Sodium Hydrogen Sulfite Pretreatment of Wood Pulp Waste for Enhancement of Enzymatic Efficiency

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Wood pulp waste is a type of industrial waste that has an enormous potential for fermentable sugar production. Efficient pretreatment is the key for enhancing the enzymatic efficiency. In this paper, sodium hydrogen sulfite pretreatment was performed on wood pulp waste to improve enzyme performance through sulfonation and hydrolysis reactions. Results showed that the enzymatic efficiency was greatly enhanced from 5.12 to 41.6% in terms of reducing sugar yield (RSY) under the optimum conditions: 6% $NaHSO_3$, 2500 P-factor, 3/1 liquid to solid ratio, and cellulase charge of 35 FPU/g substrate. Mass balance showed that approximately 73% of glucose was recovered. The mechanism of sulfonation and hydrolysis of sodium hydrogen sulfite pretreatment were confirmed by Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction (X-RD), and scanning electron microscopy (SEM).

Keywords: Wood pulp waste; Sodium hydrogen sulfite; Enzymatic efficiency; Fermentable sugar; Reducing sugar yield

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INTRODUCTION

Lignocellulosic industrial waste material is a type of raw material that can be used in bioconversion at a low cost and in an environmental friendly way (Nguyen *et al.* 2013). Wood pulp waste is a sort of industrial waste collected from the chip washing process of P-RC APMP (preconditioning refiner chemical alkaline peroxide mechanical pulping) production (Xu 2001). It has been estimated that about 8% of the total raw material can be screened out as a result of washing in the P-RC APMP production (Liu *et al.* 2013). Due to the considerable amount of water in the wood pulp waste, waste burning is not a feasible source for energy recovery. Currently, the land-filling strategy is typically adopted by the pulp mill at the cost of land and capital. Alternatively, it is a promising approach to produce fermentable sugar from these abundant industrial wastes.

The complex structure of the cell wall is composed of three major components: cellulose, hemicellulose, and lignin. This tough and compact structure (*i.e.* recalcitrant nature) makes enzymatic digestion of the woody biomass difficult. Cellulose is a homopolymer, which is linked by β-1,4-glycosidic bonds between the repeating units of β-D-glucose molecules. The linear glycan chain combined to form long packed microfibrils through intermolecular hydrogen bonds, dipole interactions, and van der Waals interaction (Harris and DeBolt 2010; Miao *et al*. 2014). Hemicellulose is a branched short chain polymer, acting as a plasticizer and coating the microfibril surface. Lignin is a three-dimensional amorphous macromolecule that functions as glue to seal the cell wall.

All these physical structures previously mentioned will hinder the enzymatic efficiency (Gomez *et al.* 2008). Moreover, the chemical structures of lignin tend to adsorb enzymes in an irreversible way, which further decreases the enzymatic efficiency. Therefore, pretreatment technology is a prerequisite to alter both physical and chemical structures of the raw material.

Recently, various pretreatment technologies have been developed (Zhou *et al.* 2013a; Bai *et al.* 2014), and include mechanical (chipping, grinding, and milling), chemical (dilute acid, alkali, ammonia steam explosion, organic solvent, and ionic liquid), and chemi-mechanical methods. The strategies of these technologies are to reduce the fiber size, decrease the cellulose crystallinity, decrease the hemicellulose and lignin content, and increase the enzyme accessibility (Vidal *et al.* 2011). In addition, some degraded organic materials, *e.g.*, furfural, 5-hydroxymethylfurfural, formic acid, and acetic acid, should be minimized as a means of improving the enzymatic hydrolysis (Dong and Bao 2010; Jahan *et al*. 2012; Shen *et al*. 2013).

Sulfite pretreatment, known as SPORL (sulfite pretreatment to overcome recalcitrance of lignocellulose), is of great interest for the scale-up potential in sulfite pulping mill (Zhu *et al.* 2009; 2010b). The advantages of such pretreatment include: (1) increased lignin hydrophilicity, (2) decreased enzyme non-productive adsorption, (3) reduced energy consumption for size reduction, (4) potential utilization of dissolved lignosulfonate, and (5) lower inhibitors amount than acid pretreatment. Results from the literature shows that the net energy output of 4.55 GJ/ton wood (before distillation) was realized when the SPORL process was performed on lodgepole pine (Zhu *et al.* 2010a).

