The Effects of Time and Temperature in Hydrothermal Pretreatment on the Enzymatic Efficiency of Wheat Straw

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An attempt to correlate biomass characteristics to its susceptibility to enzymes is often inconclusive *via* investigation of the variables of hydrothermal pretreatment. Based on an integrated analysis of physicochemical properties, cellulose bioconversion, loss of pentose sugars, formation of inhibitory products, and the cost of energy, the optimal hydrothermal operation for wheat straw (1:20 w/v%) was found. This optimal operation involved cooling the hydrolysates as soon as the temperature reached 180 °C. Finally, a total of 40.7% glucose and 70.3% sugars were recovered during subsequent enzymatic hydrolysis. Although treatment at a noticeably increased severity with a long incubation time could lead to almost 100% conversion of cellulose, the weight losses (mainly sugars) and inhibitors in the process liquid were not well suited for an industrial scale operation.

Keywords: Hydrothermal pretreatment; Enzyme hydrolysis; Variables; Wheat straw

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INTRODUCTION

Regarding petroleum supply and climate change, lignocellulosic biomass is considered an important renewable and sustainable energy source (Demirbas 2011). Furthermore, bioethanol production from cellulosic substrates has attracted great attention lately. The transformation process of second-generation bioethanol from lignocellulosic materials involves four main steps: pretreatment, enzymatic hydrolysis, yeast fermentation, and separation (Mosier *et al*. 2005). Pretreatment is a critical process that removes lignin and hemicelluloses, disrupts the crystalline structure of cellulose, and increases the porosity of the material, aiming to improve the accessibility of cellulose and, thus, the yield of fermentable sugars (Zeng *et al*. 2007). Many pretreatment technologies have been developed, including dilute acid treatment, steam explosion, wet-oxidation, and ammonia fiber explosion (AFEX), each of which has its own shortcomings. An effective pretreatment should minimize the loss of sugars, lower the required energy input, and avoid the degradation of sugars (Petersen *et al*. 2009). Hydrothermal pretreatment has recently been attracting increased attention, and it normally operates between 120 °C and 230 °C and without chemical addition (Möller *et al*. 2011; Chandra *et al*. 2012). Hydrothermal pretreatment could lead to the removal of hemicellulose after its depolymerization to oligomers and monomers, as well as the partial degradation and re-distribution of lignin in

cell walls, exposing the surfaces of inner cellulose chains (Van Eylen *et al*. 2011). Two important variables of hydrothermal pretreatment, time and temperature, affect the components and physical properties of the obtained substrate, which should be highly accessible to enzymes. A few short glucose chains present in the amorphous section of cellulose are bonded to crystalline cellulose through hydrogen bonding and may produce C_4–C_{13} oligomers when undergoing hydrothermal pretreatment at 100 °C. The minimum temperature at which the glucoside bonds of the short chains in the amorphous form underwent dissociation was determined to be *ca.* 150 °C, while the hydrolysis of a part of the crystalline structure started at *ca.* 180 °C (Petersen *et al*. 2009). The extent of hydrogen bonds and chain lengths in the crystalline and amorphous sections are different, which strongly affects the distribution of glucose oligomers in the hydrolysate (Chen *et al*. 2017). Acetic acid from released acetyl groups promotes the hydrolysis of hemicelluloses, making the polyxylose dynamics difficult to investigate with pseudo-homogeneous kinetic models; the involved reaction steps are complex. Commercial cellulase, ideally combined with β-glucosidase or β-xylosidase, could yield high glucose for subsequent yeast fermentation (Jabbour *et al*. 2013).

In this paper, a systematic optimization of temperature and incubation time of hydrothermal pretreatment was performed by evaluating the physicochemical properties of, morphological variation of, and inhibitory products produced by the substrate as well as the sugars released by enzymatic hydrolysis. Also, the dissolved oligosaccharides and lignin were also examined *via* spectrum technology. The obtained results are expected to provide some useful information for cellulosic ethanol production from wheat straw.

