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Physical and chemical characterizations of corn stover and poplar solids resulting from leading pretreatment technologies

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ABSTRACT

In order to investigate changes in substrate chemical and physical features after pretreatment, several characterizations were performed on untreated (UT) corn stover and poplar and their solids resulting pretreatments by ammonia fiber expansion (AFEX), ammonia recycled percolation (ARP), controlled pH, dilute acid, flowthrough, lime, and SO_2 technologies. In addition to measuring the chemical compositions including acetyl content, physical attributes determined were biomass crystallinity, cellulose degree of polymerization, cellulase adsorption capacity of pretreated solids and enzymatically extracted lignin, copper number, FT-IR responses, scanning electron microscopy (SEM) visualizations, and surface atomic composition by electron spectroscopy of chemical analysis (ESCA). Lime pretreatment removed the most acetyl groups from both corn stover and poplar, while AFEX removed the least. Low pH pretreatments depolymerized cellulose and enhanced biomass crystallinity much more than higher pH approaches. Lime pretreated corn stover solids and flowthrough pretreated poplar solids had the highest cellulase adsorption capacity, while dilute acid pretreated corn stover solids and controlled pH pretreated poplar solids had the least. Furthermore, enzymatically extracted AFEX lignin preparations for both corn stover and poplar had the lowest cellulase adsorption capacity. ESCA results showed that SO_2 pretreated solids had the highest surface O/C ratio for poplar, but for corn stover, the highest value was observed for dilute acid pretreatment with a Parr reactor. Although dependent on pretreatment and substrate, FT-IR data showed that along with changes in cross linking and chemical changes, pretreatments may also decrystallize cellulose and change the ratio of crystalline cellulose polymorphs ($I\alpha/I\beta$).

1. Introduction

Biomass pretreatment is an essential processing step for producing biofuels with high yields via biological routes (Chandra et al., 2007; Grethlein, 1984; Yang and Wyman, 2008). Although several pretreatment methods render biomass digestible to sugars with enzymes (Gharpuray et al., 1983; Lynd et al., 1996; Morrison, 1988; Tanaka et al., 1990; Yu et al., 1998), thermochemical pretreatments are more prone to be utilized for commercial purposes over biological or mechanical options owing to shorter processing times, higher yields, limited chemical use, and lower energy requirements (Knappert et al., 1980; Mosier et al., 2005a,b; Puri and Pearce, 1986; Weimer et al., 1986; Wyman et al., 2005b). Over the last few decades, several thermochemical pretreatments have been shown to be promising for a variety of feedstocks. As summarized elsewhere (Mosier et al., 2005b; Sun and Cheng, 2002; Yang and Wyman, 2008), these pretreatments are known for their unique features and can be put in three categories: low pH, high pH, and neutral pH pretreatments. Typically low pH pretreatments (e.g., dilute acid, flowthrough with dilute acid, and uncatalyzed and catalyzed steam explosion with either acid or SO_2 as a catalyst) remove most of the hemicellulose and a small portion of biomass lignin, Near neutral pH pretreatments such as controlled pH and flowthrough with just water remove much of the hemicellulose but leave most of the cellulose and lignin intact (Mosier et al., 2005b; Yang and Wyman, 2008). By contrast, high pH pretreatments (e.g., lime and ARP) remove a large fraction of lignin and some hemicelluloses, but AFEX is the exception that removes little of anything.

Among physiochemical changes, increased surface area, enhanced pore volume due to xylan removal, reduced cellulose degree of polymerization, increased biomass crystallinity, and melting and relocation of lignin are thought to be the most important features impacted by steam explosion and dilute acid

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pretreatments (Clark et al., 1989; Excoffier et al., 1991; Grethlein, 1984; Grous et al., 1986; Michalowicz et al., 1991; Saddler et al., 1982; Selig et al., 2007; Tucker et al., 1998; Wong et al., 1988). In addition, AFEX has been reported to decrease cellulose crystallinity and disrupt lignin-carbohydrates linkages (Chundawat et al., 2007; Laureano-Perez et al., 2005). Yet, such data is still lacking for other pretreatments, and to further tailor their processing conditions, it is vital to understand how these methods change substrate features and their impact on subsequent enzymatic hydrolysis. In addition, performance of these leading pretreatments may vary with feedstock type, and causes for this variability have not been researched well. Finally, these pretreatments have never been fully characterized using common sources of feedstock or enzymes.

