EFFICIENT FRACTIONATION OF CHINESE WHITE POPLAR BIOMASS WITH ENHANCED ENZYMATIC DIGESTIBILITY AND MODIFIED ACETONE-SOLUBLE LIGNIN

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Fractionation using concentrated phosphoric acid is a cost-effective pretreatment approach due to production of highly reactive amorphous cellulose under modest reaction conditions. Chinese white poplar biomass was used as feedstock. The effects of pretreating temperature and liquid/solid ratio of H_3PO_4/poplar (v/w, ml/g) on poplar fractionation, enzymatic hydrolysis efficiency (EHE), and supramolecular structural change were investigated. Only 31% (w/w, g/g) cellulose was retained in the solid phase at the higher liquid/solid ratio of 10:1 for 60 min, while 38 % cellulose was retained at 8:1. Temperature played an important role in lignin removal, xylan hydrolysis, and enzymatic hydrolysis, which may eventually influence cellulose conversion. More than 40% lignin could be removed after 60 min pretreatment at above 50 ^{o}C. A majority of the xylan hydrolysis could be detected in mixed rinsing liquid after 80 min pretreatment at 50 ^{o}C and liquid/solid ratio of 10:1. Up to 96.37% EHE could be obtained after 24 h enzymatic hydrolysis at 50 ^{o}C. The optimal pretreatment condition was 50 ^{o}C, liquid/solid ratio 8:1 (v/w), and 60 min. After pretreatment the *CrI* index decreased from 39.9 % to 27.7 %, suggesting a decrease of crystalline area percentage. Pyrolysis-GC-MS results of precipitated lignin indicated that nearly 48% of the lignin was phenolic, such that it can be used as a natural antioxidative material.

Keywords: Enzymatic hydrolysis; Fractionation; Cellulose recovery; Phosphoric acid; Chinese White Poplar; Pyrolysis-GC-MS

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INTRODUCTION

Lignocellulose is the most plentiful renewable biomass produced from photosynthesis. It is estimated that global annual worldwide production is in the range of 10 to 50 billion tons (Sanchez and Cardona 2008). Bioconversion of lignocellulose has the potential to produce biofuels or bulk chemicals from renewable materials with less greenhouse gas emission. Cellulosic ethanol, as one of the most important biofuels, offers unique and desirable features: a secure source of supply, limited conflict with land use for food and feed production, and lower fossil fuel inputs (Antoine and Bärbel 2009). Cellulose is a polymer of glucose, which could be fermented to make ethanol or the longer-chain alcohol butanol. Hemicelluloses are polymers of various sizes that incorporate a range of different sugars, whereas lignin has a polymer backbone made from phenolic groups, which are ring-shaped, carbon-based structures (Sanderson 2011).

Lignins are biologically synthesized from the oxidative coupling of p-hydroxycinnamyl alcohol monomers and related compounds (Vanholme et al. 2008).

The conversion of biomass to ethanol usually involves four sequential steps, including lignocellulose pretreatment, enzymatic hydrolysis, fermentation, and distillation. Various pretreatment techniques have been proposed and investigated, involving dilute acid, ammonia recycle percolation, lime, steam explosion (Hendriks and Zeeman 2009), and acidic wet oxidation (Varga et al. 2004). Pretreatment loosens the structure of lignocellulose, increasing the accessible surface area, decrystallizing and depolymerizing cellulose, dissolving part of the hemicelluloses, and solubilizing part of the lignin, which finally enhance the accessibility of cellulose to cellulase, and increase the final cellulose conversion (Antoine and Bärbel 2009).

Recently, pretreatment under moderate conditions has been investigated as a means to save energy consumption. Organic solvent-based lignocellulose fractionation is a representative pretreatment method, which separates lignocellulose components in phosphoric acid and an organic solvent environment (Li et al. 2009; Kim and Mazza 2008). Based on analysis of different solubility of cellulose, hemicelluloses, and lignin in a cellulose solvent phosphoric acid and an organic solvent acetone, the fractionation process is an effective pretreating method leading to the enhancement of enzymatic digestibility (Moxley et al. 2008; Rollin et al. 2010; Zhang at al. 2007). Systematic studies on how different pretreating factors affect fractionation and enzymatic digestibility have been lacking, and this has held back practical applications of this pretreating method.

Chinese white poplar was used as raw material in our phosphoric acid-acetone fractionation experiments. The yield rate of poplar (one kind of short-rotation and perennial crop) is about 7 times higher compared to natural forests (U. S. DOE 2009), which justifies its being characterized as a dedicated energy crop for cellulosic ethanol production. Effects of pretreating temperature, pretreating time, pretreating liquid/solid ratio on distributions of cellulose, xylan, lignin in solid residual and glucose, xylose, and lignin in liquid were studied in detail in this work. Biomass recovery and enzymatic hydrolysis efficiency were monitored to evaluate the pretreatment systematically. SEM, XRD, and pyrolysis-GC-MS were employed to illustrate the supramolecular structure, the percentage of crystalline area, and lignin monomer change of acetone-soluble lignin, respectively.

