Hydrothermal Treatment and Enzymatic Saccharification of Corncobs

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Hydrothermal pretreatment of corncobs in aqueous media under non-isothermal conditions is an effective means for solubilizing hemicellulose fractions and improving cellulose hydrolysis. The effects of a range of pretreatment severities (temperatures of 170 to 230 °C) on the conversion of corncobs into fermentable sugars were examined. The major differences between the conversions of untreated and pretreated corncobs were the dissolution of hemicelluloses into the prehydrolyzate and the partial removal and relocation of lignin on the external surface of biomass particles (in the form of recondensed droplets) in the pretreated corncobs. Hemicellulose dissolution increased with pretreatment temperature. The maximum sugar recovery (272.3 g/kg raw material) and the minimum accumulation of inhibitory compounds in the prehydrolyzate were observed following treatment at 190 °C. While the fibrils of the untreated raw material remained largely intact, serious disruption of the cell wall was observed in SEM images of the surfaces of pretreated samples. Accordingly, the cellulose digestibilities of residues increased from 26.8% for the raw material to almost 100% for the 190 °C-treated sample. It was concluded that low severity hydrothermal pretreatment can be successfully applied to corncobs to obtain high cellulose digestibility while operating at low enzyme charges.

Keywords: Hydrothermal pretreatment; Enzymatic hydrolysis; Corncobs; Cellulose digestibility

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INTRODUCTION

Concerns regarding the future availability of fossil fuels, as well as the problems stemming from their extensive use, have motivated studies of the production of gaseous and liquid biofuels from biomass. Biomass has been identified as an important source of biofuels and other value-added products. It is considered economically, environmentally, and socially sustainable (Demirbas 2011; Nigam and Anoop 2011; Sahare *et al*. 2012). Biomass includes the biodegradable fraction of products, wastes, and residues from agriculture (including vegetable and animal substances), forestry, and related industries, as well as the biodegradable fraction of industrial and municipal wastes. These are the world's most abundant carbohydrates in the form of lignocellulosic materials (LCM) and they have the potential to be a major source of fermentable sugars for the production of second-generation fuel ethanol. The LCM cell wall, which accounts for 80 to 90% of the dry weight of lignocellulosic material, is composed mostly of cellulose, hemicelluloses, and lignin. Cellulose is a crystalline, linear polymer of D-glucose molecules bound by β-1,4-glycosidic linkages with a high degree of polymerization. Hemicelluloses are

branched, amorphous heteropolymers made up of a variety of sugars. These sugars can be substituted with uronic acid moieties, esterified phenolic acids, or acetyl groups. Hemicelluloses have a degree of polymerization only a fraction of that of cellulose. Lignin is a three-dimensional, amorphous polymer with a complex structure. It is made up of different phenyl-propane units. The rest of the components within LCM (including ash, extractives, pectins, and proteins) account for only a small proportion of their mass (Romaní *et al*. 2010). Cellulose is organized into microfibrils and is surrounded by hemicelluloses and encased inside a lignin matrix. This complex structure, combined with the crystallinity of cellulose, limited binding sites available to enzymes, as well as the physical barrier provided by the hemicellulose and lignin surrounding cellulose, gives lignocellulose its recalcitrant nature. This restricts the efficiency of the enzymatic hydrolysis of the parent biomass. Thus, a pretreatment step is critical for the success of the overall biochemical conversion process (Nitsos *et al*. 2013). The goals of pretreatment are to remove the recalcitrant barriers and increase the enzymatic digestibility of cellulose by altering the chemical compositions and physical structures of biomass feedstock (Zhao *et al*. 2012). At the same time, pretreatment should increase the production of fermentable C5 and C6 sugars from hemicelluloses and minimize the formation of degraded products (such as furfural and 5-hydroxymethylfurfural (HMF)) that inhibit the subsequent fermentation processes. Various methods and processes have been investigated to pretreat LCM, including the use of dilute acid, bases, organosolv, steam explosion, and ammonia fiber explosion (AFEX) (Vancov and Mclntosh 2011; Mclntosh and Vancov 2012; Pan *et al*. 2006; Wang *et al*. 2009; Alizadeh *et al*. 2005). However, these pretreatments sometimes require corrosion-resistant reactors and their products must be neutralized or recycled to reduce their negative impacts on the environment and on subsequent processes (for example, fermentation).