EXPERIMENTAL

Materials

The wheat straw was obtained from Hebei Province, Zhangjiakou, China. After drying under ambient conditions and grinding, the particles that passed through a 20-mesh screen were collected. The chemical composition of these particles was then determined using high performance anion-exchange chromatography (HPAEC; Thermo Fisher, Waltham, USA) as 29.7% cellulose (estimated as glucose), 29.2% hemicelluloses (including 24.6% xylose, 3.8% arabinose, and 0.8% galactose), and 26.0% lignin (including 20.5% acid-insoluble and 5.5% acid-soluble lignin), according to National Renewable Energy Laboratory (NREL) standard analytical procedures (Sluiter *et al*. 2005). The moisture content of the starting material was 4.6%, and the ash content was 7.4%. Commercial cellulase (145 FPU/g) was kindly supplied by Novozymes Investment Co., Ltd. (Beijing, China) and all other chemicals were of analytical grade from Sinopharm Chemical Reagent Beijing Co., Ltd., unless otherwise mentioned.

Hydrothermal pretreatment

The hydrothermal pretreatments were performed in a laboratory-scale autoclave batch reactor (Parr Instrument Company, Moline, IL, USA) with a maximal volume of 1000 mL. The 20 g samples were mixed with 400 mL of deionized water and heated at designated temperatures (120 °C, 140 °C, 160 °C, and 180 °C) with different incubation times (0 min and 180 min) with the heating rate of 4 °C/min. As the planned condition was achieved, tap water was immediately opened to through the stainless steel cooling ring inside the reactor to reduce the inside temperature to room temperature. The temperature

dropped rapidly at the start of cooling process and the cooling time varied depending on the pretreatment temperature. The severity of the pretreatments ($\log R_0$) ranged from 0.6 to 4.6 (Table S1). After pretreatment, the solid fractions were collected by filtration and completely washed (moisture contents were between 79.7% and 88.9%). The liquid was also collected for analysis of the degraded products (formic acid, acetic acid, furfural, and 5-HMF) *via* high-performance liquid chromatography (HPLC; Agilent 1200 series, Agilent Technologies, Santa Clara, USA) and for analysis of molecular weight distributions *via* gel permeation chromatography (GPC) on a PL Aquagel-OH mixed column (300 mm × 7.5 mm), as well as the dissolved aromatic products *via* a ultraviolet (UV)-visible (Vis) spectrophotometer (UV2600; Techcomp (holdings) Limited, Shanghai, China).

Methods

Physical characterization

Analysis of the crystalline structures of the solid samples was performed *via* X-ray diffraction (XRD-6000; Shimadzu, Tokyo, Japan), scanning from 5° to 45° 2θ at a scanning speed of 2 °/min with a Cu K_α radiation source (λ = 0.154 nm) at 40 kV and 30 mA. The crystallinity index (CrI) was calculated with Eq. (1) (Segal *et al*. 1959),

$$\text{CrI}\ (\%) = [(I_{\text{crystalline}} - I_{\text{amorphous}}) / I_{\text{crystalline}}] \times 100 \quad (1)$$

where $I_{\text{crystalline}}$ is the intensity value around 2θ = 22.4° and $I_{\text{amorphous}}$ is the intensity value around 2θ = 18°. The SEM images of all samples were performed on a Hitachi S-3400 N II (Hitachi, Tokyo, Japan) instrument after being coated with a thin layer of gold (Hitachi, Tokyo, Japan).

Enzymatic hydrolysis

According to the moisture contents, approximately 24.6 g to 45.1 g (equivalent to 5 g of dried sample) of solid were distributed in 100 mL sodium acetate buffer (50 mM, pH 5.5). After the addition of cellulase (20 FPU/g substrate), the mixtures were incubated in a rotary shaker (50 °C, 150 rpm), and the hydrolysate was sampled every 6 h and analyzed with HPAEC system.