In this study, we sought to understand physiochemical changes resulting from pretreatments by leading options of AFEX, ARP, controlled pH, dilute acid, flowthrough, lime, and SO_2 with corn stover, an agriculture residue, and poplar, a woody biomass. Chemical characterizations were performed to determine glucan, xylan, lignin, and acetyl contents for untreated and pretreated corn stover and poplar solids resulting from the leading pretreatments. Physical characteristics including cellulose crystallinity, cellulose degree of polymerization (DP), copper number, cellulase adsorption capacity of biomass solids and enzymatically extracted lignin solids, and changes in surface elemental composition were also measured.

2. Methods

2.1. Materials

Pure cellulose, Avicel PH-101, was purchased from FMC Corporation, Philadelphia, PA (Cat 11365, Lot 1094627). Regenerated amorphous cellulose (RAC) was prepared from Avicel PH 101 according to a method reported by Zhang and Lynd (2005). Bacterial cellulose was a commercially available product called CHAOKOH® (coconut gel in syrup, Thep. Padung Porn Coconut Co. Ltd, Bangkok, Thailand) that was further purified according to a procedure reported elsewhere (Kipper et al., 2005; Väljamäe et al., 1999). Dewaxed cotton linters were provided by Dr. Rajesh Gupta at Auburn University. Other chemicals and reagents were purchased from Fisher, Pittsburgh, PA and Sigma Chemicals, St. Louis, MO. The National Renewable Energy Laboratory (NREL) in Golden, CO generously provided unpretreated Kramer corn stover and poplar. Our partners in the Biomass Refining Consortium for Applied Fundamentals and Innovation (CAFI) generously provided solids resulting from pretreatment of the same NREL sources of corn stover and poplar as follows: AFEX by Michigan State University, ARP by Auburn University, controlled pH by Purdue University, dilute acid pretreatment with the Sunds pilot reactor and steam gun for corn stover and poplar, respectively, by NREL, lime by Texas A&M University, and sulfur dioxide by the University of British Columbia. John Bardsley graciously prepared poplar solids at Dartmouth College using flowthrough pretreatment and corn stover solids using batch dilute acid pretreatment with a 1L Parr reactor. Pretreatment conditions and feedstocks are summarized in Table 1.

2.2. Enzymes

Spezyme® CP cellulase (lot 301-04075-034; 59 ± 5 FPU/ml, 123 ± 10 mg protein/ml) along with data on its activity and protein numbers was generously provided by the Genencor Division of Danisco US, Inc. (Rochester, NY, USA).

2.3. Delignification

Delignification of pretreated poplar solids was performed in a water bath in a fume hood using sodium chlorite and acetic acid at 70 °C (Yoshida et al., 2008). To 80 ml of hot DI water, app. 2.5 g dry weight of pretreated poplar solids, 0.5 ml of acetic acid, and 1 g of sodium chlorite were added, and then 0.5 ml of fresh acetic acid and 1 g of sodium chlorite were introduced every hour for 6 h. Samples were left overnight in a water bath and then washed extensively until the filtrate pH was neutral. To determine the impact of the chlorite delignification method on cellulose chain length, filter paper and Avicel cellulose along with poplar solids were also taken through the delignification procedure. The solids were dried at 50 °C prior to determining their composition according to NREL LAP 002 (NREL, 2004), as reported in Table 2.

Table 2
Major fractions in delignified poplar solids (%) prepared by the pretreatments studied.