Hydrothermal pretreatment is often considered an environmentally friendly technology because the treatment medium contains only LCM and water (Garrote *et al*. 1999; Romaní *et al*. 2010; Xiao *et al*. 2012). Hydrothermal pretreatment is carried out at elevated temperatures (160 to 240 °C) and pressures (around 1 to 3.5 MPa) over time periods ranging from a few minutes to several hours. Typical solids concentrations are below 20 wt. % (Alvira *et al*. 2010). Liquid water (at high temperature) produces an elevated level of hydronium ions that act as an acid and catalyze the hydrolysis and deacetylation of hemicelluloses. Acetic acid and other organic acids generated by autohydrolysis can further catalyze the hydrolysis reaction (Holopainen-Mantila *et al*. 2013; Kim *et al*. 2009; Liu 2008). During hydrothermal pretreatment, hemicelluloses are decomposed into oligosaccharides, monosaccharides, and sugar-degradation products, whereas residues rich in cellulose and lignin can be separated by further processing. This allows for a variety of possible applications including the production of fermentable sugars and fuels (Saha *et al*. 2013; Cara *et al*. 2008).

The objective of this study was to investigate the influence of hydrothermal pretreatment on the physical and chemical features of corncobs and on the yields of sugars following pretreatment and enzymatic saccharification. Following pretreatment, the contents of monosaccharides, soluble oligosaccharides, and insoluble sugars were determined along with the contents of the yeast-inhibiting compounds furfural and HMF. The morphologies and crystallinity indices (CrI) of untreated and pretreated corncobs were characterized by scanning electron microscopy (SEM) and X-ray diffraction (XRD), respectively. Finally, enzymatic hydrolysis was carried out to evaluate the efficiency of hydrothermal pretreatment.

EXPERIMENTAL

Materials

The corncobs were collected from a corn field in Hebei province, China. The corncobs were ground to pass through a 40- to 80-mesh sieve, and the powder was dewaxed with a toluene and ethanol (2:1, v/v) mixture for 6 h. Next, it was dried completely at 60 °C for 16 h in an oven. The dried materials were stored in sealed bags at room temperature until further processing. The chemical composition of the corncobs was determined to be 36.1 ± 0.2% glucan, 26.6 ± 0.1% xylan, 5.1 ± 0.1% arabinan, 4.4 ± 0.2% acetyl groups, 14.0 ± 0.3% Klason lignin, 3.2 ± 0.2% acid-soluble lignin, and 10.6 ± 0.2% others, according to the standard Laboratory Analytical Procedures for biomass analysis provided by the National Renewable Energy Laboratory (NREL) (Colorado, USA) (Sluiter *et al*. 2008a). The data presented are the averages from two separate experiments.

Hydrothermal Pretreatment

Hydrothermal pretreatment was performed in a Parr reactor with a 1-L internal volume (Parr Instruments Company, Moline, IL) fitted with six blade turbine impellers. The reactor was heated by an external fabric mantle and cooled by the circulation of tap water through an internal loop. A mixture of corncobs and water (15 g/150 mL) was stirred at 150 rpm and heated to reach the desired maximum temperature (in the range of 170 to 230 °C), while the maximum pressure varied within the range of 119 to 416 psi. Once the target temperature was reached, the media were immediately cooled to 30 °C by cooling water circulation.

The intensity of hydrothermal pretreatment is defined as the logarithm of the severity factor R_o (Lavoie *et al*. 2010) and was calculated using the expression

$$\log R_o = \log[R_{o\,HEATING} + R_{o\,COOLING}] \quad (1)$$

$$= \log\left[\int_0^{t_{MAX}} \exp\left(\frac{T(t) - T_{REF}}{\omega}\right) \cdot dt + \int_{t_{MAX}}^{t_F} \exp\left(\frac{T'(t) - T_{REF}}{\omega}\right) \cdot dt\right]$$

According to Eq. (1), log R_o accounts for the combined effects of temperature and the durations of the heating and cooling periods on the pretreatment severity. In the expression, t_{MAX}(min) is the time needed to achieve T_{MAX} (°C), and t_F (min) is the time needed for the whole heating-cooling period, whereas $T(t)$ and $T'(t)$ represent the temperature profiles of the heating and cooling processes, respectively. Calculations were made using values reported in the literature for ω and T_{REF} (14.75 °C and 100 °C, respectively).

The reaction mixtures were separated into solid residues and prehydrolyzates by vacuum filtration. The residues were thoroughly washed with distilled water until the filtrate was neutralized and were then dried for solid yield determination (expressed as g solid residue recovered/100 g raw material, on dry basis). The chemical compositions of the solid residues were analyzed using the same methods employed for the raw corncobs. The prehydrolyzates were filtered through 0.22-μm membranes and used to determine the contents of monosaccharides, oligosaccharides, and degraded products. These

measurements were made according to the NREL analytical methods for the determination of sugar, byproduct, and degraded product contents in the liquid fraction of process samples (Sluiter *et al*. 2008b).