EXPERIMENTAL

Raw Material Preparation

Poplar branches were collected from Changping District, Beijing. Air-dried poplar branches were hammer-milled and screened. The -40/+70 mesh fraction was collected and stored at room temperature. Moisture, ash content, and benzene/ethanol extractives were determined according to corresponding Chinese standards as follows: moisture (GB/T 2677.2-1993), ash (GB/T 2677.3-1993), and benzene/ethanol extractives (GB/T 2677.6-1994). Determinations of structural carbohydrate and lignin of the starting

biomass materials were followed by standard analytical methods (Sluiter et al. 2008; NREL/TP-510-42618).

Pretreating and Rinsing Procedures

Based on the results of preliminary work, one gram of air-dried poplar was pretreated with 85 wt.% phosphoric acid in a 50 mL centrifuge tube (shown in Fig. 1) at a liquid/solid ratio of 8:1 to 10:1 (v/w, ml/g) and temperature of 40 °C to 60 °C. Samples were mixed with a glass rod and incubated in an air bath for 40 to 100 min at 100 rpm. 20 mL of pre-chilled acetone was added into the tube to precipitate the slurry. The samples were centrifuged at 10000 rpm for 10 min at room temperature. The pellets were resuspended by adding 20 mL of pre-chilled acetone and centrifuged twice. After decanting the supernatant, solid pellets were washed with 20 mL deionized water twice and centrifuged at 10000 rpm for 10 min. The residual solid pellets were stored at 4 °C for following compositional analysis and enzymatic hydrolysis tests.

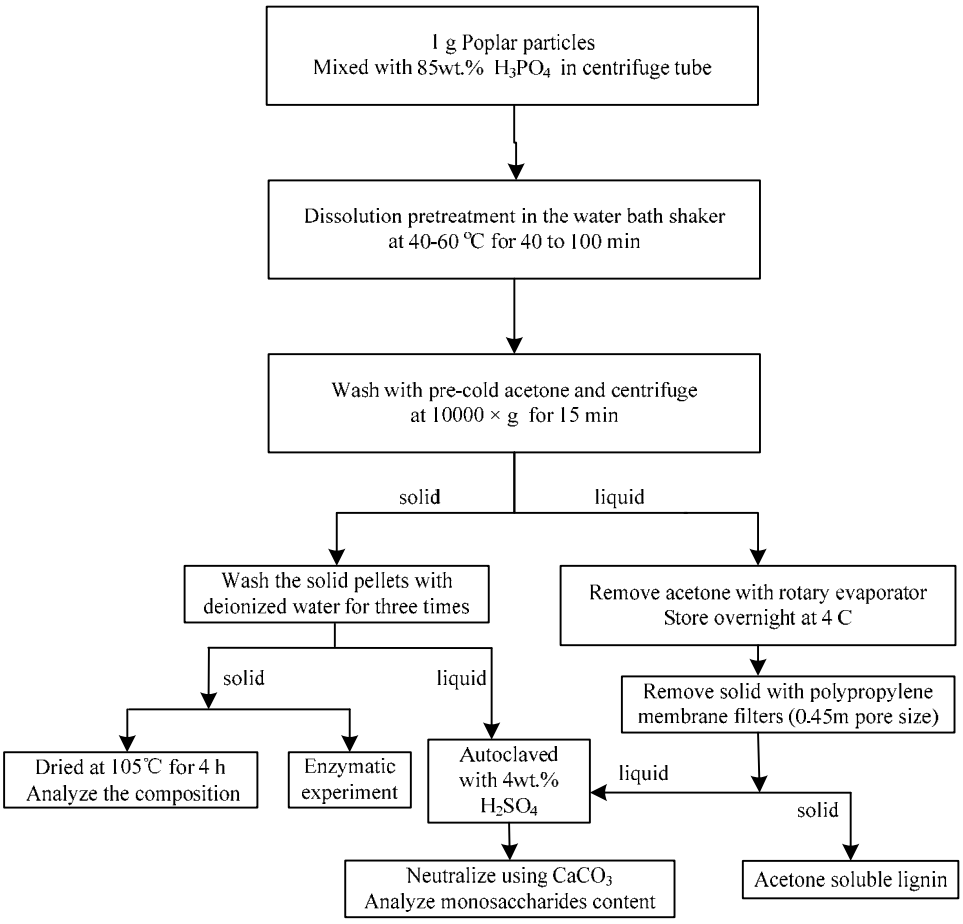

Fig. 1. Flow scheme of pretreating process

As for the phosphoric acid-acetone supernatant, rotary evaporators were used to separate acetone through vacuum distillation at 0.08 Mpa and 50 °C. After acetone evaporation, 50 mL of deionized water was added to acetone-free phosphoric acid, then

precipitating lignin for 10 h. The precipitated lignin was filtered through a polypropylene membrane (0.45 μm pore size) in a vacuum filter holder, dried at 105 °C for 2 h, and finally weighed. The combined acetone-free delignified supernatant and water-washing supernatant were retained at 4 °C for further analysis.

