ± 1.0
BC: Bacterial cellulose; RS: Rice straw; CKK: *Caragana korshinskii Kom.*; SE: Steam explosion.				

The BC, RS, and CKK had very different chemical compositions. The cellulose content of BC was more than 99%, while the cellulose contents of untreated RS and CKK were 33.3% and 30.6%, respectively. There was a large amount of lignin and hemicellulose

in the RS and CKK. These components form a dense, three-dimensional structure with cellulose, which limits the enzymatic digestibility of cellulose. When RS and CKK were subjected to SE pretreatment, most hemicelluloses and part of the ash content were removed (mainly cellulose and lignin were left). The hemicellulose content of RS and CKK after SE pretreatment was decreased from 21.7% to 4.9% and 17.9% to 6.2%, respectively. The cellulose content of RS and CKK was increased to 45.1% and 38.4%, respectively. The results indicated that the SE pretreatment could increase the percent of cellulose and decrease the hemicellulose and ash for the different lignocelluloses.

Enzymatic Hydrolysis

The BC, as well as the untreated and SE pretreated samples were enzymatically hydrolyzed using commercial Cellulast 1.5 L coupled with *β*-glucosidase and xylanase at 50 °C for 72 h. Glucose and xylose yields of these substrates during enzymatic hydrolysis are shown in Fig. 1. The BC had the highest enzymatic hydrolysis rate and yield. In all cases, the enzymatic conversion during the first 24 h was quick and slowed down gradually. The glucose yield of BC reached 85.9% at 24 h and continued to increase to 93.1% at 72 h. However, only 40.0% and 23.5% glucose yield were observed for the untreated RS and CKK after 72 h of enzymatic hydrolysis, respectively. The SE pretreatment increased the glucose and xylose yields of RS and CKK. 82.6% and 45.5% glucose yield, and 49.7% and 39.1% xylose yield were obtained for SE pretreated RS and CKK at 72 h. These results indicated that SE pretreatment could improve glucose and xylose yields of lignocellulosic substrates. The enzymatic hydrolysis yield of RS was higher than CKK regardless of pretreatment. This is consistent with the previous studies that woody biomass had higher lignin content and stronger recalcitrance than herbaceous biomass (Zhu and Pan 2010; Zhao *et al*. 2012). Combined with the results of the composition analysis, although the compositions of different substrates were diverse, the hydrolysis rate of all substrates during the enzymatic hydrolysis process were gradually reduced. The higher cellulose content led to the higher enzymatic digestibility.