Enzymatic Hydrolysis

The residues were enzymatically hydrolyzed with 5% substrate (w/v) in 15 mL of 0.05 M sodium acetate buffer (pH = 4.8) using an air-shaking incubator at 150 rpm for 48 h. The temperature was maintained at 50 °C. An enzyme cocktail of cellulase (6 FPU/g substrate) and β-glucosidase (3.59 IU/FPU) supplied by Youtell Biochemical Co., Ltd. (Shanghai, China) was used for all hydrolysis experiments. Samples were withdrawn at time points specified in the text and immediately deactivated the enzymes in boiling water for 5 min, centrifuged at 10000 g for 10 min, and filtered for sugar analysis (using the method cited above). The cellulose digestibility (%) was calculated as the mass of glucose in the enzymatic hydrolyzate expressed as a percentage of the total cellulose in the substrate.

Analytical Procedures

The structural carbohydrates in the solid samples and the monosaccharides in the liquids were analyzed by a high-performance anion exchange chromatograph (HPAEC) (Dionex, ICS 3000, USA) equipped with an amperometric detector, an AS50 autosampler, a CarbopacTM PA-20 column (4 × 250 mm, Dionex), and a guard PA-20 column (3 × 30 mm, Dionex). Neutral sugars were separated by a 5 mM NaOH (carbonate-free and purged with nitrogen) for 20 min followed by a 0 to 75 mM NaAc gradient in 5 mM NaOH for 15 min. The columns were then washed with 200 mM NaOH to remove carbonate for 10 min, followed by 5 min of elution with 5 mM NaOH to reequilibrate the column before the next injection. The total analysis time was 50 min, and the flow rate was 0.4 mL/min. Calibration was performed with standard solutions of L-arabinose, D-glucose, D-xylose, D-mannose, D-galactose, glucuronic acid, and galacturonic acid. The concentrations of inhibitors were quantitatively analyzed at 50 °C using an HPLC system (Agilent 1200 series, Agilent Technologies, U.S.) equipped with a refractive index detector (RID). The sample separation was performed using an Aminex HPX-87H column (300 mm × 7.8 mm) (Bio-Rad Laboratories Inc., Hercules, CA) with 0.005 M sulfuric acid as the eluent at a flow rate of 0.6 mL/min. The morphologies of the raw material and residues were studied by scanning electron microscopy (SEM) using a Hitachi S-3400N II (Hitachi, Japan) instrument at 15 kV. Images of the surfaces of the samples were taken at magnifications of 1000× and 3000×. Prior to generating the images, the specimens were sputter-coated with a thin layer of gold. The crystallinity indices (CrI) of the raw material and residues were measured by the powder method of X-ray diffraction using an XRD-6000 instrument (Shimadzu, Japan) with a Cu Kα radiation source (λ = 0.154 nm) at 40 kV and 30 mA. Samples were scanned over a range of 2θ from 5° to 60° at a speed of 2°/min. The CrI was calculated according to the formula proposed by Segal *et al*. (1959), which is shown below,

$$CrI = 100 \times [(I_{002}\text{-}I_{am}) / I_{002}] \quad (2)$$

where I_{002} is the intensity of the 002 peak (at $2\theta = 22°$) and I_{am} is the intensity at $2\theta = 18°$. The I_{002} peak corresponds to the crystalline fraction, and I_{am} corresponds to the amorphous fraction.

RESULTS AND DISCUSSION

Compositions of Solid Residues Resulting from Pretreatment

The solid yields and the compositions of the solid residues resulting from hydrothermal pretreatment are summarized in Table 1. The hydrothermal pretreatment dissolved hemicelluloses from the corncobs, thus lowering the solid yields. The solid yields decreased rapidly from 69.4 g/100 g at 170 °C to 48.7 g/100 g at 190 °C, then further decreased to 48.5 g/100 g at 200 °C and 47.0 g/100 g at 230 °C. These results suggest that the solubilization of corncob residues increased continuously with temperature, although less notably at temperatures above 190 °C. On the other hand, at higher pretreatment temperatures, solid yields decreased, likely due to the partial degradation of cellulose, a behaviour that can be justified by the decrease of the cellulose content of residues obtained at T_{MAX} values of 210 to 230 °C.

From Table 1, it also can be seen that cellulose and Klason lignin were the major chemical components of the solid residues and that the content of hemicelluloses was relatively low. The cellulose content of the residuals first increased from 38.2 g/100 g residue (at a T_{MAX} of 170 °C) to 50.3 g/100 g residue (at a T_{MAX} of 200 °C), then decreased to 44.4 g/100 g residue in the experiment performed at a T_{MAX} of 230 °C. At a T_{MAX} of 210 °C, partial cellulose degradation began to take place, resulting in decreases in the amount of bound glucose within the solid residues. This observation was in agreement with those of other reports regarding the hydrothermal pretreatment of *Eucalyptus globulus*, *Tamarix ramosissima*, and *Olea europaea* trimmings (Romaní *et al*. 2010; Xiao *et al.* 2012; Requejo *et al.* 2012). The observed increase in the cellulose content in the pretreated material (with a maximum of 50.3%) in comparison to the untreated substrate (36.1%) indicates one important advantage of biomass pretreatment for the subsequent enzymatic hydrolysis.