Although many studies related to sulfite and bisulfite pretreatment on woody material have been conducted, the pretreatment of wood pulp waste from the washing process of P-RC APMP production has not been reported; this results in the unique characteristic of a swollen and small dimension. The objective of this study was to enhance the enzymatic efficiency of the industrial waste in terms of reducing sugar yield (RSY). In addition, the chemical and physical structure changes of biomass after pretreatment were characterized by Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (X-RD), and scanning electron microscopy (SEM).

EXPERIMENTAL

Materials

Wood pulp waste was collected from the chip washing stage of P-RC APMP production process of Shandong Chenming Paper Co., Ltd. (China), and stored in a cold room (-10 °C).

Sodium hydrogen sulfite and sulfuric acid were purchased from Sinopharm Chemical Reagent Co. (China). Cellulase L-10 with an activity of 150 FPU/mL (determined as Filter Paper Unit) was kindly provided by KDN Biotech Group Co., Ltd. (Tsingtao, China).

Methods

Pretreatment

Pretreatment of the wood pulp waste was conducted in 60-mL high-pressure, stainless steel vessels. The desired temperature was maintained with a thermostat-controlled silicone oil bath. The treatment conditions were: liquid to solid ratio of 3 to 6, temperature of 160, 170, 180, 190, 200, and 210 °C, and time of 5,10, 15, 20, 25, 30, 35, 40, 50, and 60 min. Initially, 5 g of oven-dried wood pulp waste and the required amount of sodium hydrogen sulfite solution were added to the vessels, which were then were immersed into a pre-heated oil bath. Once the reaction was completed, the vessels were removed immediately from the oil bath and cooled under cold tap water. Finally, the substrate and hydrolyzate were separated using filter paper and stored at -4 °C.

Enzymatic hydrolysis

A 0.5-g amount of oven-dried pretreated substrate was placed in a 100-mL Erlenmeyer flask. Then, the required amount of cellulase solution was added to the flask, and the reaction was performed in a shaking water bath (Zhicheng Co., Shanhai). The cellulase hydrolysis conditions were according to the authors' previous work (Liu *et al*. 2011): substrate concentration of 2% (w/v), pH 4.8 (with 0.1 M acetate buffer), cellulase dosage of 35 FPU/g (oven-dried pretreated substrate), 50 °C, 48 h, and 160 rpm. Once the reaction was finished, the hydrolyzate and residual were filtered by filter paper.

Chemical analysis

The chemical composition of biomass was measured using high performance liquid chromatography (HPLC; Agilent 1100, USA) after two-stage acid hydrolysis. Prior to chemical analysis, the wood pulp waste was milled and screened at 40 to 60 mesh (0.38 to 0.25 mm). Initially, 0.3 g of sample was hydrolyzed with 3.0 mL of 72% (w/v) H_2SO_4 for 1 h at 30 °C. After that, the hydrolysate was diluted in 4% (w/w) H_2SO_4 with distilled water and then hydrolyzed for 1 h at 121 °C (Yang *et al*. 2013a). Klason lignin was measured gravimetrically after washing and drying the solid residue from the acid hydrolysis according to TAPPI T222 (1988). Benzene-ethanol extractive and ash content were also measured based on the gravimetric method according to TAPPI T204 (1988) and T211 (1991), respectively. The composition of the reducing sugar in the enzymatic hydrolyzate was analyzed using HPLC after acid hydrolysis at 121 °C and 1 h.

For fast analysis, the reducing sugar in the spend liquor was determined by UV-Vis spectrophotometry at a wavelength of 540 nm according to the DNS method (Pala *et al*. 2001). The reducing sugar yield (RSY) was calculated using Eq. 1,

$$\mathrm{RSY} = \frac{CV}{m(1-\omega/100)} \times n \times 100\% \tag{1}$$

where m is the pretreated substrate weight (g), ω is the moisture content (%), C is the concentration of reducing sugar (g/L), V is the total volume of hydrolysate (L), and n is the number of dilutions.