HPLC Analysis

High performance liquid chromatography (HPLC) (using LC20-AD, Shimadzu Corporation, Japan) was carried out as per the NREL procedure (Sluiter et al. 2006, NREL/TP-510-42623). A Bio-Rad Aminex 87H column (300×7.8 mm, Bio-Rad Laboratories, Hercules, CA) was used to determine the monosaccharide in the liquid phase. Percent component recovery in solid was calculated with Eqs. 1 and 2 based on the component weight in raw poplars, respectively. Percent monosaccharide or lignin in liquid was calculated with Eqs. 3 and 4, respectively.

$$\text{Component recovery}(\%)=\frac{\text{solid residual(g)} \times \text{component (\%) in pretreated sample}}{\text{component(g) in raw material}}\times 100\% \quad (1)$$

$$\text{Total sugar recovery}(\%)=\frac{\left\{\begin{array}{l}\left[\text{glucose recovery}(\%)+\text{cellulose recovery}(\%)\right]\times \\ \text{cellulose(g) in raw material} + \text{xylan(g) in raw material} \\ \times\left[\text{xylose recovery}(\%)+\text{xylan recovery}(\%)\right]\end{array}\right\}}{\left[\text{cellulose(g)} + \text{xylan(g)}\right]\text{in raw material}}\times 100\% \quad (2)$$

$$\text{Monosaccharide recovery}(\%)=\frac{\text{soluble monosaccharide(g) in liquid} \times 0.9}{\text{corresponding polysaccharide(g) in raw material}}\times 100\% \quad (3)$$

$$\text{Acetone-soluble ligin recovery}(\%)=\frac{\text{lignin(g) precipitated from acetone}}{\text{lignin(g) in raw material}}\times 100\% \quad (4)$$

Enzymatic Digestibility

0.15 g solid samples were subjected to enzymatic hydrolysis. Enzymatic hydrolysis was conducted in 5.0 mL citrate buffer (0.05 M, pH 4.8, containing 0.25% sodium azide) for 72 h at 50 °C and 150 rpm as per NREL procedure (Selig et al. 2008, NREL/TP-510-42629). Cellulase (NS50013 at 70 FPU/ml) and *β*-glucosidase (NS50010 at 250 CBU/ml) were kindly provided by Novozymes (Beijing, China). The cellulase loading was 15 FPU/g cellulose, and the *β*-glucosidase loading was 30 CBU/g cellulose. Samples extracted from the hydrolysis were kept in boiling water to inactivate the enzymes. Supernatant solution was transferred into a centrifuge tube and centrifuged at 10000 rpm for 15 min. The final supernatant was filtered through a 0.45 μm membrane and stored at

4 °C for subsequent HPLC analysis. All assays were performed in duplicate. The enzymatic digestibility and cellulose conversion were calculated with Eqs. 5 and 6, respectively.

$$\text{Enzymatic hydrolysis efficiency}\left(\text{EHE}\right)(\%)=\frac{\text{hydrolyzed glucose(g)} \times 0.9}{\text{cellulose(g) in pretreated sample}}\times 100\% \quad (5)$$

$$\text{Cellulose conversion}(\%)=\frac{\begin{array}{c}\text{cellulose percentage}(\%)\text{ in pretreated sample} \times \\ \text{solid residual(g)} \times \text{enzymatic hydrolysis efficiency}(\%)\end{array}}{\text{cellulose(g) in raw material}}\times 100\% \quad (6)$$

Characterization

Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) was performed in JEOL microscope (JSM-6460LV, Japan Electronics Co. Ltd, Japan) with acceleration voltage of 120 kV.

X-ray diffraction (XRD)

X-ray diffraction (XRD) patterns were collected on a powder X-ray diffracto-meter (RICOH, D/max TTR-Ⅲ, Japan), with the operating conditions set at 40 mA and 40 mV. It was run over a 2θ range of 5 to 30° at 0.02° step intervals, with a step time of 0.4 s (Ozgul et al. 2010). Crystallinity index (*CrI*) is defined as the percentage of crystallinity material in the biomass, and was calculated with Eq. 7 (Segal et al. 1959),

$$CrI(\%)=\frac{I_{002}-I_{am}}{I_{002}} \times 100\% \quad (7)$$

where I_{002} is the maximum intensity of 002 peak at $2\theta = 22.5°$ and I_{am} is the intensity at $2\theta = 18.7°$.

Pyrolysis GC-MS (PY-GC-MS)

Pyrolysis was performed in a CDS Pyroprobe 5200 pyrolyser (Chemical Data Systems). An analytical balance (readability of 0.01 mg) was used to weigh raw poplar and precipitated lignin accurately (0.50 mg ± 0.02 mg). The pyrolysis temperature was set at 600 °C and held for 20 s, with a heating rate of 20 °C/ms. The injector temperature of GC (Clarus 560, Perkin Elmer) was kept at 300 °C. The chromatographic separation was performed in an Elite-35MS capillary column (30 m × 0.25 m i.d., 0.25 μm film thickness). Helium (99.999%) was used as the carrier gas with a constant flow rate of 1 mL/min and a 1:80 split ratio. The oven temperature was programmed from 40 °C to 180 °C (3 min) with a heating rate of 4 °C/min and then to 280 °C (4 min) with a heating rate of 10 °C/min. The temperature of the GC/MS interface and source temperature was held at 280 °C, and the mass spectrometer was operated in EI mode at 70 eV. The mass spectra were obtained from m/z 20 to 400. Chromatographic peaks were identified

according to NIST MS (National Institute of Standards and Technology, Mass Spectral) library and literature data of pyrolysis products.