The content of hemicelluloses (including xylan, arabinan, and acetyl groups) exhibited similar behaviour, decreasing steadily with the severity of treatments. This trend was more marked in the case of xylan. More specifically, the xylan content in the residue was only 1 g/100 g residue at 230 °C. This phenomenon could be attributed to the physical impediment of the outer edges of the cell wall, where xylan accumulated before being hydrolyzed. Simultaneously, lignin recondensation and precipitation onto cellulose during the pretreatment also inhibited the isolation of hemicelluloses. The hydrophobic nature of lignin impedes acid access to the ether linkages of the xylooligomers in the hydrolyzate, leaving a small amount of xylan in the residues (Yang *et al*. 2012). Arabinan and acetyl groups were completely released at 200 °C and 220 °C, respectively. These observations were in good agreement with previous studies on the evaluation of the hydrothermal pretreatment to reduce biomass recalcitrance using different raw materials for enhanced enzymatic digestibility, such as beech (Nitsos *et al*. 2013), hybrid polar (Kim *et al*. 2009), soybean straw (Wan *et al*. 2011), and wheat straw (Merali *et al*. 2013).

The residues were found to have a notably high level of Klason lignin content as compared to the raw material. The Klason lignin content increased as pretreatment severity increased to between 16.7 and 41.7 g/100 g residue. These values were similar to those reported in other studies, which attributed the high lignin yield (114% at 230 °C) to the formation of "pseudo-lignin", an acid-insoluble polymer formed by condensation reactions between lignin and sugar-derived products under higher pretreatment severities (Mittal *et al*. 2009; Ramos *et al*. 2003).

Table 1. Severity Factors Corresponding to Maximal Temperature, Solid Yield, and Composition Data for Residues from Hydrothermal Treatment of Corncobs

T (°C)	log R_0	Solid yield	Chemical composition of residues (g/ 100 g residue)				
			Glucan	Xylan	Arabinan	Acetyl group	Klason lignin
0	0.00	100.0±0.0	36.1±0.2	26.6±0.1	5.1±0.1	4.4±0.2	14.0±0.3
170	2.31	69.4±1.4	38.2±0.5	37.9±0.0	2.9±0.1	2.6±0.2	16.7±0.2
180	2.45	53.3±0.6	42.4±0.8	27.2±0.3	1.4±0.2	1.7±0.1	19.3±0.3
190	2.71	48.7±0.5	48.6±0.0	12.8±0.4	0.5±0.2	0.8±0.1	24.2±0.2
200	2.96	48.5±0.7	50.3±0.6	8.7±0.6	0.0±0.0	0.5±0.1	28.7±0.1
210	3.29	48.1±0.0	47.0±0.8	3.2±0.5	0.0±0.0	0.4±0.1	33.6±0.0
220	3.71	47.9±0.0	43.8±0.0	1.4±0.8	0.0±0.0	0.0±0.0	41.7±0.3
230	3.91	47.0±0.4	44.4±0.4	1.0±0.6	0.0±0.0	0.0±0.0	41.7±0.1
* All data in this table are mean value of duplicate experiments							

Table 2. Concentrations of Degradation Products Generated during Hydrothermal Pretreatment (g/L)

Fraction	T_{MAX} (°C)						
	170	180	190	200	210	220	230
Acetic acid	1.03±0.10	1.53±0.11	2.46±0.04	3.97±0.07	4.39±0.21	3.04±0.31	5.00±0.13
HMF	0.00±0.00	0.07±0.01	0.15±0.10	0.59±0.02	0.76±0.14	0.68±0.10	1.27±0.10
Furfural	0.00±0.00	0.20±0.02	1.12±0.24	3.03±0.30	5.13±0.20	2.76±0.15	3.86±0.10
Formic acid	0.42±0.11	0.72±0.14	1.30±0.00	2.49±0.25	2.24±0.15	1.44±0.30	2.15±0.23
* All data in this table are mean values of duplicate experiments							

The residues obtained in pretreatments at high temperatures contained almost exclusively cellulose and lignin, which indicates that hemicelluloses can be selectively removed from corncobs by hydrothermal pretreatment.