Characterization methods

Fourier transform infrared spectroscopy was performed with a MAGNA-IR 550 FTIR (Thermo Nicolet, USA). The spectrum was set at 500 to 4000 cm^{-1} using a potassium bromide (KBr) disc. The background spectrum was suppressed before sample analysis.

An X-ray diffractometer (D8-FOCUS, Bruker, Germany) was used to determine the crystallinity of samples with Cu Kα radiation (λ = 0.154 nm) at 10 kV. The X-RD data was collected from 2θ = 10 to 60° at a scan rate of 2°/min. The sample was ground into 80 to 120 mesh. The crystallinity (Xc) was calculated based on Eq. (2):

$$X_c = \frac{A_{cr}}{A_{cr} + A_{am}} \times 100\% \tag{2}$$

where A_{cr} and A_{am} are the integrated area of the crystalline and amorphous phases, respectively.

Scanning electron microscopy (SEM) was performed with an S-3400 (Toshiba, Japan) at an accelerating voltage of 15 kV to observe the morphology of the fiber surface. Prior to being placed in the microscope, the samples were sputter-coated with platinum for good conductivity to avoid sample charging.

RESULTS AND DISCUSSION

Chemical Components of Raw Material

Figure 1 shows the morphology of poplar wood pulp waste and poplar chips. It can be clearly seen that the wood pulp waste had smaller dimensions than the chips. Table 1 lists the chemical components of wood pulp waste, which is rich in glucan, implying the great potential for bioconversion. However, the deconstruction of cell wall to facilitate enzyme access is necessary for enhancing the enzymatic efficiency. The sodium hydrogen sulfite pretreatment was adopted for this purpose.

Fig. 1. Morphology of wood pulp wastes (left) and chips (right)

Table 1. Wood Pulp Waste Chemical Components

Composition	Glucan	Xylan	Klason lignin	Benzene-ethanol extractives	Ash
Content (%)	49.2±0.5	21.2±0.2	23.6±0.2	1.10±0.0	2.63±0.02

Effect of Liquid /Solid Ratio

Table 2 shows the effect of liquid to solid ratio on the sodium hydrogen sulfite pretreatment. It can be seen that the solid recovery greatly dropped after pretreatment, implying the dissolving of the chemical components. As a result, the RSYs were greatly increased by about 300 to 400% from the untreated sample. Alternatively, the lower liquid-to-solid ratio yielded a lower solid recovery and higher RSY. This can be explained by the lower final pH and higher chemical concentration, which enhances the acid hydrolysis process during the pretreatment.

Table 2. Effect of Liquid /Solid Ratio of Pretreatment

Liquid /solid ratio	Initial pH	Final pH	Solid recovery (%)	RSY (%)
3	1.50±0.00	1.74±0.02	66.8±0.5	21.1±0.2
4	1.52±0.01	1.97±0.01	68.5±0.7	17.8±0.1
5	1.53±0.00	2.04±0.01	69.2±0.4	17.0±0.2
6	1.56±0.01	2.09±0.02	71.5±0.7	13.9±0.1
Untreated sample			100	5.12±0.05

Note: Pretreatment conditions as follows: $NaHSO_3$ 3.0% and H_2SO_4 1.1% (based on wood pulp waste), 180 °C, and 30 min. Enzymatic hydrolysis: cellulase 35 FPU g^{-1} (based on pretreated substrate), 48 h, pH 4.8, and 50 °C

Effect of P-factor

The P-factor has been introduced to the pretreatment process by combining pretreatment temperature and time (Gütsch *et al*. 2012). The formula is given as Eq. 3,

$$\text{P-factor} = \int_0^t \exp(43.2 - \frac{E_a}{RT})dt \tag{3}$$

where T refers to the pretreatment temperature (Kelvin), R is the constant (8.314 J/(mol K)), E_a is the activation energy (134 kJ/mol), and t is the pretreated time.