RESULTS AND DISCUSSION

Compared with the raw material, the treated samples exhibited more fragments, holes, and droplets as the severity of the treatment process was increased (namely by prolonging residence time and elevating treatment temperature) (Fig. 1). Meanwhile, the appearance of the treated samples became dark and had the scent of burnt sugar, resulting from the Mailard browning products between furfural from C_5 sugar degradation and peptide amino groups (Liavoga *et al*. 2007). The lignin component could be melted and coalesced on the surface of the fibers as debris of perfectly granular and globular droplets. However, flattened disks and irregular droplets from the reshaping process of lignin could also be found in Fig. 1, which were likely due to the nonuniform distribution of lignin in cell walls (*i.e.* across the delamination layer, cell corner, pit, *etc.*) and the casual aggregation of the hydrophobic lignin in a hydrophilic environment (Donohoe *et al*. 2008).

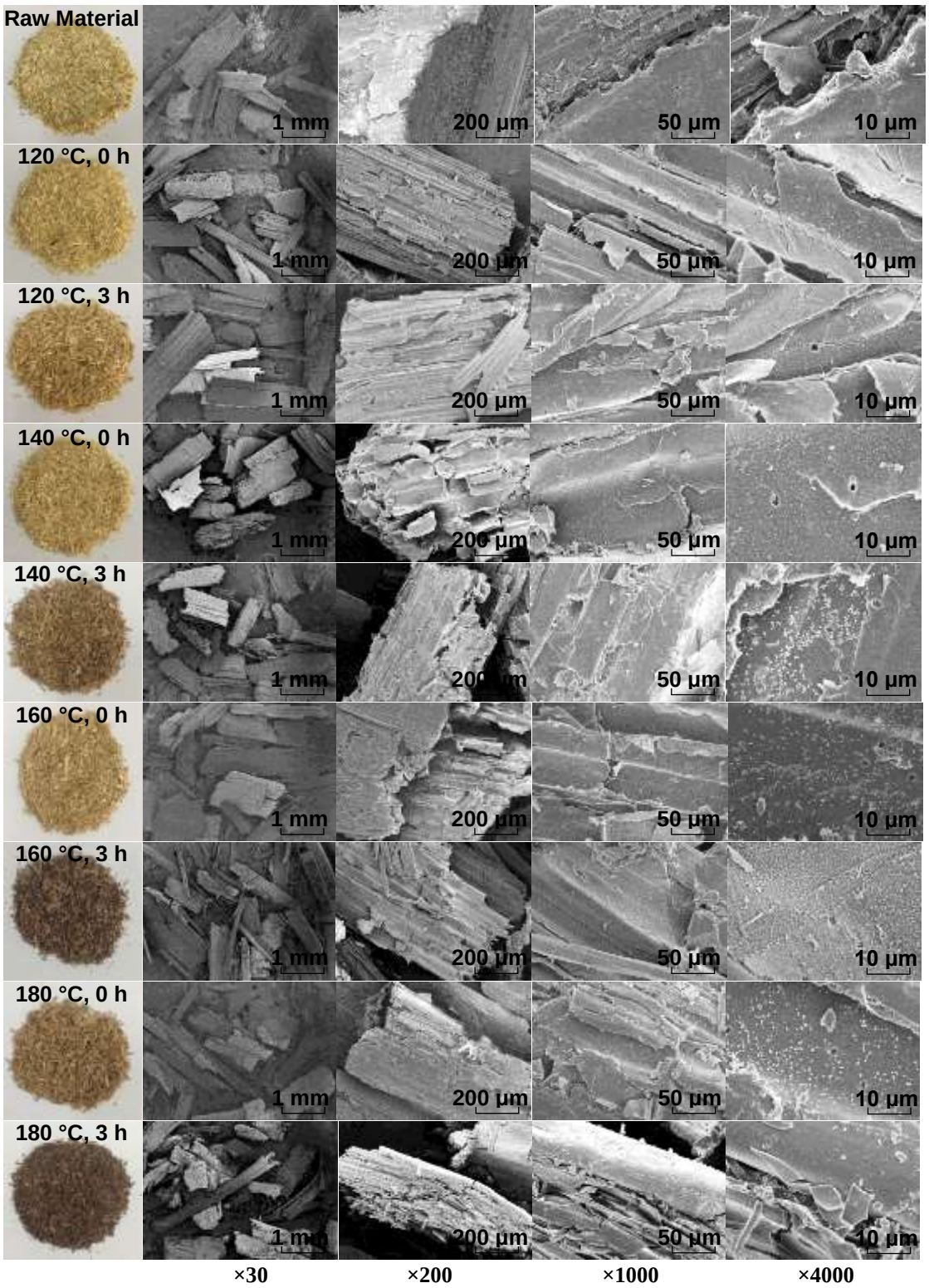

Fig. 1. SEM images of the raw and hydrothermally treated wheat straw with increasing temperatures and incubation periods