Pretreatment	Glucan	Xylan	Lignin
AFEX	75.8 ± 1.6	23.3 ± 0.14	2.4 ± 0.72
ARP	72.9 ± 4.1	20.9 ± 0.9	3.0 ± 0.8
Controlled pH	88.4 ± 0.6	11.5 ± 0.1	1.9 ± 0.4
DA	91.7 ± 2.4	5.9 ± 0.0	3.2 ± 1.3
Lime	72.6 ± 2.0	23.4 ± 1.0	2.2 ± 0.6
SO_2	92.7 ± 0.8	9.7 ± 0.0	1.5 ± 0.2

Table 1
Pretreatment methods, conditions, and severity levels for pretreatments of corn stover and poplar solids.

Pretreatment	Corn stover		Poplar	
	Pretreatment conditions	[a]Severity, Log R_0	Pretreatment conditions	Severity, Log R_0
AFEX	90 °C,220 psi, 1:1 NH_3 to Biomass, 5 min	0.40	180 °C,700 psi, 2:1 NH_3 to Biomass, 30 min, and 233% moisture content (dwb)	3.83
ARP	170 °C, 325 psi, 3.33:1 NH_3:Dry wt., 20 min, and 3.3 ml/g of corn stover	3.36	185 °C,400 psi, 3.66:1 NH_3 to Biomass (dry wt.), 27.5 min and 23.49% solid	3.94
Controlled pH	190 °C, 15 min (+ 5 min heat up)	3.83	200 °C, 10 min (+5 min heat up)	3.94
Dilute acid	Sunds System 180 °C, 0.03H_2SO_4:Dry wt, 90s, 25% solids	2.53	[b]190 °C, 0.02H_2SO_4:Dry wt., 70 s, 30% solids	2.72
	Parr Reactor 160 °C, 0.005 H_2SO_4:Dry wt., 20 min, 5% solids	3.07		
Flowthrough	nd	nd	190 °C, 0.05% H_2SO_4, 20 ml/min, 15 min	3.83
Lime	55 °C, 0.5:1 $Ca(OH)_2$ to Biomass (dry wt.), 4 weeks, water loading- 10 g/ g dry biomass	3.28	65 °C, 0.5:1 $Ca(OH)_2$ to Biomass (dry wt.)	3.57
SO_2	190 °C, 5 min, 3% SO_2-steam explosion	3.35	200 °C, 5 min, 3% SO_2-steam explosion	3.64

[a] Severity parameters included only time and temperature; Log R_0 = Log [time exp(($H - R$)/14.75], where time is in minutes, H-pretreatment temperature in °C, R is a reference temperature, 100 °C.

[b] Dilute acid poplar solids were prepared with a bench scale steam gun at NREL.

2.4. Chemical characterizations

Compositional data were determined by our CAFI partners or in our laboratory according to NREL Laboratory Analytical Procedure 002 (NREL, 2004). The acetyl contents of corn stover and poplar solids were determined as per NREL LAP002 using glacial acetic acid as a calibration standard (NREL, 2004).

2.5. Physical characterizations

Untreated corn stover and poplar, their pretreated solids, and delignified poplar solids were dried at 50 °C for several days and then pulverized using a coffee blender (KitchenAid, model # BCG1000B1). Samples with particle sizes less than 150 μm, greater than 125 μm, and less than 125 μm were collected for further physical characterizations.

2.6. Biomass crystallinity

Wide angle X-ray diffraction (WAXD) on pure Avicel glucan and untreated and pretreated corn stover and poplar solids was conducted according to a method described by Chang and Holtzapple (2000). Samples of particle size less than 125 μm were scanned at a speed of 1°/min, range from $2\theta = 0–40^\circ$, and with a step size of 0.04° at room temperature by positioning the samples on a quartz sample holder using a Rigaku Miniflex diffractometer in conjunction with a Cu K_α radiation source ($\lambda = 0.154$ nm) operated at 30 kV. Biomass crystallinity as expressed by crystallinity index (CrI) was determined according to a method by Segal et al. (1959)) as follows:

$$\mathrm{CrI} = 100 \times \left[(I_{002} - I_{\mathrm{amorphous}})/I_{002}\right]$$

in which, I_{002} is the intensity for the crystalline portion of biomass (i.e., cellulose) at about $2\theta = 22.5$ and $I_{\mathrm{amorphous}}$ is the peak for the amorphous portion (i.e., cellulose, hemicellulose, and lignin) at about $2\theta = 16.6$. In this study, the second highest peak after $2\theta = 22.5$ was at $2\theta = 16.2$ and was assumed to correspond to the amorphous region; however, the amorphous peak is reported to occur at around $2\theta = 18.7$ in the literature.