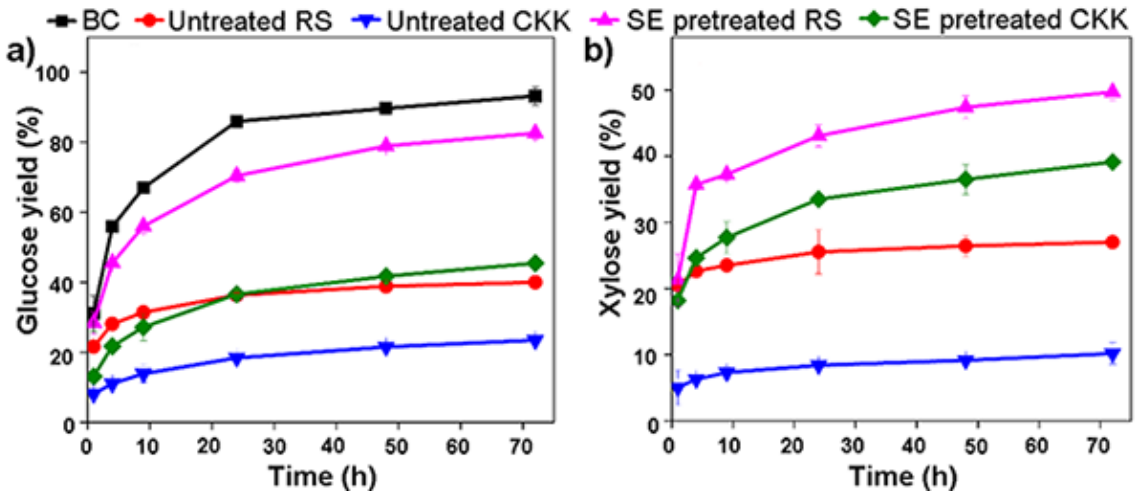

Fig. 1. Glucose and xylose yield of different cellulose substrates during enzymatic hydrolysis; a) glucose yield; b) xylose yield. BC: Bacterial cellulose; RS: Rice straw; CKK: *Caragana korshinskii* Kom.; SE: Steam explosion

Enzyme Adsorption

The purpose of studying enzyme adsorption is to investigate the common features or changes that could be associated with the reduced rates of hydrolysis. The adsorption performance of the enzyme on the substrate was investigated (Palonen *et al.* 2004). The enzyme in the solution needs to be adsorbed on the substrate first and will then subsequently carry out the enzymatic hydrolysis. Therefore, the amount of free enzymes in the hydrolytic supernatant was measured and subtracted from the initial added enzymes to calculate the portion of adsorbed enzymes. Although adsorption of enzymes during enzymatic hydrolysis of lignocellulose containing lignin has been extensively studied, less results using pure cellulose substrates have been published. Figure 2 showed BC, as well as untreated and pretreated RS and CKK adsorption performances with the enzyme during enzymatic hydrolysis. BC adsorbed 69.8% of the enzyme during the 1 hour of enzymatic hydrolysis. The untreated RS and CKK only adsorbed 20.7% and 14.3% of the enzyme at 1 h, respectively. SE pretreatment effectively improved the adsorption capacity of substrates on the enzyme. The adsorption of the enzyme on the pretreated substrate was increased to 79.7% (RS) and 48.3% (CKK) within 1 h. This is most likely because most hemicelluloses were removed, and the surface areas of the substrates were increased (Palonen *et al*. 2004; Kumar *et al.* 2009). After the initial rapid adsorption period, the adsorbed enzyme of BC was gradually released into the enzymatic hydrolysis supernatant. As a result, the free enzymes in the supernatant of BC enzymatic hydrolysate was increased to 69.1% after 24 h of enzymatic hydrolysis. As for the SE pretreated RS and CKK, the free enzyme in supernatant of SE pretreated CKK was decreased continuously with the processing of enzymatic hydrolysis, while that of SE pretreated RS slightly increased after 1 h and then remained almost unchanged after 10 h. At the same time, although SE pretreated CKK adsorbed higher enzymes than untreated RS (Fig. 2), its enzymatic yield at 72 h was only slightly higher than untreated RS (even lower than untreated RS before 24 h). This might be due to the fact that the lignin of pretreated lignocellulosic biomass was unproductive and it irreversibly adsorbed the enzyme, and the adsorption of hardwood lignin was different from herbaceous lignin (Tu *et al.* 2009; Guo *et al.* 2014).

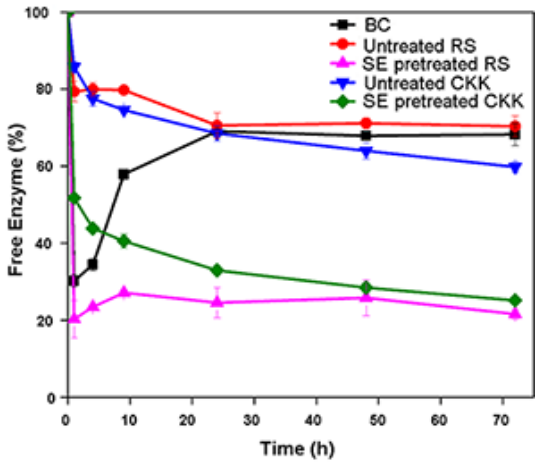

Fig. 2. Enzyme adsorption during the enzymatic hydrolysis of different cellulose substrates. BC: Bacterial cellulose; RS: Rice straw; CKK: *Caragana korshinskii* Kom.; SE: Steam explosion