Figures 2a and 2b show the solid recoveries and RSYs as a function of P-factor, respectively. As can be seen, the solid recoveries decreased dramatically from 75 to 62% at the first 2500 h, and then leveled off. This can be explained by the fact that the hemicellulose and part of lignin were sensitive to the pretreatment, while the cellulose was rather stable at a high P-factor. After the pretreatment, the enzymatic hydrolysis was conducted on all the substrates. Accordingly, the RSYs increased quickly with elongation of P-factor to 2500 h, and then remained stable at high levels. It can be seen that both solid recoveries and RSYs were well described by the P-factor at the given chemical charge (Figs. 1a2 and 1b2). The P-factor of 2500 h was the critical point of initial fast phase and second slow phase. A similar study reported that the solid recovery decreased rapidly at the initial pre-extraction stage when using green liquor to extract Loblolly pine (Yoon and Heiningen 2010).

Effect of $NaHSO_3$ and H_2SO_4 Charge

Because it is a branched short chain polymer, hemicellulose can easily be attacked by hydrogen ions to form oligo/mono sugars, acetic acid, and furfural during the pretreatment process (Yang *et al*. 2013b; Zeng *et al*. 2014). Lignin can also form soluble fragments or altered chemical structures through sulfonation and degradation reactions through sulfite or bisulfite ion attack.

The effect of chemical charge on solid recoveries and RSYs are shown in Table 3. As shown, the solid recoveries gradually decreased with increased chemical charge, especially for the sulfuric acid charge, implying that the acid charge played a major role in the degradation of components. However, the RSY decreased at the high sulfuric acid charge (2.2%). A decrease in the enzymatic efficiency at high acid charge was also observed by Zhu *et al*. (2009) when using SPORL to convert spruce into biofuel.