2.7. Cellulase adsorption capacity of pretreated solids and enzyme extracted lignin

Enzyme extracted lignin [EnzL] solids were prepared by following a procedure described elsewhere (Kumar and Wyman, in press-a, 2009a). Cellulase adsorption was determined by a nitrogen method as reported in detail previously (Kumar and Wyman, 2008). Cellulase adsorption parameters for pretreated solids and EnzL were characterized by fitting adsorption data to the Langmuir equation via a nonlinear regression with Polymath software (Kumar and Wyman, 2008; Lynd et al., 2002).

2.8. Copper number

Braidy's method reported by Holtzapple (1981) was used to determine the copper number. In brief, a 10 ml of solution containing 1 part of reagent B (Copper solution – copper (II) sulfate · $5H_2O$ [100 g/l] in DI water) and 19 parts of reagent A (Alkali – sodium carbonate [anhydrous 130 g/l] and sodium hydrogen carbonate [50 g/l] in DI water) was added to a culture tube followed by addition of 0.25 g of dry substrate with a particle size between 125 and 150 μm. Sealed culture tubes were boiled in water for three hours with intermittent mixing. Then, the samples were filtered through a coarse glass crucible, and the tubes were washed with water to remove remaining particles. The crucibles were then washed with a hot solution of water and alkali (1:1 water: reagent A) followed by washing two times with hot water. Furthermore, copper (I) oxide trapped in the substrate was dissolved with two 5 ml portions of reagent C (Ferric iron – $(NH_4)_2SO_4 \cdot Fe_2\,(SO_4)_3 \cdot 24H_2O$ [100 g/l] and 93% sulfuric acid [140 ml/l] in DI water), and the filtrate was collected in Erlenmeyer flasks. The substrate was further washed with 10 ml of reagent F (2 N sulfuric acid), and the filtrate was collected in the same flask. Two to three drops of Ferroin indicator were added to the flasks containing filtrate collected from the previous two steps. This wash was then titrated with 0.01 N of reagent D (Cerric ammonium sulfate, 0.04 N – cerric ammonium sulfate [25.3 g/l] and 93% sulfuric acid [30 ml/l] in DI water), and the volume in ml needed to change color of the wash from pale orange to pale green was noted. Then the copper number was calculated as follows:

$$\text{Copper number} = 0.06354 * \mathrm{v/w}$$

where v is the volume of 0.01 N reagent D (ml) and w is the weight of substrate (g)

2.9. Degree of polymerization

The average degree of polymerization (DPv) of cellulose was measured using a conventional Cannon Fenske viscometer by measuring flow time (up to 1/100 of second) of a solution using a stop watch according to ASTM standard D 1795 (ASTM, 1986). First, with continuous nitrogen flushing, a small[2] amount of dried and pulverized fine powder (particle size <125 μm) of untreated or pretreated corn stover solids containing about 10 mg of glucan was added to a 0.5 M copper diethylene amine (CED; Fisher chemicals, Pittsburgh, PA) solution in a 125 ml Erlenmeyer flask. Then to solublize the solids, the flasks were mixed by shaking the solution for 30 min at 70 °C in a thermostated shaker water bath unit. Unsolublized solids were filtered out from the solution by using a 45 μm Steriflip (Cat SE 1M002M8, Millipore, Billerica, MA) and weighed, and the solublized fraction was determined and used in further calculations by assuming that both solublized and unsolublized fractions had similar compositions.