It was found that the substrates with high enzymatic hydrolysis yield (BC and SE pretreated RS) would first rapidly adsorb the enzyme and desorb subsequently, while the

substrates with low enzymatic hydrolysis yield (untreated RS and CKK) had weak adsorption capacity. The time course of enzyme adsorption indicated that BC had both strong enzymes adsorb and desorption ability compared to lignocellulose, which was attributed to the absence of irreversible adsorption of lignin or hemicellulose. It was obviously that BC could be used as substrate for the study of the process of pure cellulose enzymatic hydrolysis, which avoided the interference of lignin or hemicellulose. The evaluated distribution of cellulase during enzymatic hydrolysis can be used to improve the cellulase recycling in further study (Tu *et al*. 2007; Rodrigues *et al.* 2014).

SEM Analysis

The micro-morphology of the untreated and SE pretreated biomass was observed by SEM to examine the apparent changes of substrates during pretreatment. As shown in Fig. 3a-b, rigid and highly ordered fibrils were observed for the untreated RS and CK. The surface of untreated CKK looks rougher than in the untreated RS. The SE treatment would result in morphological changes of RS and CKK. The morphologies of pretreated lignocellulose were quite different from untreated lignocellulose. The cellulose fibers were separated and exposed after SE pretreatment, and the external surface area and porosity were increased (Fig. 3c-d). This could lead to higher susceptibility of attack by enzymes in pretreated lignocellulose. Moreover, many droplets were found on the surface of pretreated samples, which could be from the migration and redeposition of lignin from the cell walls (Donohoe *et al.* 2010). Compared to the scattered structure of SE pretreated RS (Fig. 3c), the SE pretreated CKK showed a more compact structure (Fig. 3d). These results demonstrated that CKK had a stronger resistibility to SE pretreatment. This was most likely due to large amounts of lignin contained in the CKK.

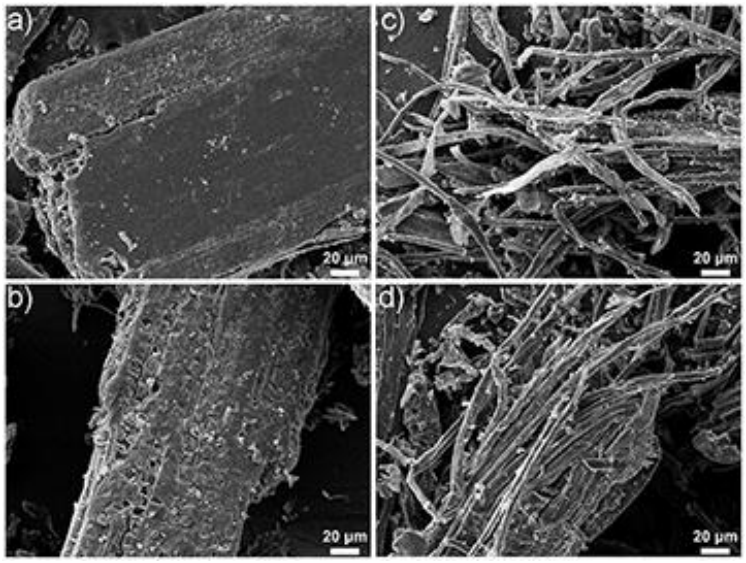

Fig. 3. SEM micrographs of untreated and SE pretreated lignocellulose: a) untreated RS; b) untreated CKK; c) SE pretreated RS; and d) SE pretreated CKK. RS: Rice straw; CKK: *Caragana korshinskii* Kom.; SE: Steam explosion