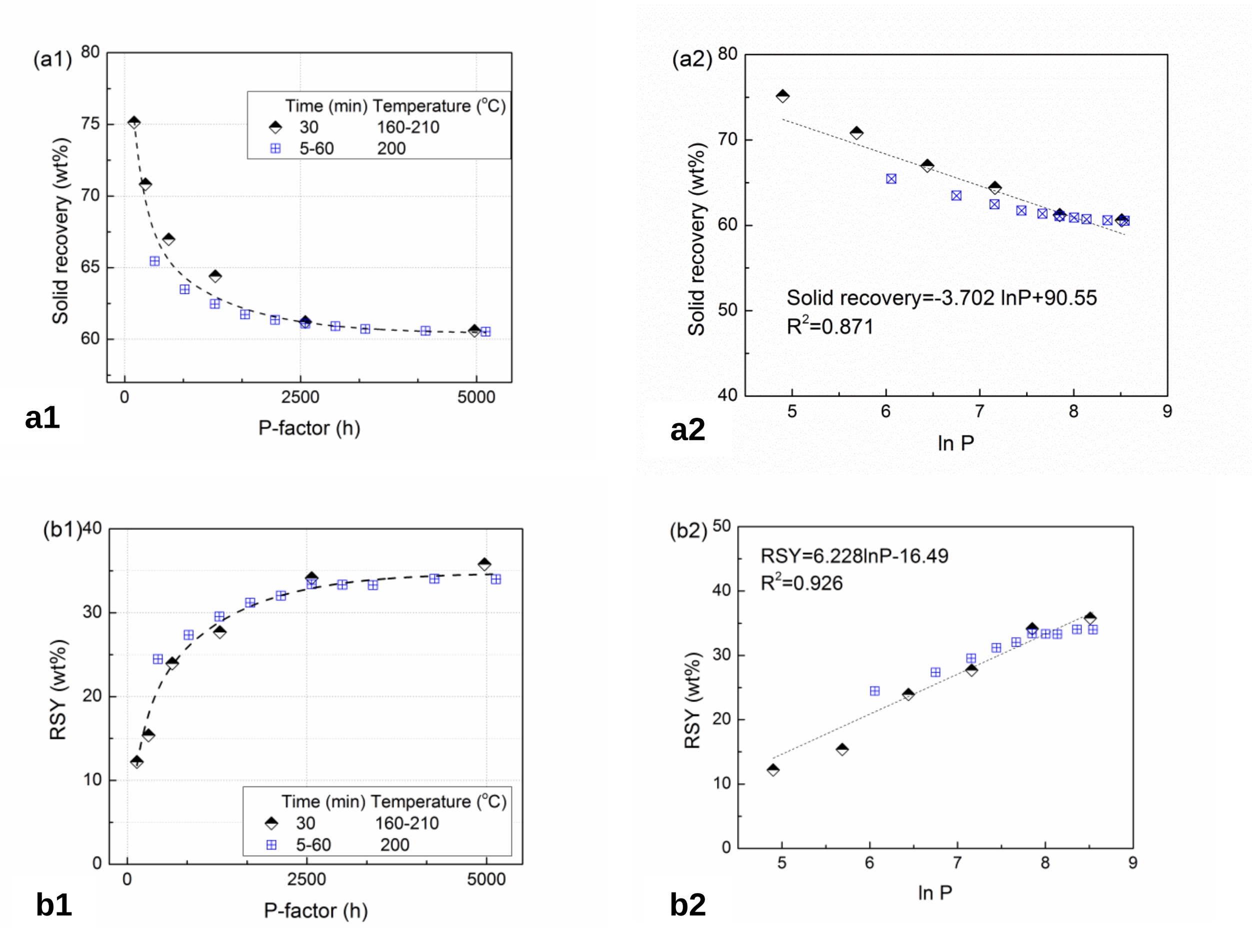

Fig. 2. (a) Pretreated solid recovery *versus* P-factor and (b) RSY (reducing sugar yield based on oven dried pretreated substrate) *versus* P-factor.
Pretreatment conditions: $NaHSO_3$ 3.0% and H_2SO_4 1.1% (based on wood pulp waste), Liquid /solid ratio 3/1. Enzymatic hydrolysis: cellulase 35 FPU g^{-1} (based on pretreated substrate), 48 h, pH 4.8, and 50 °C

The RSYs were greatly improved by increasing the sodium hydrogen sulfite charge, suggesting that the lignin sulfonation and degradation increased the enzymatic efficiency. Moreover, the non-productive adsorption of cellulase to lignin can be decreased by lignin sulfonation (Zhou *et al*. 2013b). Varnai *et al*. (2010) claimed that the lignin content was the key factor affecting the enzymatic hydrolysis for the pretreatment technology. Therefore, the optimum condition of 6% $NaHSO_3$, 2500 P-factor, 3/1 liquid to solid ratio was selected for the following experiment.

Mass Balance of Optimized Pretreatment Process

Figure 3 summarizes the mass balance of 1000 g wood pulp waste bioconversion process under optimum conditions. The dash line was not conducted in this study. It can be seen that hemicellulose was substantially removed in the pretreatment stage, which was up to 91% of the total xylose. Meanwhile, approximately 29% of lignin and 24% of cellulose were degraded and dissolved into the pretreatment liquor. The amount of inhibitors such as furfural, HMF, and formic acid in the hydrolyzate was low. After enzymatic hydrolysis, 357 g of cellulose was recovered from enzymatic hydrolysis liquor, which represented 73% of the total glucose in the wood pulp waste. About 70% of total lignin remained in enzymatic hydrolysis residue. For the bioconversion process, about 15% of mass was not accounted for during the bioconversion process, which may be a result of being lost either as gaseous or unidentified compounds.

Table 3. Effect of Chemical Charge on RSY

H_2SO_4 Charge (%)	$NaHSO_3$ Charge (%)	Initial pH	Final pH	Solid recovery (%)	RSY (%)
0.00	0	2.96±0.02	3.77±0.04	66.2±0.46	33.5±0.24
	1	2.83±0.02	3.58±0.03	64.6±0.45	34.0±0.34
	3	2.63±0.01	3.75±0.04	61.6±0.62	36.9±0.27
	6	2.52±0.02	3.72±0.04	56.4±0.56	41.6±0.32
0.55	0	1.37±0.00	3.13±0.03	64.6±0.45	31.2±0.21
	1	1.38±0.01	3.20±0.03	64.1±0.44	32.9±0.23
	3	1.48±0.01	3.14±0.03	61.7±0.52	34.9±0.35
	6	1.59±0.01	3.22±0.03	54.3±0.54	39.5±0.30
1.10	0	1.12±0.00	2.25±0.02	61.3±0.51	29.1±0.25
	1	1.13±0.00	2.31±0.02	60.7±0.41	31.0±0.31
	3	1.21±0.01	2.62±0.03	59.8±0.50	33.2±0.23
	6	1.36±0.01	2.50±0.03	55.0±0.55	38.2±0.28
2.20	0	0.80±0.01	1.47±0.01	44.4±0.44	4.65±0.05
	1	0.80±0.00	1.52±0.02	44.4±0.54	9.12±0.09
	3	0.78±0.01	1.49±0.02	42.8±0.33	25.2±0.15
	6	0.77±0.01	1.55±0.02	40.4±0.40	33.9±0.24