Although there was minimal morphological variation, the chemical components changed accordingly with severity (Table 1). The immediate cooling process did not fully display the effect of high-temperature water on the autohydrolysis of wheat straw, corresponding to a slight decrease in yield (from 84.6% to 75.2%) and increase in glucose content (from 35.9% to 37.3%). In contrast, long incubation times noticeably changed the chemical components of the raw materials and values of yields; glucose and xylose contents suddenly varied when temperatures higher than 140 °C were used. After treatment under the most severe condition ($T180_3$), only 57.6% starting material remained, and 51.3% glucose was detected in the treated samples. The hemicellulosic carbohydrates were evidently degraded, and the relative content of lignin was reasonably increased. The absorption values from ultraviolet (UV) analysis gradually increased as the severity was increased, especially as the temperature reached 180 °C (Fig. S1). The typical peak near 270 nm originated from the phenolic hydroxyls conjugated to α-carbonyls, carbon-carbon double bonds, or biphenyl groups in lignin (Wang *et al*. 2010). However, the other expected absorption peak at approximately 320 nm from associated hydroxycinnamic acid (*p*-coumaric acid and ferulic acid) was seldom detected, indicating that the connecting units could not be released under the current conditions. Moreover, the relative increase in cellulose together with the decrease in amorphous hemicelluloses and part of lignin resulted in a gradual increase of crystallinity index (CrI), regardless that the XRD pattern was maintained. In comparison, the crystallinity of the cellulosic substrate was maintained as the pretreatment temperatures rose above 140 °C, and even slightly decreased, as part of the cellulose in the crystalline region started to hydrolyze.

Table 1. Yields, Relative Weight Percentages of Monomeric Sugars and Lignin Content, and Crystallinity Values of the Pretreated Wheat Straw at Different Hydrothermal Pretreatment Severities

	Yield[a]	Main Components[b] (wt%, w/w)						Crystalline
	(%)	Glc	Xyl	Ara	Gal	AIS	ASL	(%)
Raw Material	100	29.7	24.6	3.8	0.8	20.5	5.5	16.6
$T120_0$[c]	84.6	35.9	29.6	4.4	0.9	24.3	4.9	27.7
$T120_3$	80.5	36.2	30.1	3.8	0.7	24.7	4.6	28.2
$T140_0$	85.2	36.0	29.2	4.0	0.7	25.4	4.7	29.0
$T140_3$	75.1	38.3	29.3	2.1	0.4	26	3.9	29.1
$T160_0$	80.1	35.7	28.4	3.3	0.6	27.5	4.5	29.5
$T160_3$	55.7	48.6	18.9	0.5	0.2	28.8	3.0	28.3
$T180_0$	75.2	37.3	24.7	1.8	0.3	31.8	4.2	29.3
$T180_3$	51.6	51.3	5.5	--	--	39.4	3.9	28.8

[a]Weight % based on the starting materials; [b]Glc: Glucose; Xyl: Xylose; Ara: Arabinose; Gal: Galactose; AIS: Acid insoluble lignin; ASL: Acid soluble lignin. [c] This column denotes the severity/pretreatment conditions used in each row

The liquid fraction from the hydrothermal process was analyzed in this study, including its molecular distribution (Table 2 and Fig. S2) and the further-degraded products (Table 2). The dissolved oligosaccharides were mainly divided into high-molecular and low-molecular fractions, and the immediate cooling process, from 120 °C to 180 °C, did not obviously affect the molecular distribution of degraded polysaccharides in hydrolysis, which resulted in the limited variation of M_w values from 7670 Da to 8320 Da. The slightly-increased M_w values (T140 and T160), together with two samples after a 3 h incubation