To demonstrate the microstructure changes of cellulose substrates during the enzymatic hydrolysis, SEM was employed to observe the solid residues after 0 h, 4 h, 24 h, and 72 h of hydrolysis. Figure 4a reveals that the surface of BC, compared with freeze-

dried BC, oven-dried and pulverized BC was more compact and intact (Penttilä *et al.* 2018). There were many unsmooth pits after 4 h of enzymatic hydrolysis (Fig. 4b). As the enzymatic hydrolysis proceeded, the monolithic structure was eventually degraded into small particles (Fig. 4c-d). For the SE pretreated RS and CKK, the microfibers were gradually degraded into shorter fibers and continued degradation into small particles (Fig. 4e-l). After 72 h of enzymatic hydrolysis, the size of the hydrolyzed solid residues of SE pretreated RS (Fig. 4h) was smaller than that of SE pretreated CKK (Fig. 4l). These results suggested that the cellulase could randomly cut the substrate into small pieces during the enzymatic process.

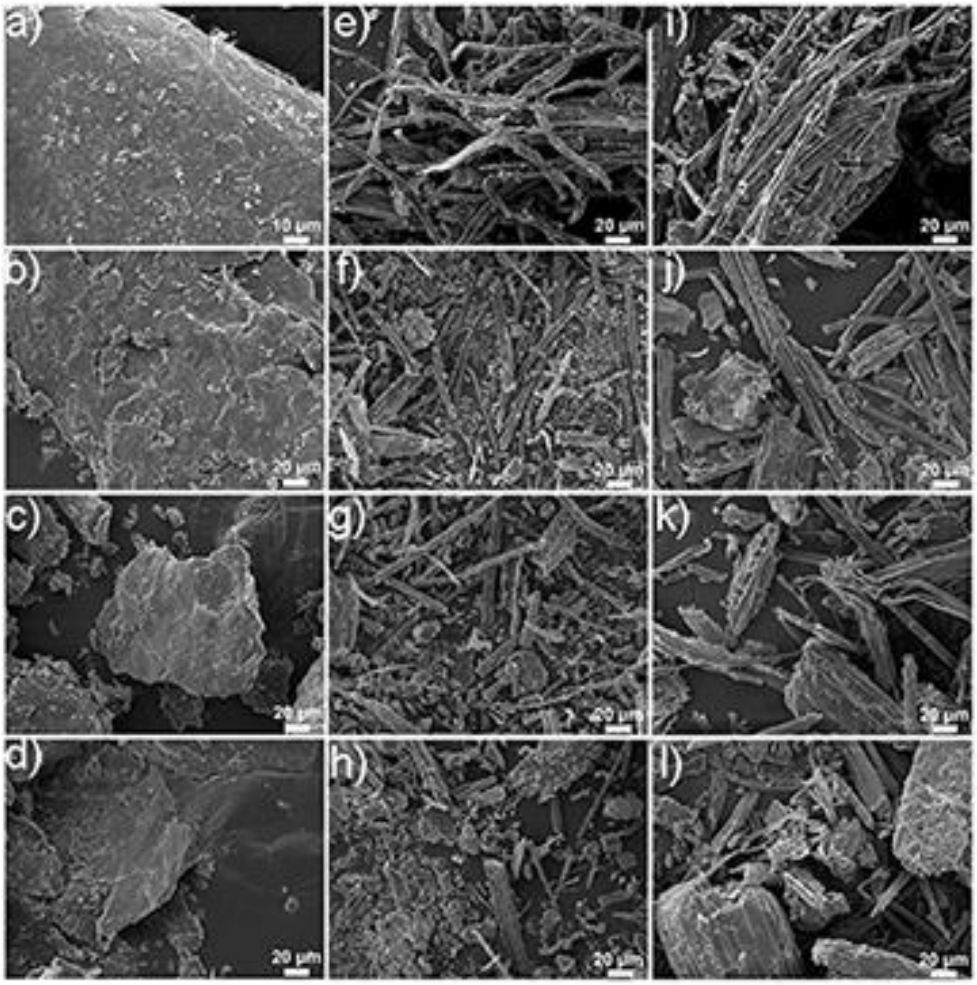

Fig. 4. SEM micrographs of solid residues after 0 h, 4 h, 24 h and 72 h of enzymatic hydrolysis of BC (a-d), SE pretreated RS (e-h), and SE pretreated CKK (i-l), respectively. BC: Bacterial cellulose; RS: Rice straw; CKK: *Caragana korshinskii* Kom.; SE: Steam explosion