RESULTS AND DISCUSSION

Compositional Analysis of Poplar

Compositional analysis is crucial for verifying fractionation effects and enzymatic digestibility. Based on the whole-cell-wall analysis of NREL procedures (Sluiter et al. 2008, NREL/TP-510-4261 8), peeled branch of poplar was immersed in 72 wt. % sulfuric acid at 30 ^{o}C for 1 h, then the solution was diluted with water to reach the final concentration of 4 wt. % sulfuric acid and autoclaved for 1 h. The hydrolyzate was subjected to monosaccharide quantification with HPLC.

Table 1. Composition of Poplar Branches (stored in air) and Poplar Wood

Component	Mean[a] (%)	Mean[b] (%) (Wyman et al. 2009)	Mean[c] (%) (Wyman et al. 2009)
Ash	0.92	0.8	1.1
Moisture	1.91	-	-
Benzene-ethanol extractives	2.33	3.4	3.6
Acid-insoluble lignin	25.04	21.4	29.1
Cellulose	40.77	45.1	43.8
Xylan	18.62	17.8	14.9

[a] based on an air-dried sample
[b] based on a dry weight. Planted in Arlington, Wisconsin site, harvested in 2004
[C] based on a dry weight. Planted in Alexandria, Minnesota, harvested in 2004

Table 1 shows the chemical composition of poplar samples. Compositions of baseline material poplar (Wyman et al. 2009) obtained from farms in Alexandria (Minnesota, USA) and in Arlington (Wisconsin, USA) are also shown for comparison. Cellulose, xylan, and lignin accounted for 40-45%, 15-18%, and 21-29% of the raw material respectively. There was no obvious discrepancy between our results and those of the other studies mentioned above.

Effect of Liquid/Solid Ratio (H_3PO_4/Poplar)

The effects of liquid/solid ratio of H_3PO_4/poplar in fractionation are shown in Table 2. Liquid/solid ratios of 8:1 and 10:1(v/w) were employed to investigate the distributions of glucose, xylose, and lignin during poplar pretreatment for 40 to 100 min at 50 ^{o}C. The calculated composition recovery is shown in Table 3. Pretreatment with the higher ratio of 10:1 resulted in more cellulose loss in solid residual. Only 0.31 g cellulose could be obtained at liquid/solid ratio of 10:1 for 60 min with 1 g raw poplar, while 0.38 g cellulose was obtained at 8:1.

Glucose and xylose were detected in combined acetone-free delignified supernatant and water-washing supernatant simultaneously. By contrast, Moxley et al. (2008)

detected only xylose but no glucose in water-washing supernatant in previous research involving phosphoric-acetone fractionation. In our preliminary experiments glucose and xylose were detected in both delignified phosphoric liquid and water-washing supernatant (data not shown). Cellulose and xylan dissolved more at higher liquid/solid ratio. The explanation could be that dissolved cellulose and xylan are partially hydrolyzed into monosaccharide with excessive phosphoric acid soaking, so that the glucose content in combined supernatant at liquid/solid ratio of 10:1 was 6 times (0.06 g vs 0.01 g) of that at 8:1 (in Table 2).

Table 2. Effect of H_3PO_4/Poplar (v/w, ml/g) Ratio on Pretreatment at 50 °C Based on 1g Raw Poplar

Component content (g)	Liquid/solid ratio (v/w, ml/g)								Untreated poplar (g)
	8:1				10:1				
	Pretreatment time (min)								
	40	60	80	100	40	60	80	100	
Solid residual	0.73	0.69	0.64	0.59	0.65	0.60	0.57	0.49	1.00
Cellulose [a]	0.39	0.38	0.36	0.32	0.35	0.31	0.28	0.26	0.41
Xylan [a]	0.15	0.13	0.09	0.09	0.10	0.08	0.07	0.07	0.19
Lignin [a]	0.15	0.14	0.14	0.12	0.17	0.16	0.19	0.15	0.25
Glucose [b]	0.01	0.01	0.02	0.06	0.04	0.06	0.08	0.09	
Xylose [b]	0.04	0.05	0.08	0.07	0.09	0.10	0.10	0.10	
Precipitated lignin [c]	0.09	0.11	0.12	0.13	0.07	0.10	0.06	0.10	

[a] Component content in solid residual after fractionation
[b] Monosaccharide content in the combined acetone-free delignified supernatant and water-washing supernatant
[c] Precipitated lignin from acetone-free phosphoric acid

About 18% of total projected cost for biological production of cellulosic ethanol can be attributed to pretreatment (Yang and Wyman. 2008). The comprehensive utilization of sugars may offset the cost of pretreatment, making the cellulosic ethanol more competitive. Based on the fractionation process in this work, the concentration of glucose and xylose in combined supernatant were only 0.1 to 1.8 g/L and 0.4 to 2.0 g/L, respectively. The recycling of combined supernatant could enhance the concentration of the sugars. However, the feasible application of these sugars may reside in the cultivation of micro-algae or biogas production in view of energy consumption. Moreover, the recovery of glucose and xylose in combined supernatant at a liquid/solid ratio of 8:1 were lower than that at 10:1, resulting in over 22% glucose transferring into the liquid phase with only 63% cellulose recovery in solid phase for the latter case. Therefore, samples pretreated at 8:1 resulted in higher cellulose recovery and total sugar recovery than at 10:1 for the same pretreating time. Therefore, the ratio 8:1 was judged to be preferable for a poplar phosphoric-acetone fractionation process.