($T120_3$ and $T140_3$), could all probably be ascribed to the dissolved biomacromolecules after acidic hydrolysis of the inner polysaccharides (hemicelluloses and cellulose). However, further increasing the pretreatment temperature to 160 °C and 180 °C and the incubation time to 3 h caused a sharp decrease in M_w values (approximately 2000 Da), which corresponded to the notably left-shifted peaks of molecular distribution for the $T160_3$ and $T180_3$ samples. The appearance of 5-hydroxymethylfurfural (5-HMF) and furfural in the process liquid, which strongly inhibits the following bioconversion (Kont *et al*. 2013), was not surprising (Table S1).

Table 2. Weight/Number Molecular Weight (Da) and Polydispersity (M_w/M_n) Recovered in the Process Liquid at Different Hydrothermal Pretreatment Severities

	$T120_0$	$T120_3$	$T140_0$	$T140_3$	$T160_0$	$T160_3$	$T180_0$	$T180_3$
Mw	7670	8970	8230	8610	8320	2040	7820	2430
Mn	80	78	63	90	65	70	94	161
Mw/Mn	96	115	131	96	128	29	83	15

After enzymatic hydrolysis, the originally loosened structure of the treated samples was further disfigured, increasing holes and fragments on the surface. The bioconversion process released the combinations between cellulose chains and shortened fiber lengths, resulting in the destruction of the original structure (Kont *et al*. 2013). For bioconversion efficiency, increasing the severity of hydrothermal pretreatment clearly improved the concentration of glucose in the hydrolysate; the maximum was 32.5 mg/L for the $T180_3$ sample, which almost corresponded to the 100% conversion of cellulose (Fig. 2). The enhancement of cellulose biodegradation was attributed to the destruction of plant cell walls by moving or solubilizing lignin, breaking the bonds of lignin-carbohydrate connections (LCC), and hydrolyzing a proportion of hemicellulosic moieties (Mosier *et al*. 2005; Cybulska *et al*. 2009). Compared to elevating the hydrothermal temperature, prolonging the incubation time appeared to be more efficient for cellulose bioconversion as reported by Yu *et al*. (2010). However, the data in this study indicated that both temperature and incubation time had similarly positive effects on the bioconversion of cellulose under the relatively low temperatures (120 °C and 140 °C), leading to twice the increase of glucose release. At a higher temperature, increasing the incubation time from 0 h to 3 h almost further doubled the bioconversion efficiency, which was limited by elevating hydrothermal temperatures. The bioconversion efficiency of xylan exhibited a similar trend to that of cellulose (Fig. S3). However, the maximum xylose concentration was not found at the highest bioconversion efficiency because a large amount of hemicelluloses was dissolved during the hydrothermal process. Considering both the weight balances and bioconversion efficiencies, high temperature (180 °C) and short incubation time (0 h) was selected as the optimal condition for achieving the maximum recovery of sugars.