FTIR Analysis

The chemical structure changes of different cellulose substrates before and after enzymatic hydrolysis were characterized by FTIR (Fig. 5). The peaks at 1058 cm^{-1}, 1108 cm^{-1}, 1164 cm^{-1}, 1370 cm^{-1}, and 3350 cm^{-1} were typically related to the special absorption of cellulose (Adapa *et al.* 2009; Hsu *et al.* 2010). The peaks at 1058 cm^{-1} and 1108 cm^{-1} were assigned to the C–O vibration and the C–O–C pyranose ring skeletal vibration. The

absorption band at 1164 cm^{-1} was assigned to the antisymmetric bridge C–O–C stretching in cellulose (Silva *et al.* 2012; Chen *et al.* 2016). The band at 1370 cm^{-1} is associated with the C–H stretch of cellulose. The peak at 3350 cm^{-1} was attributed to the O–H stretching vibrations of cellulose. The intensity of these peaks in BC was higher than that in untreated RS and CKK. This was due to BC being almost pure cellulose, while the cellulose of RS and CKK were encapsulated by hemicellulose and lignin. The absorption peaks in the untreated RS and CKK were like that in the SE pretreated solid, which indicated that the chemical structure of the cellulose in RS and CKK were not disrupted by pretreatment. For the untreated RS and CKK, the FTIR spectra showed two shoulder peaks at 1732 cm^{-1} and 1245 cm^{-1}, which corresponded to the C=O and C-O bond stretching of hemicellulose, respectively (Adapa *et al.* 2009; Hsu *et al.* 2010). However, the intensity of these two peaks in SE pretreated RS and CKK were much lower. These results confirmed the results that most of the hemicellulose was removed by SE pretreatment, which was obtained in the composition analysis. The absorption peaks at 1512 cm^{-1} and 1463 cm^{-1} in the untreated RS and CKK were assigned to the aromatic skeletal vibrations in lignin-like structures (Adapa *et al.* 2009; Zhou *et al.* 2011; Monteil-Rivera *et al.* 2013). After enzymatic hydrolysis, the peaks at 1512 cm^{-1} and 1463 cm^{-1} of SE pretreated RS and CKK were increased. The peaks at 1058 cm^{-1}, 1108 cm^{-1}, and 1164 cm^{-1} in BC and SE pretreated RS were decreased, while no noticeable change in the SE pretreated CKK. The results indicated that the cellulose content in the substrate was gradually decreased during the enzymatic hydrolysis, while the lignin content was increased.