Prehydrolyzate Composition

Figure 1 shows the composition of the prehydrolyzates obtained after hydrothermal pretreatment on the basis of 1 kg of raw material. Because hemicelluloses were the main biomass component removed by the hydrothermal pretreatment, xylooligosaccharide (XOS) and xylose were the most abundant sugars detected in the prehydrolyzates (Fig. 1a, b). The highest XOS yields (181.07 to 177.27 g/kg raw material) were obtained at a T_{MAX} of 180 to 190 °C, followed by a sharp decrease at higher severities, which indicates that as much as 68.07% of the xylan in the raw material can be

recovered as XOS under relatively mild pretreatment conditions. XOS were hydrolyzed into xylose monomers and degraded into furfural under more severe conditions (see Fig. 1a). Glucooligosaccharides (GlcOS) generated from cellulose reached the highest yield (14.05 g/kg raw material) at a T_{MAX} of 190 °C, accounting for about 3.9% of the initial cellulose. The arabinosyl groups linked to oligomers (ArbOS) yields decreased steadily from 21.90 g/kg raw material under the mildest conditions to 0 g/kg raw material at a pretreatment temperature of 220 °C. Changes in the oligosaccharides' yields in this study were in agreement with the results of previous studies (Gullón *et al*. 2010; Requejo *et al*. 2012).

Figure 1b shows the curves for recovered monosaccharides in prehydrolyzates as a function of the hydrothermal pretreatment severity. As expected, with increases in pretreatment severities, the yields of xylose in prehydrolyzates first increased from 2.67 to 58.40 g/kg raw material between 170 and 200 °C, then quickly decreased to 0.44 g/kg raw material at a T_{MAX} of 230 °C. This decrease in xylose in the prehydrolyzates could be attributed to the degradation of xylose into furfural. The released amounts of glucose were generally below 4.50 g/kg raw material at all hydrothermal pretreatment temperatures. Arabinose was rapidly released from hemicelluloses and reached the highest yield of 11.07 g/kg raw material at 190 °C, demonstrating that this component was highly susceptible to hydrolysis reactions (Gullón *et al*. 2010; Saha *et al*. 1999). At 220 °C, no arabinose was detected in the prehydrolyzates, which was likely due to further degradation generating inhibitory products. The total saccharides (including all the sugars present in the prehydrolyzates), total xylose (combined monomers and oligomers), total glucose (combined monomer and oligomers), and total arabinose (combined monomer and oligomers) had trends similar to those of the oligosaccharides, because of the abundant oligosaccharides concentrations and low monosaccharides concentrations in the prehydrolyzates.

The main degradation products encountered in the prehydrolyzates were acetic acid, furfural, 5-hydroxymethylfurfural (HMF), and formic acid. Furfural is a pentose dehydration product. Under acidic hydrothermal conditions, it can be further degraded either through hydrolytic fission of the aldehyde group to formic acid or through oxidation to 2-furoic acid. HMF is a dehydration product of hexose sugars (glucose, mannose, and galactose) that can be further converted, under acidic conditions, into equimolar amounts of levulinic and formic acid (Nitsos *et al*. 2013). The concentrations of these inhibitory compounds increased steadily with T_{MAX}, reaching maxima of 2.49 g/L, 5.13 g/L, 5.00 g/L, and 1.27 g/L for formic acid, furfural, acetic acid, and HMF, respectively (Table 2). Interestingly, under the operational conditions leading to the maximum sugar yield in prehydrolyzate, the respective concentrations of the degradation products were comparatively low (acetic acid, 2.46 g/L; HMF, 0.15 g/L; furfural, 1.12 g/L; and formic acid, 1.30 g/L at a T_{MAX} of 190 °C).

Morphological Structure of Corncobs

SEM images of the different corncob samples were obtained to show the fiber morphology before and after hydrothermal pretreatment (Fig. 2). The untreated sample had the typical regular and compact surface structure, with highly fibrous and intact morphology (Fig. 2A). In the residues generated *via* treatment at 170 and 180 °C, considerable disruption of the fibers could be clearly seen (Figs. 2B and C).