Pretreating time also has impacts on fractionation process. Component recovery in solid residual decreased gradually along the progress of pretreatment. In particular, in the liquid phase xylose recovery increased from 47% (pretreated for 40 min) to 53%

(pretreated for 100 min) at a liquid/solid ratio of 10:1. By contrast, xylose recovery was much higher than glucose recovery in the liquid phase.

Table 3. Recovery of Cellulose, Xylan, Lignin in Pretreated Solid Residual and Glucose, Xylose, Lignin in Liquid Phase (at 50 °C)

Pretreatment		Glucose recovery (%)		Xylose recovery (%)		Lignin recovery (%)		Total sugar recovery (%)
Liquid/ solid ratio	Time (min)	In solid residual	In liquid	In solid residual	In liquid	In solid residual	Precipi-tated	
8:1	40	95.12	2.44	78.95	21.05	60.00	36.00	98.33
	60	92.68	2.44	68.42	26.32	56.00	44.00	95.00
	80	87.80	4.88	47.37	42.11	56.00	48.00	91.67
	100	78.05	14.63	47.37	36.84	48.00	52.00	90.00
10:1	40	85.37	9.76	52.63	47.37	68.00	28.00	96.67
	60	75.61	14.63	42.11	52.63	64.00	40.00	91.67
	80	68.29	19.51	36.84	52.63	76.00	24.00	88.33
	100	63.41	21.95	36.84	52.63	60.00	40.00	86.67

Total sugar recovery decreased in the course of pretreating time from 40 to 100 min. Long fractionation time facilitates dissolution of cellulose and xylan, as well as promoting delignification. What should be mentioned is that hydrolysis of dissolved cellulose and xylan is also enhanced with increasing pretreating time. In most cases over 40% of the lignin was dissolved into the acetone phase. The delignification fluctuation may be caused by lignin repolymerization occurring after 1 h of pretreatment.

Effect of Pretreating Temperature

The experiments were conducted at 40, 50, 60 °C for 40 to 100 min to investigate the effect of temperature on delignification, xylan removal, and cellulose recovery during phosphoric-acetone fractionation. The cellulose recovery decreased slightly with the increase of pretreating temperature (in Table 4).

The hydrolysis of dissolved cellulose and xylan was enhanced with increasing pretreating temperature. Compared to cellulose, xylan is characterized by its low degree of polymerization. This heteropolymer is composed primarily of β-1,4-linked xylose with various amounts of arabinose, glucose, galactose, uronic acids as sidegroups, and it does not have the rigid crystalline region structure of cellulose. So xylan is more liable to dissolve in phosphoric acid. Further, more than half of dissolved xylan is hydrolyzed into the mixed liquid phase at 60 °C for more than 40 min. Xylan hydrolysis increased from 52.6% (40 min) to 73.7% (100 min) at 60 °C. By contrast, the minimum value of cellulose recovery in solid residual was still 70.7% at 60 °C after 100 min.

Table 4. Effect of Pretreating Temperature on Component Transferring in Pretreated Solid Residual Based on 1 g Raw Poplar (liquid/solid ratio of 8:1 (v/w))

Component content (g)	Pretreating temperature (°C)												Un-treated poplar (g)
	40				50				60				
	Pretreatment time (min)												
	40	60	80	100	40	60	80	100	40	60	80	100	
Solid residual	0.84	0.79	0.75	0.70	0.73	0.69	0.64	0.59	0.64	0.57	0.51	0.48	1.00
Cellulose [a]	0.41	0.40	0.39	0.37	0.39	0.38	0.36	0.32	0.38	0.36	0.32	0.29	0.41
Xylan [a]	0.17	0.15	0.12	0.11	0.15	0.13	0.09	0.09	0.09	0.06	0.04	0.02	0.19
Lignin [a]	0.23	0.20	0.21	0.22	0.15	0.14	0.14	0.12	0.14	0.12	0.10	0.11	0.25
Glucose [b]	ND	ND	0.01	0.03	0.01	0.01	0.02	0.06	0.01	0.01	0.03	0.04	
Xylose [b]	0.02	0.04	0.06	0.07	0.04	0.05	0.08	0.07	0.10	0.12	0.14	0.14	
Precipitated lignin [c]	0.02	0.04	0.05	0.03	0.09	0.11	0.12	0.13	0.12	0.13	0.13	0.13	

[a] Component contents in solid residual after pretreatment
[b] Monosaccharide content in the combined acetone-free delignified supernatant and water-washing supernatant
[c] Precipitated lignin from acetone-free phosphoric acid