Note: Pretreatment conditions: P-factor 2500, Liquid/solid ratio 3/1. Enzymatic hydrolysis: cellulase 35 FPU g^{-1} (based on pretreated substrate), 48 h, pH 4.8, and 50 °C

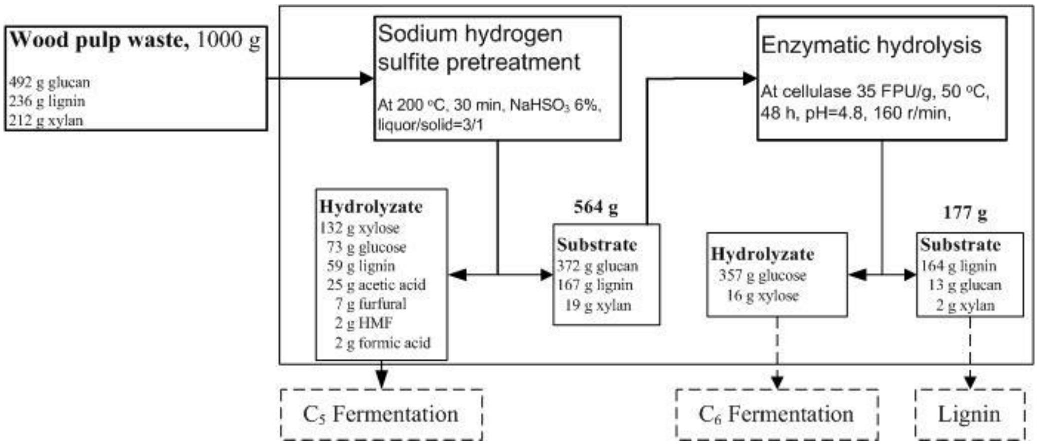

Fig. 3. Mass balance for the bioconversion of wood pulp waste

Characterization of Wood Pulp Waste and Pretreated Substrate

Fourier transform infrared spectroscopy provides information on chemical structure, molecular conformation, and hydrogen bonding patterns. The FTIR spectra of wood pulp waste and pretreated substrate are shown in Fig. 4a. The bands at 1168 and 1190 cm^{-1} were attributed to the absorption of sulfonic group (Bu *et al.* 2012), which did not appear in the spectrum of the untreated sample. This indicated that the lignin sulfonation occurred during the pretreatment. Bu *et al.* (2012) reported that the sulfonation of the lignin was attributable to the reduction of non-specific protein binding, which contributed to an increase of enzymatic hydrolysis efficiency. The bands at 1430 and 893 cm^{-1} were attributed to crystallized and amorphous cellulose, respectively. The decrease of the ratio (A1430/A893) reflected the reduction of crystallinity.

Furthermore, X-RD was employed to determine the changes of crystallinity (Fig. 4b). Based on Eq. (2), the crystallinity index of pretreated substrate was 65.7%, which was higher than the untreated wood pulp waste (55.4%), which agreed with the FTIR results. This can be explained by the substantial removal of the amorphous regions, including xylan, glucan, and part of lignin during the pretreatment. The exposure of cellulose was beneficial for the cellulose accessibility increase, thus enhancing enzymatic efficiency.