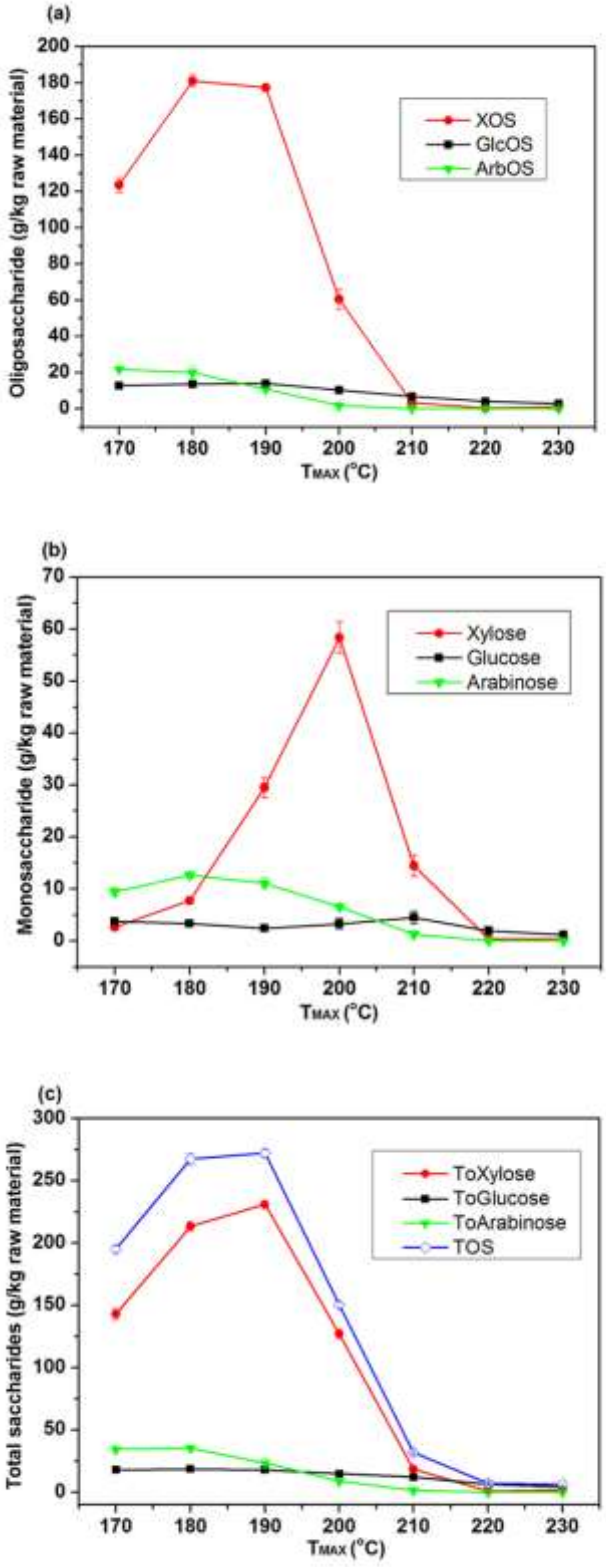

Fig. 1. Production of oligosaccharides (a), monosaccharides (b), and total saccharides (c) by hydrothermal pretreatment of corncobs, as a function of temperature (GlcOS, glucooligosaccharide; XOS, xylooligosaccharide; ArOS, arabinosyl groups linked to oligomers; TOS, total oligosaccharides). Values for log R_o are: 2.31, 2.45, 2.71, 2.96, 3.29, 3.71, and 3.91, for T_{MAX} values of 170,180, 190, 200, 210, 220, and 230 °C, respectively.

Most of the particles yielded by pretreatment at 190 °C changed from fibrous to spherical shapes and their surfaces were rugged. These particles also formed aggregates (Fig. 2D).