Table 5. Recovery of Cellulose, Xylan, Lignin in Pretreated Solid Residual and Glucose, Xylose, Lignin in Liquid Phase (liquid/solid ratio of 8:1 (v/w))

Pretreatment		Glucose recovery (%)		Xylose recovery (%)		Lignin recovery (%)		Total sugar recovery (%)
Temperature (°C)	Time (min)	In solid residual	In liquid	In solid residual	In liquid	In solid residual	Precipitated	
40	40	100.00	ND	89.47	10.53	92.00	8.00	100.00
	60	97.56	ND	78.95	21.05	80.00	16.00	98.33
	80	95.12	2.44	63.16	31.58	84.00	20.00	96.67
	100	90.24	7.32	57.89	36.84	88.00	12.00	96.67
50	40	95.12	2.44	78.95	21.05	60.00	36.00	98.33
	60	92.68	2.44	68.42	26.32	56.00	44.00	95.00
	80	87.80	4.88	47.37	42.11	56.00	48.00	91.67
	100	78.05	14.63	47.37	36.84	48.00	52.00	90.00
60	40	92.68	2.44	47.37	52.63	56.00	48.00	96.67
	60	87.80	2.44	31.58	63.16	48.00	52.00	91.67
	80	78.05	7.32	21.05	73.68	40.00	52.00	88.33
	100	70.73	9.76	10.53	73.68	44.00	52.00	81.67

As for the delignification process, a fluctuation phenomenon was also observed during the fractionation process. However, the overall delignification was coincident with the amounts of glucose and xylose in the liquid phase with increasing temperature. The delignification fluctuation may be caused by lignin depolymerization and subsequent lignin polymerization. Removal of lignin and xylan will further improve the cellulase accessibility to cellulose.

Degradation of hydrolyzed glucose and xylose may influence the total sugar recovery and ethanol yield. In this regard, temperature, liquid/solid ratio, and pretreating time all have important effects on fractionation process. These three factors must be controlled to enhance total sugar recovery.

Enzymatic Hydrolysis Efficiency (EHE) and Cellulose Conversion

Pretreated poplar samples were hydrolyzed by *Trichoderma* cellulase at 50 °C. The enzyme loading was 15 FPU/g cellulose supplemented with *β*-glucosidase of 30 CBU/g cellulose. The hydrolysis rates of pretreated samples were much higher for the first 9 h, as shown in Fig. 2. EHE of pretreated samples amounted up to 60% after 10 h enzymatic hydrolysis and increased to 67% after 24 h enzymatic hydrolysis. Raw material showed only 18.42% EHE after 24 h enzymatic hydrolysis. EHE increased with the increase of pretreating temperature. EHE difference between samples pretreated at 40 °C and 50 °C was much greater than that between 50 °C and 60 °C. EHE enhancement could be attributed to more delignification and xylan dissolution at higher temperature. Polysaccharide not only swelled, it even dissolved in 85 wt.% phosphoric acid during fractionation process (Zhang et al. 2006). In this work it was demonstrated that samples pretreated at above 50 °C had greater susceptibility to interaction between cellulose and cellulase, so the dissolved cellulose could produce precipitated amorphous cellulose. After 24 h enzymatic hydrolysis, EHE of samples pretreated at 40, 50, and 60 °C were 67.91, 93.36, and 96.37%, respectively. After 24 h enzymatic hydrolysis, further EHE increase was slight.

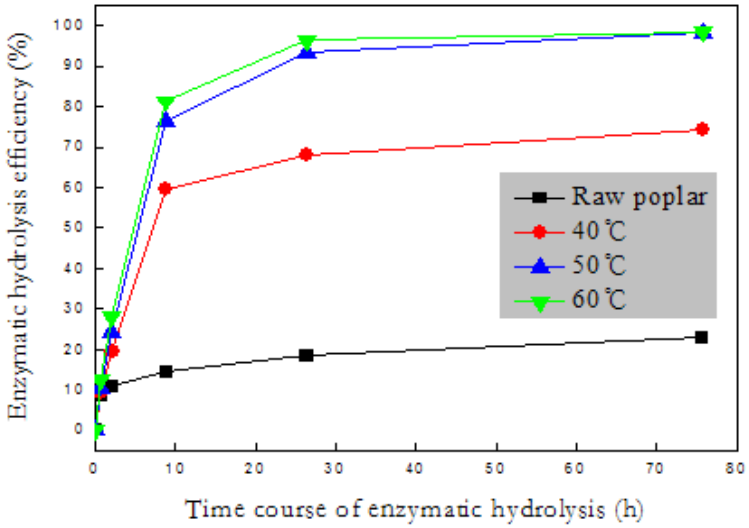

Fig. 2. Enzymatic hydrolysis profile of poplar samples pretreated at different temperature (for 60 min, at liquid/solid ratio of 8:1, cellulase loading is 15 FPU/g DM, β-glucosidase loading is 30 CBU/g DM)

Combining the process of pretreatment and enzymatic hydrolysis, whole cellulose conversion of raw material only accounted for one fifth of the available cellulose in the raw material. When pretreating temperature increased from 40 °C to 50 °C, whole cellulose conversion increased greatly from 67.64% to 89.82% after 24 h of enzymatic hydrolysis. However, the cellulose conversion slightly decreased to 87.98% at 60 °C after 24 h of enzymatic hydrolysis. Considering both cellulose recovery and enzymatic hydrolysis efficiency, pretreatment at 50 °C and liquid/solid ratio of 8:1 for 60 min was judged to be the optimal condition. In Table 6, cellulose conversion data in this work exhibited comparable enzymatic hydrolysis efficiency with leading technologies reported before (Wyman et al. 2005).