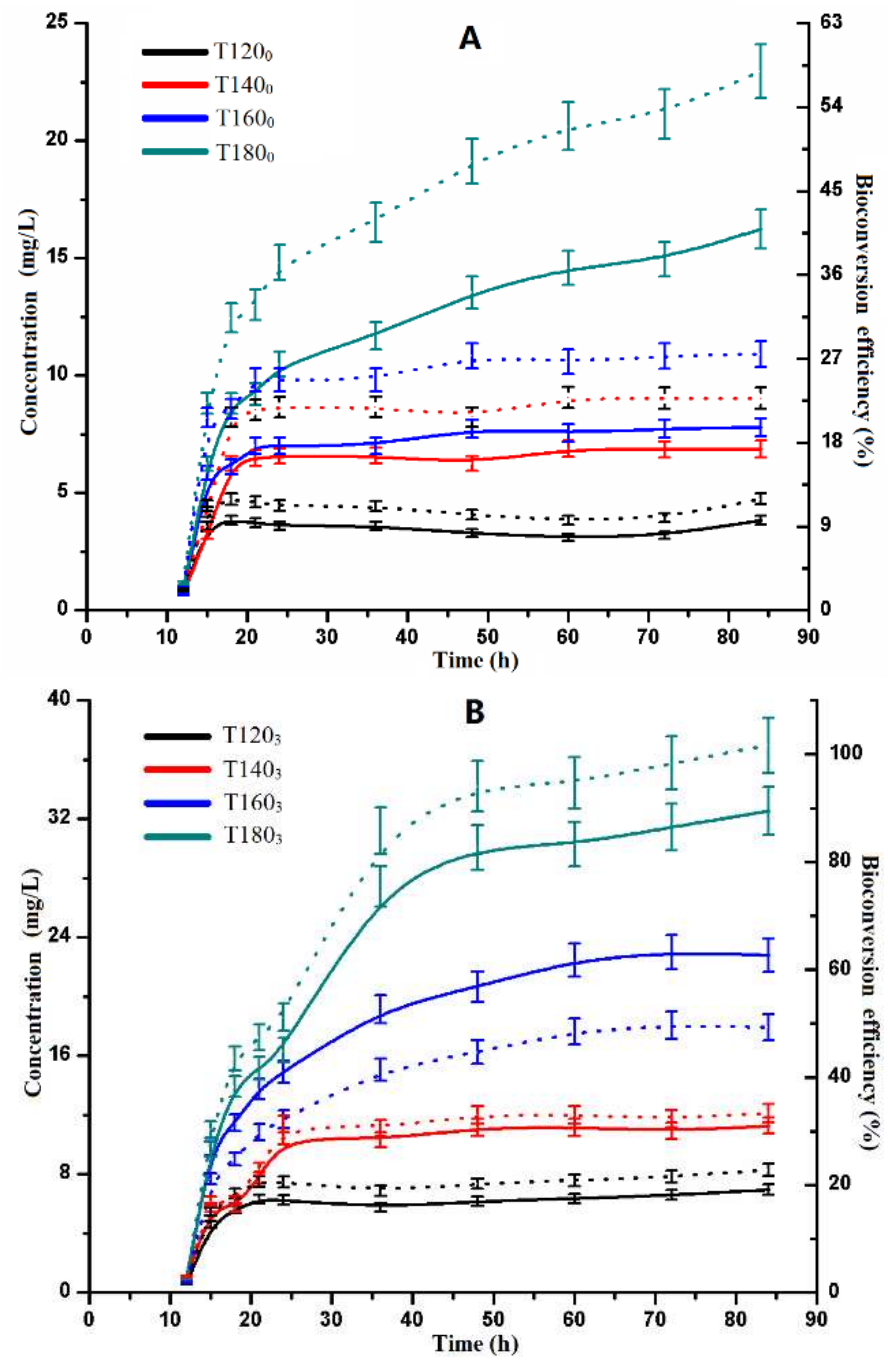

Fig. 2. Variation of glucose concentration (solid lines, left axis) and bioconversion efficiency (dotted lines, right axes) during 72 h enzyme hydrolysis for different pretreatment temperatures and incubation periods (A: incubation time =0 h; B: incubation time =3 h)

CONCLUSIONS

1. Two variables (temperature and time) were selected to analyze the effect of hydrothermal pretreatment on the substrate characteristics, inhibitory products, and bioconversion efficiency of wheat straw.

2. Although the expected transformation of cellulose was achieved at the most severe condition ($\log R_0 = 4.6$), the optimal condition for sugar recovery was chosen as the immediate cooling upon reaching 180 °C.