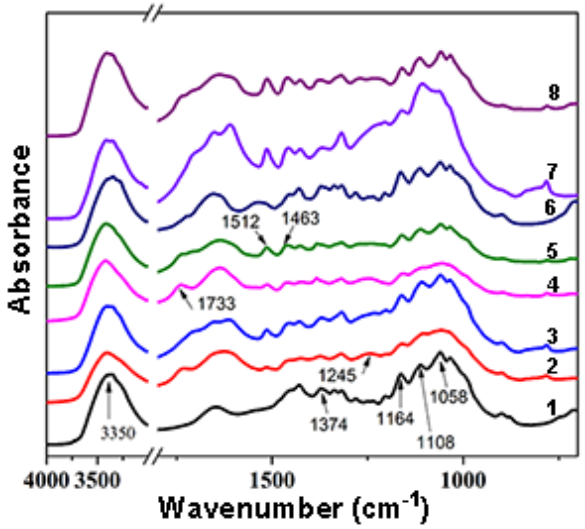

Fig. 5. FTIR spectra of different cellulose substrates before and after enzymatic hydrolysis (line 1, BC; line 2, untreated RS; line 3, SE pretreated RS; line 4, untreated CKK; line 5, SE pretreated CKK; line 6, BC after 24 h enzymatic hydrolysis; line 7, SE pretreated RS after 72 h enzymatic hydrolysis; line 8, SE pretreated CKK after 72 h enzymatic hydrolysis). BC: Bacterial cellulose; RS: Rice straw; CKK: *Caragana korshinskii* Kom.; SE: Steam explosion

XRD Analysis

To evaluate the relationship of cellulose crystallinity with the enzymatic hydrolysis, the XRD spectra of different substrates at different time points of the enzymatic hydrolysis process was measured (Fig. 6, Table 2). The diffraction peaks appeared at 2θ of 14.20°,

16.42°, and 22.36° of BC represent a typical (Iα) crystalline structure of cellulose. The large diffraction peak at approximately 22.36° represents the presence of a highly organized 'crystalline' cellulose structure, which was also observed in the XRD spectra of the untreated RS and CKK (Fig. 6b-c). However, this peak in untreated RS and CKK was wider and rounder than in BC as the presence of hemicellulose and lignin. After SE pretreatment, the peak of RS and CKK at 22.36° gets sharper and narrower. This reflected the higher crystallinity in SE pretreated RS and CKK. After enzymatic hydrolysis, the hydrolyzed solid showed a similar XRD spectra to that of the initial substrates. As a result, the cellulosic crystal style in hydrolyzed solid residues did not change during enzymatic hydrolysis.

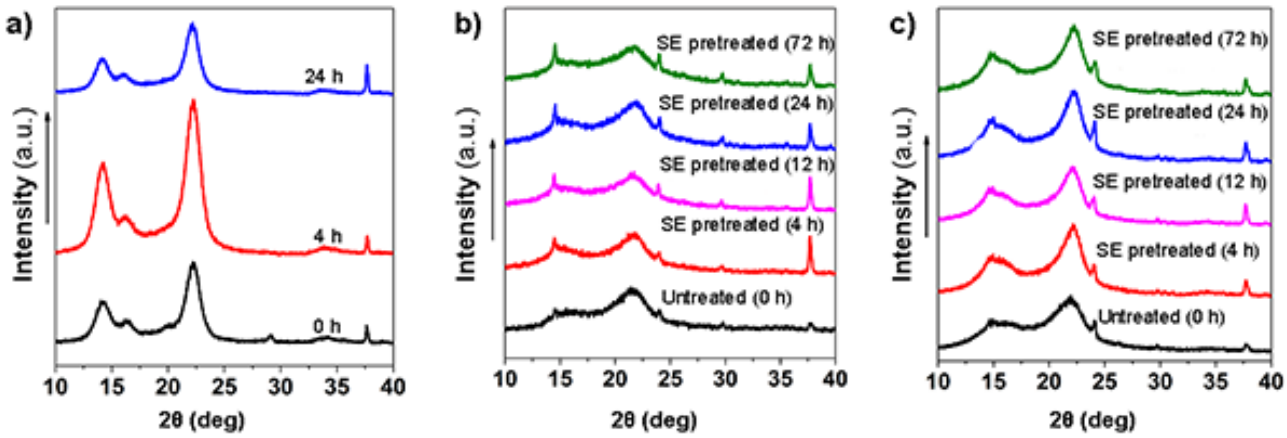

Fig 6. XRD spectra of BC(a), RS (b), and CKK (c) during enzymatic hydrolysis. BC: Bacterial cellulose; RS: Rice straw; CKK: *Caragana korshinskii* Kom.; SE: Steam explosion