Table 6. Comparison of Two-stage Glucose Yields between Mainstream Pretreating Method[a] and Our Investigation

Pretreatment System	Glucose yields [b] (%)		
	pretreatment	enzymatic digestion [c]	Total glucose
Dilute acid	6.26	85.39	91.65
Flowthrough	7.06	88.60	95.67
Partial flow pretreatment	6.74	82.18	88.92
Controlled pH	0.32	84.91	85.23
AFEX	-	95.99	95.99
ARP	-	90.05	90.05
Lime	0.48	91.49	91.97
This work	2.44	89.82	92.26

[a] data source (Wyman et al. 2005)
[b] glucose yield, the percentage of glucose produced in original cellulose in poplar
[c] with a loading of 15 FPU/g cellulose in original corn stover

Characterization

SEM of raw and pretreated poplar

Scanning electron microscopy was used to reveal the effect of phosphoric-acetone fractionation on the surface morphology of milled raw material. Figure 3 shows SEM images of raw poplar particles and samples pretreated at 40 °C, 50 °C, and 60 °C. Some cracked micro-fibrils could be seen outside the fibrous structure (Fig. 3A). Milling to +70/-30 mesh was able to slightly destroy the surface of raw poplar cell wall, but vascular bundles and fibril structure of cell wall were almost intact and are easily discernible.

When raw poplar was pretreated at 40 °C, readily apparent fractures and small caves on the cell wall appeared (Fig. 3B). However, the gross supramolecular fibril structure changed slightly, which is due to limited removal of lignin and xylan. When it was pretreated at 50 °C, more swellings and indentations appeared on the outer surface (Fig. 3C). Schistose textures peeled off and the whole cellulosic structure was severely altered when pretreated at 60 °C; therefore, the fibrous structures were hard to recognize (Fig. 3D).

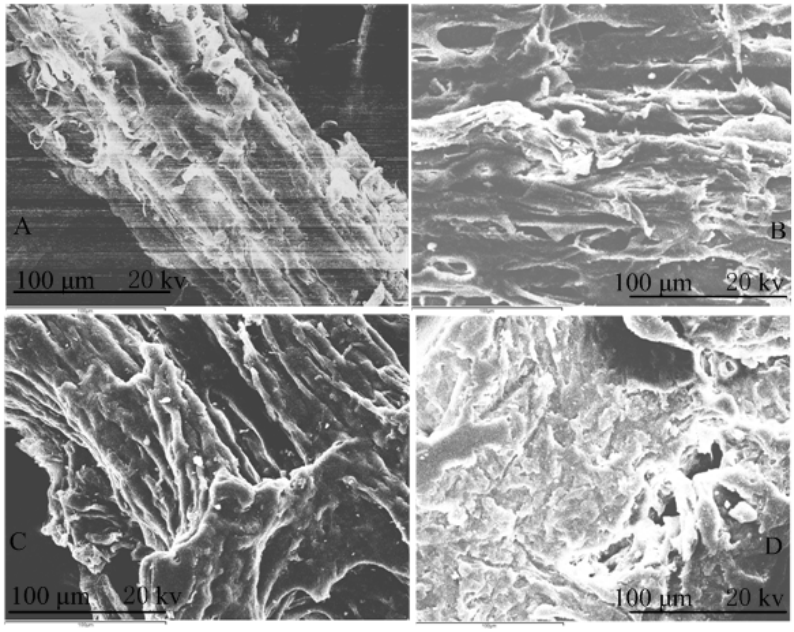

Fig. 3. SEM observations of poplar particles: (A) Raw poplar; (B) Sample pretreated at 40 °C; (C) Sample pretreated at 50 °C; (D) Sample pretreated at 60 °C

XRD of raw and pretreated poplar

The crystallinity of poplar is one of the most important factors affecting enzymatic hydrolysis efficiency. X-ray diffraction was used to investigate the *CrI* of raw poplar particle and regenerated poplar sample. As shown in Fig. 4, raw poplar showed a typical cellulose-I diffraction angle around 22.5°, but once the pretreated poplar particle was precipitated from acetone, it exhibited a maximum peak at the diffraction angle of around 20°, which is considered to be the characteristic angles for cellulose-*II*. This subtle peak position movement is attributed to the change of crystalline type from cellulose-*I* to cellulose-*II* (Howell et al. 2009). Also, Cellulose-*II* is much more liable to be hydrolyzed with cellulase in comparison with cellulose-*I* (Kuo and Lee 2009).