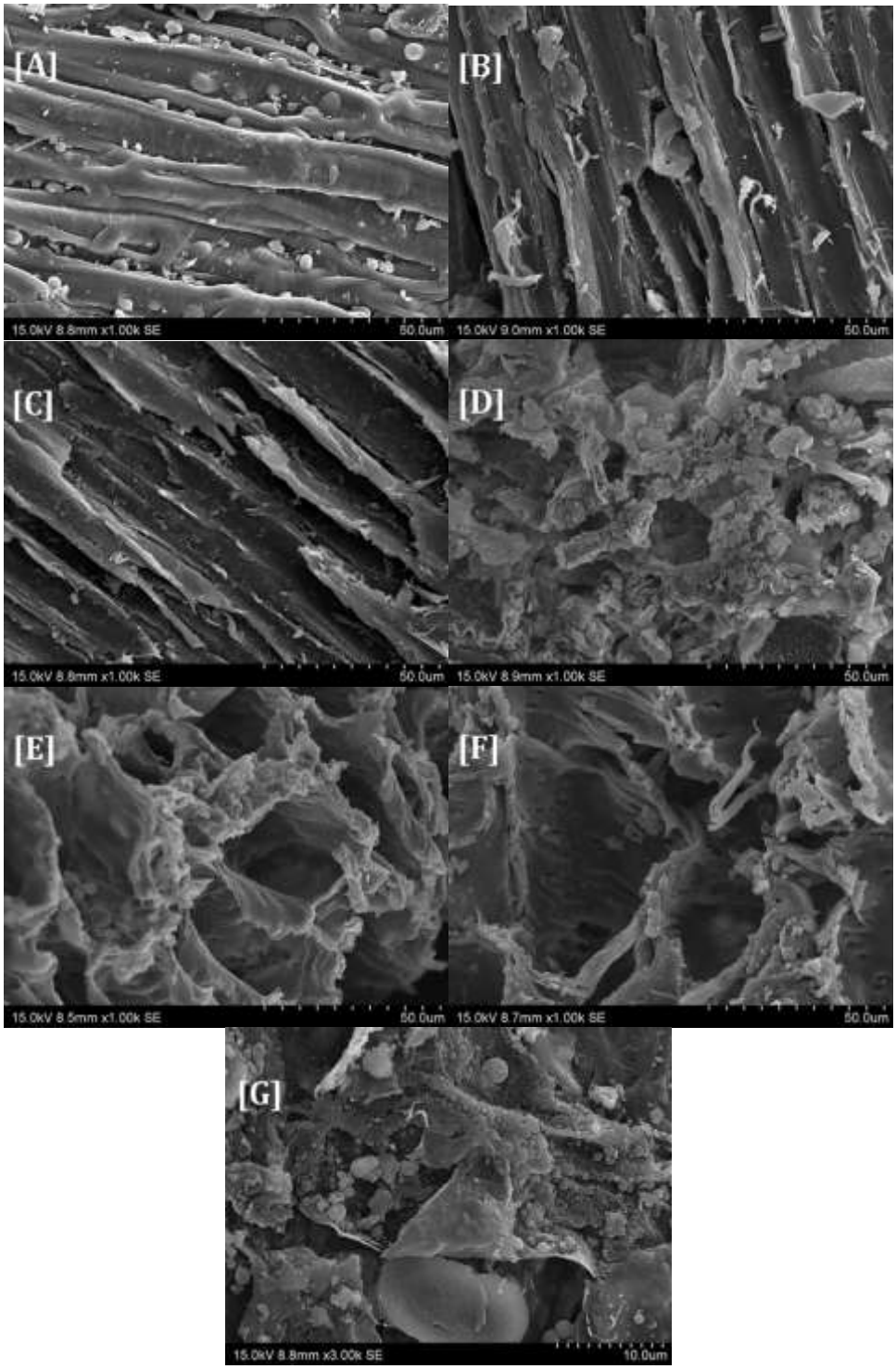

Fig. 2. SEM images at 1000 × magnification for untreated and pretreated corncobs; [A] untreated corncobs at 1000 ×, [B] 170 °C at 1000 ×, [C] 180 °C at 1000 ×, [D] 190 °C at 1000 ×, [E] 200 °C at 1000 ×, [F] 230 °C at 1000 ×, and [G] 200 °C at 3000 ×

As expected, surface exposures were more prominent under harsher pretreatment conditions, which can be seen in the images of the solid residues yielded by pretreatment temperatures over 190 °C (Figs. 2E and F). Based on the differences in SEM images, it is evident that hydrothermal pretreatment resulted in the disruption of the biomass's structure to some extent (at 170 and 180 °C), reduced fiber length (at 190 °C), and completely disrupted the biomass structure (at above 190 °C). Lignin droplets appeared on the surface of residues (at 200 °C) and were visible at 3000× magnification (Fig. 2G), suggesting that lignin melted under the high temperature and pressure and subsequently condensed. Donohoe *et al*. (2008) also observed a range of droplet morphologies that appear on and within cell walls of pretreated biomass, as well as the specific ultrastructural regions that accumulate the droplets. They thought that thermochemical pretreatments reaching temperatures above the range for lignin phase transition cause lignins to coalesce into larger molten bodies that migrate within and out of the cell wall, and can redeposit on the surface of plant cell walls. Therefore, the lignin droplets observed when the corncobs were exposed to hydrothermal pretreatment at high temperature were in accordance with the observations of other reports (Donohoe *et al*. 2008; Kaparaju and Felby 2010; Kristensen *et al*. 2008; Xiao *et al*. 2011).

Effect on Crystallinity Index

The crystallinity of cellulose is one of the most important structural factors affecting the rate of enzymatic hydrolysis. Hydroxyl groups exist in cellulose macromolecules, so they form various ordered, crystalline arrangements with numerous intra- and intermolecular hydrogen bonding patterns. The strength of the hydrogen bonding networks may limit enzyme accessibility to the glycosidic bonds in cellulose and may negatively affect the interaction of enzymes with cellulose. It has been demonstrated that the cellulase attack is principally initiated within the more easily accessible amorphous portions of cellulose, which is readily degraded before hydrolysis of the less accessible crystalline portion (Sahare *et al*. 2012; Yang *et al*. 2012). Table 3 shows the values of the CrI of untreated and pretreated corncobs yielded by different pretreatment severities. It was found that the CrI of pretreated samples (at temperatures lower 200 °C) increased slightly, probably due to the removal of hemicelluloses and the contribution of the glycosidic bond hydrolysis reaction in the amorphous portion of the cellulose. When the corncobs were pretreated under more severe conditions, degradation of crystalline portion during the pretreatment seemed to occur, resulting in successive decreases in the CrI.