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APPENDIX

Supplementary Material

Table S1. Hydrothermal Pretreatment Experimental Conditions and the Concentrations (Mg/L) of the Main Inhibitors Recovered in the Process Liquids

	Temperature (°C)	Time[a] (h)	logR_0[b]	Main Inhibitors			
				Formic Acid	Acetic Acid	5-HMF	Furfural
$T120_0$	120	0	0.6	--	0.1	--	--
$T120_3$	120	3	2.8	--	0.2	--	--
$T140_0$	140	0	1.2	--	0.2	--	--
$T140_3$	140	3	3.4	0.2	0.5	--	--
$T160_0$	160	0	1.8	0.1	0.3	--	--
$T160_3$	160	3	4.0	0.5	0.8	0.1	0.3
$T180_0$	180	0	2.4	0.3	0.5	--	--
$T180_3$	180	3	4.6	--	0.9	0.2	1.2

[a]The incubation time was recorded as the designed temperature was reached; [b] For logR_0 calculation ($logR_0 = \log\left[t \times exp\left(\frac{T-100}{14.75}\right)\right]$) (t is for the resident time (min), and *T* is for temperature (°C)) of the immediate-cool process, the reaction time (t) was defined as 1 min

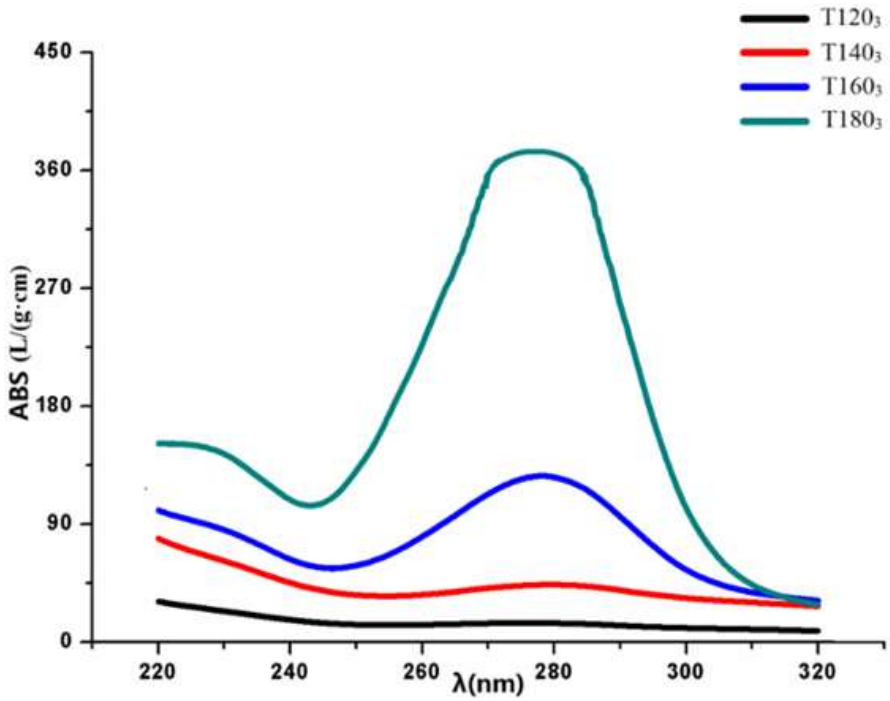

Fig. S1. UV patterns of the selected liquid samples

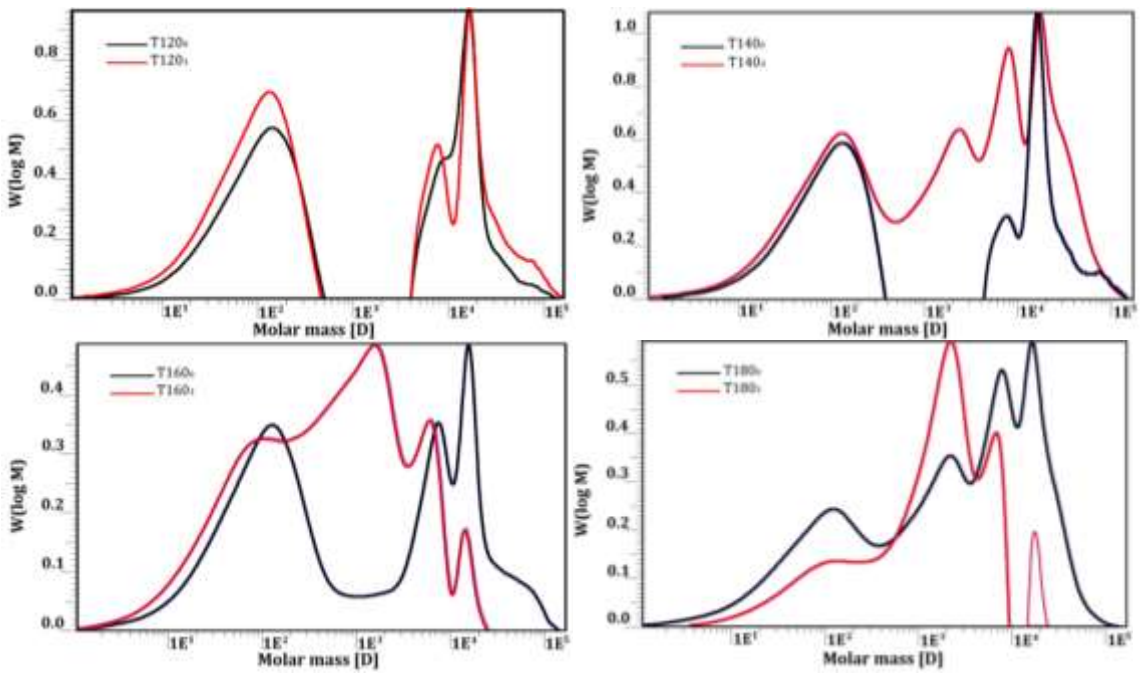

Fig. S2. Molecular weight distributions of the liquid samples

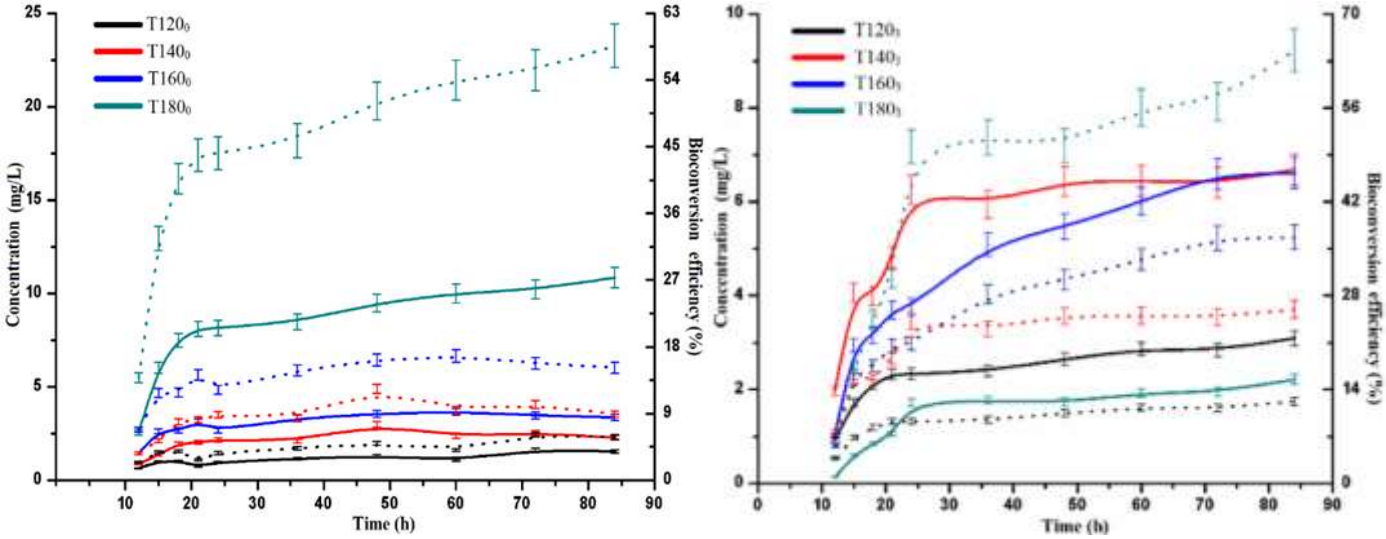

Fig. S3. Variation of xylose concentrations (solid line, left coordinate) and bioconversions (dot line, right coordinate) during 72 h enzyme hydrolysis