Table 2 shows the *CrI* that was calculated from the X-ray diffraction patterns. The data demonstrated that there was no direct correlation between crystallinity and enzymatic hydrolysis. The previous studies had shown that the crystallinity would hinder the enzymatic hydrolysis (Mittal *et al.* 2011). BC used here was regarded as a pure and high crystalline cellulose, and one can easily study the relationship between crystallinity and enzymatic hydrolysis to exclude the influence of lignin and hemicellulose. In this study, the value of *CrI* was 91.0%, 59.9%, and 65.9% for BC, untreated RS, and CKK, respectively. BC has the highest crystallinity in all samples, but its enzymatic hydrolysis yield was the highest as well. After SE pretreatment, the *CrI* of RS and CKK was increased to 70.3% and 78.8%, respectively, and the enzymatic hydrolysis was enhanced at the same time. The increase of *CrI* for lignocellulose after acid, alkali, or SE pretreatment has also been reported in previous studies (Kapoor *et al.* 2015; Chen *et al.* 2016; Lotfi Aski *et al.* 2018). This might be due to the *CrI* of lignocellulose being sensitive to other components such as hemicellulose, lignin, and amorphous cellulose domains (Chen *et al.* 2016). The increase in *CrI* could be attributed to the removal of hemicellulose and ash, or the disruption of amorphous cellulose during SE pretreatment. Besides, the *CrI* of the BC, SE pretreated RS, and SE pretreated CKK did not show any obvious changes during the enzymatic hydrolysis. Mansfield also found there was no discernible difference in the value of *CrI* for unbleached Kraft pulp before and after enzymatic hydrolysis (76.5% and 76.8% crystalline for the samples before and after enzymatic hydrolysis, respectively) (Mansfield

et al. 1997). Wang *et al.* (2010) found *CrI* only increased by 2% after 6 days of enzymatic hydrolysis in cotton fibers. This demonstrated that the enzymatic hydrolysis might not preferentially hydrolyze amorphous cellulose. The combined effect of crystallinity and composition was the key to determining the yield of enzymatic hydrolysis (Park *et al.* 2010; Li *et al.* 2014).

Table 2. The Crystallinity Index (CrI) of Different Cellulose Substrates

	CrI of Substrates (Mean ± SD, %)		
	RS	CKK	BC
Untreated	59.9 ± 1.3	65.9 ± 4.2	91.0 ± 0.7
SE pretreated	70.3 ± 2.3	78.8 ± 1.6	N.D.
4 h hydrolysed sample of SE pretreated	68.8 ± 0.4	80.8 ± 2.6	91.6 ± 0.2
24 h hydrolysed sample of SE pretreated	71.2 ± 1.3	84.1 ± 0.7	91.3 ± 0.9
72 h hydrolysed sample of SE pretreated	71.3 ± 1.3	79.5 ± 2.8	N.D.
BC: Bacterial cellulose; RS: Rice straw; CKK: *Caragana korshinskii Kom.*; SE: Steam explosion. N.D., Not detected.			

CONCLUSIONS

1. Although the composition and enzymatic yield of different substrates varied, their enzymatic hydrolysis rates showed a similar trend during hydrolysis. The enzymatic hydrolysis rates declined with enzymatic hydrolysis. The adsorption efficiency of cellulose to the enzyme was maximized at the initial enzymatic stage.
2. Bacterial cellulose (BC) and rice straw (SE) pretreated rice straw (RS) could firstly adsorb and subsequently desorb the enzyme, while the untreated RS and *Caragana korshinskii* (CKK) had weak adsorption capacity. Untreated RS and CKK would also form irreversible adsorption with the enzyme.
3. There is no direct correlation between the crystallinity and the enzymatic hydrolysis yield of cellulose in this study. FTIR results showed that the cellulose was continuously released, and lignin was retained in the enzymatic residue during enzymatic hydrolysis.

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