Table 3. Crystallinity Index of Untreated and Pretreated Corncobs

Severity (°C)	untreated	170	180	190	200	210	220	230
CrI (%)	28.41	32.35	33.26	33.81	33.13	33.01	32.72	31.60

Enzymatic Hydrolysis

Native corncobs and residues were used as substrates for enzymatic hydrolysis to assess the cellulose digestibility of various substrates derived from the different pretreatment conditions examined. Figure 3 shows the experimental results expressed in terms of cellulose digestibility (%). It should be noted that mild hydrothermal pretreatment resulted in substrates with higher cellulose digestibility and enabled

complete (or almost complete) cellulose conversion for substrates pretreated at T_{MAX} values of 180 to 190 °C.

The untreated corncobs reached only 26.8% cellulose digestibility under the enzymatic hydrolysis conditions. However, the substrates pretreated at T_{MAX} values of 170 to 180 °C achieved cellulose conversions in the range of 83.4 to 97.8%, and the sample pretreated at a T_{MAX} of 190 °C reached complete conversion. These results were in accordance with the other effects of hydrothermal pretreatment on corncobs identified and discussed above, including the removal of hemicelluloses, the partial removal of lignin, the partial relocation of lignin, and the increased surface exposure. The maximum cellulose digestibility of corncobs was in accordance with the result from dilute acid pretreated sample (Wang *et al*. 2011) and slight higher than that after alkali pretreatment (Sahare *et al*. 2012). However, the residues obtained under the severest conditions, at T_{MAX} values of 200 to 230 °C, exhibited the opposite behavior, in which the enzymatic digestibility decreased from 91.9 to 61.5 %. This phenomenon could be attributed to the pronounced deposition of dissolved and recondensed lignin on the external surface of the biomass particles, thereby inhibiting the interaction between cellulose and the enzymes, as revealed by the SEM images for residues obtained at a pretreatment temperature of 200 °C at 3000 magnification (Fig. 2G) (Nitsos *et al*. 2013). The extrusion of lignin from the cell wall may also cause adhesion of adjacent cellulose microfibrils to each other *via* hydrogen bonding, which could have a detrimental effect on the digestion of cellulose (Yang *et al*. 2012). Overall, excellent cellulose digestibility were obtained following hydrothermal pretreatment at T_{MAX} values of 180 to 190 °C.

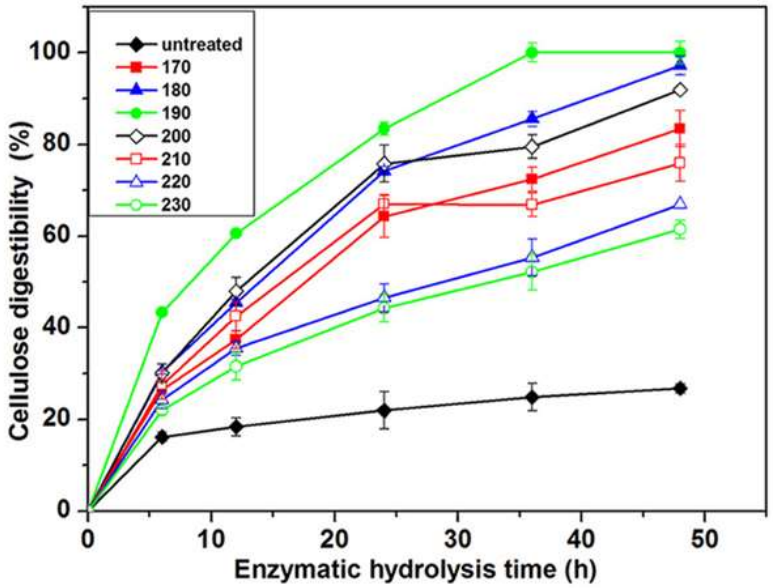

Fig. 3. Cellulose digestibility of untreated and pretreated corncob

CONCLUSIONS

1. The main effect of the hydrothermal pretreatment was the removal of hemicelluloses, which resulted in enriched cellulose and lignin content.

2. The maximum yield of sugars in the prehydrolyzate (272.3 g/kg raw material) was obtained at a pretreatment temperature of 190 °C. This prehydrolyzate contained only a small amount of inhibitory products.
3. The obvious disruption of fiber in the intensely pretreated corncob residues led to a high level of surface exposure. Recondensed lignin droplets were relocated onto the external surface of biomass particles pretreated at a T_{MAX} of 200 °C.
4. Enzymatic saccharification of hydrothermal pretreated corncobs (at a T_{MAX} of 190 °C) resulted in 100% cellulose digestibility, which implies that hydrothermal pretreatment can be successfully applied to corncobs to obtain high cellulose digestibility while operating at low enzyme charges.

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