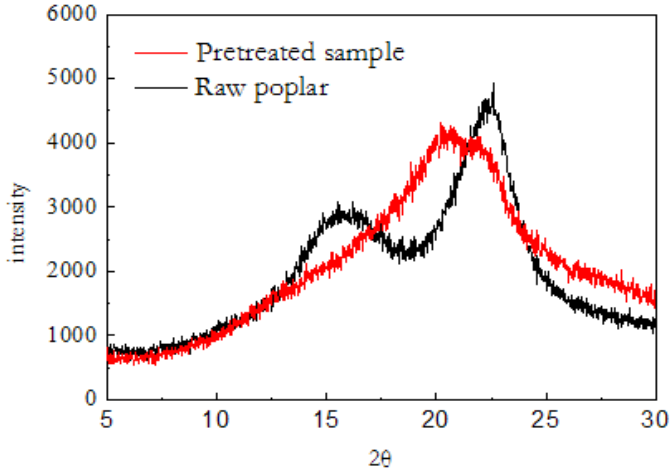

Fig. 4. XRD patterns of raw poplar particle and precipitated poplar particles (pretreated at 50 °C for 60 min with liquid/solid ratio of 8:1, then regenerated from acetone-water rinsing solution)

The empirical *CrI*, which represents the relative crystalline content, was also calculated from Fig. 4 (data not shown). After phosphoric-acetone fractionation, *CrI* decreased from 39.9% to 27.7%, suggesting a decrease of crystalline area percentage. Subsequently, relative lower *CrI* and crystalline type transition can both explain the enhancement of enzymatic hydrolysis.

PY-GC-MS of raw poplar and precitipated lignin

Pyrolysis-GC-MS was performed at 600 ^{o}C to compare the structure change from raw poplar to precipitated lignin. Based on ion chromatograms, the peak percentage values of involved compounds were calculated through integral calculation by means of the NIST MS library (Lu et al. 2010). The pyrolytic products were classified into nine groups (Table 7). Compared to raw poplar, the phenolic compounds peak percentage accounted for 48% in the precipitated lignin, and the anhydrosugar compounds peak percentage decreased by 68%. The peak percentage changes of phenols and anhydrosugars together indicate that when acetone-soluble lignin is precipitated from acetone-free dilute phosphoric acid, the functional group structure of the precipitated lignin is completely different from that in the starting raw poplar.

Table 7. Composition of Pyrolytic Products of Raw Poplar and Precipitated Lignin (peak percentage area %)

Sample name	Phenols (%)	Phenyls (%)	Alcohols (%)	Carbonyls [a] (%)	Acids (%)	Furans (%)	Anhydro-Sugars (%)	Hydro-Carbons (%)	Others (%)
Raw poplar	23.73	1.83	2.01	41.47	8.07	0.68	5.79	1.13	15.29
Precipitated lignin [b]	48.00	7.56	0.83	16.43	13.86	0.15	1.83	3.38	7.96

[a] including ketone, aldehyde, ester
[b] lignin precipitated from acetone-free phosphoric acid

The amounts of three lignin monomers were also analyzed, so that monomer fractions of phenols could be determined. In raw poplar, the peak percentage values of guaiacol-type, syringol-type, and phenol-type were 53.38%, 29.86%, and 2.65%, respectively. The phenol-type became the predominant lignin monomer in the precipitated lignin, with a peak percentage value of 47.34% (Table 8).

Table 8. Molar Fraction of Phenols from Pyrolysis at 600 ^{o}C for Raw Poplar and Precipitated Lignin

Compound name of phenols	Raw poplar (peak area %)	Precipitated lignin (peak area %)
Syringol-type	29.86	21.96
Guaiacol-type	53.38	20.75
Phenol-type	2.65	47.34

Therefore, after this fractionation process, methoxyl groups removal is the main modification of acetone-soluble lignin, with the percentage of phenol-type monomer amounting to nearly half of lignin monomers. Methoxyl groups on the aromatic ring are active substituent groups, and free-radical chain-reaction is the main reaction leading to lignin cracking (Shen et al. 2010). These results indicate that acetone-soluble lignin was modified to only a slight extent without much monomer degradation. The potential application of phenol-type rich lignin from this process could reside in preparation of phenol-type natural antioxidative materials (Mitsuo et al. 2005).

CONCLUSIONS

1. Higher liquid/solid ratio and excessive temperature will cause the hydrolysis of dissolved cellulose and xylan, such that hydrolyzed monomeric sugar can be detected in mixed rinsing liquid.
2. Under the optimal pretreatment condition of 50 ^{o}C, a liquid/solid ratio of 8:1 (v/w), and 1 h pretreatment duration, the highest cellulose conversion of 89.82% could be obtained after 24 h enzymatic hydrolysis.
3. When raw poplar was pretreated at 50 ^{o}C, more signs of swellings and cavity formation appeared on the outer surface. Further, schistose textures of pretreated samples peeled off, and the whole cellulosic structure was severely altered when pretreated at 60 ^{o}C.
4. After phosphoric-acetone fractionation, the *CrI* index decreased from 39.9% to 27.7% suggesting a decrease of crystalline area percentage.
5. Phenolic compounds accounted for almost half of the precipitated lignin.

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