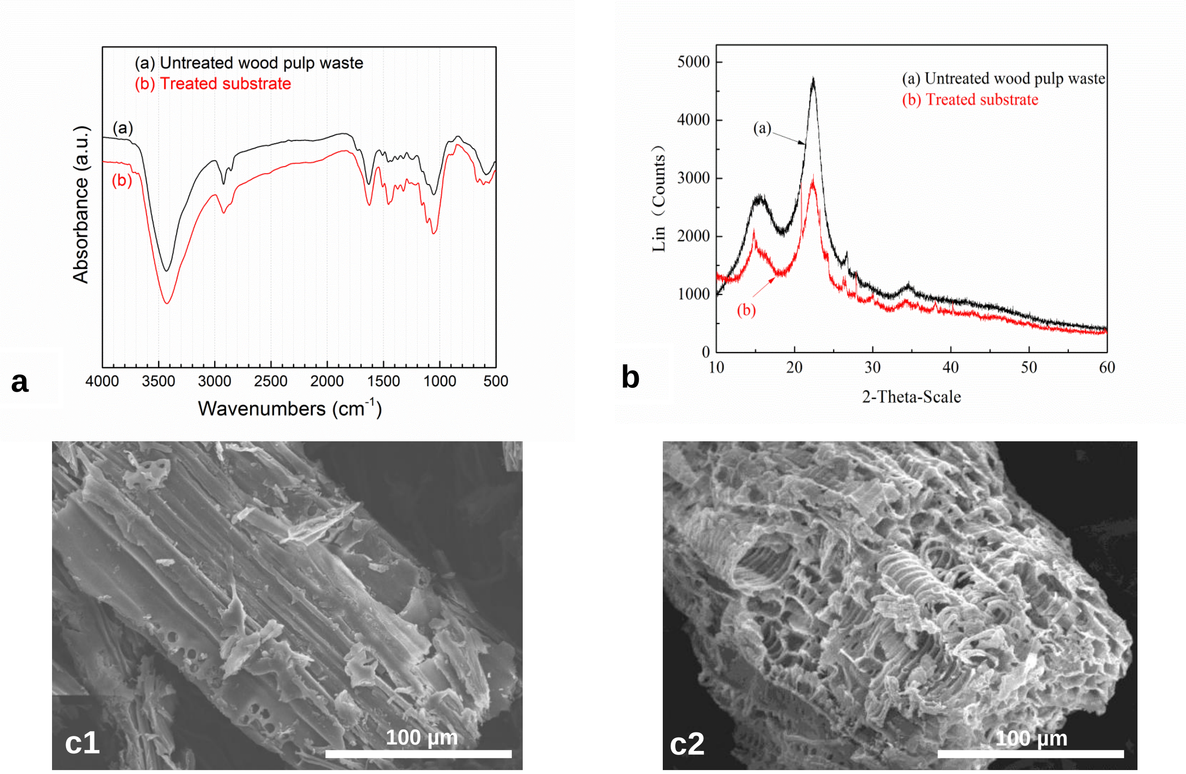

Fig. 4. (a) FTIR and (b) X-RD spectra of un-treated and pretreated samples. SEM micrographs of (c1) un-treated and (c2) pretreated samples.
Pretreatment conditions: $NaHSO_3$ 6% (based on wood pulp waste), P-factor 2500, and a liquid to solid ratio 3/1

In addition, SEM analysis was performed on the biomass and pretreated substrate (Fig. 4c). The untreated sample displayed a compact structure, showing ordered and smooth surfaces of fibrils. The pretreated fibers had many openings, which were generated through swelling and dissolving reaction. The porous fiber structures facilitated the enzyme accessibility, which enhanced the enzymatic efficiency. Sindhu *et al.* (2011) also observed the obvious changes on fiber surface of biomass after pretreatment for bioethanol production.

CONCLUSIONS

1. Sodium hydrogen sulfite pretreatment was performed on wood pulp waste from P-RC APMP production process to enhance the enzymatic efficiency. The experimental results showed that the enzymatic efficiency was greatly improved from 5.12 to 41.6% in terms of reducing sugar yield by the pretreatment.
2. Pretreatment P-factor of 2500 was the critical point between initial fast phase and second slow phase. The enzymatic efficiency was further improved through increasing sodium hydrogen sulfite charge. Mass balance showed that about 73% of the total glucose was recovered under the optimum condition.
3. The mechanism of sulfonation and hydrolysis during the pretreatments enhanced the enzymatic efficiency, which was confirmed by FTIR, X-RD, and SEM analysis.

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