Nitrogen was flushed through the filtered solution, and the solution intrinsic viscosity (dl/g) was determined according to ASTM standard D 1795 (ASTM, 1986). The cellulose degree of polymerization (DPv) was then calculated based on the intrinsic viscosity values via a relation that takes into account the effect of hemicellulose on viscosity (Heiningen et al., 2004):

$$\mathrm{DP} = ((1.65 * [\eta] - 116 * \mathrm{H})/\mathrm{G})^{1.11}$$

where $[\eta]$ is the intrinsic viscosity (cm^3/g) and H and G are the mass fractions of hemicellulose[3] and glucan, respectively.

The solids were delignified to determine the degree of polymerization of cellulose, DPv, in poplar solids using sodium chlorite and acetic acid because the very low solubility of untreated and pretreated solids in CED. Then the DPv of the delignified solids (<3.0% lignin) was determined by the same procedure as for corn stover except that the filtration step could be eliminated because holocellulose[4] solids were completely solublized in the CED solution. For pure cellulose samples, about 0.1 g of substrate was used with the rest of the procedure the same as for delignified poplar solids.

2.10. Electron spectroscopy of chemical analysis (ESCA)

ESCA surface chemical characterization was carried out using a Physical Electronics PH15400 ESCA electron spectrometer equipped with a non-monochromatic Mg Kα (15 V, 300 W) X-ray source for untreated and pretreated samples of size less than <125 μm. A

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[2] A small amount of solids was taken to minimize the lignin effect on viscosity.

[3] For both feed stocks, xylan was a major hemicellulose component thus only xylan mass fraction was taken into account.

[4] Cellulose + hemicellulose.

250 × 250 μm square area with a take off angle of 45° was analyzed, and peak intensities were determined by peak area integration. Curve fitting to C_1s and O_1s peaks was carried out with a Lorentzian–Gaussian curve-fitting program (Chundawat et al., 2007)

2.11. FTIR-ATR spectroscopic analysis

To investigate and quantify chemical changes in pretreated biomass, a Spectrum One FTIR system (Perkin Elmer, Wellesley, MA) with a universal ATR (Attenuated Total Reflection) accessory was used at Michigan State University, as described in more detail elsewhere (Chundawat et al., 2007). In brief, the samples were pressed uniformly against the diamond surface using a spring-loaded anvil, and mid-IR spectra were obtained by averaging 16 scans from 4000 to 400 cm^{-1} at 2 cm^{-1} resolution. Baseline and ATR corrections for penetration depth and frequency variations were applied using Spectrum One software supplied with the equipment.

2.12. Scanning electron microscopy (SEM)

SEM pictures were taken of untreated and pretreated poplar solids (dried powder of particle size <125 μm) at different magnifications such as 200, 500, 100, 2000, and 3000 times using LEO 982 SEM at 5 kV in the centralized facility at Stevens Institute of Technology, NJ. Prior to taking pictures, the samples were sputter-coated with a thin layer of gold.

3. Results

3.1. Compositional analysis

Compositions, preparation conditions, and severity parameter values are reported in Table 1 for untreated and pretreated solids, and the amount of each component left after pretreatment for both corn stover and poplar are presented in Table 3. Although no direct relation was found between severity level (shown in Table 1) and xylan removal by pretreatment, low pH pretreatment removed a major portion of xylan, and xylan removal increased with severity for a given pretreatment. The high pH pretreatments of ARP and lime removed a large fraction of the biomass lignin, but AFEX, the other high pH approach, removed virtually nothing. Lignin removal during ARP pretreatment was lower for poplar (40%) than corn stover (70%) even though pretreatment severity was higher for the former.

3.2. Acetyl content

The removal of acetyl groups that are side chains on the xylan backbone from biomass has been reported to enhance enzymatic digestion owing to enhanced cellulose/xylan accessibility and enzyme effectiveness (Chang and Holtzapple, 2000; Grohmann et al., 1989; Kim and Holtzapple, 2006; Pan et al., 2006; Rivard et al., 1992; Wood and McCrae, 1986). Mitchell et al. (1990) reported that with increased degree of acetylation, the extent of inhibition of xylanase and debranching enzymes increased. A study in our laboratory showed that acetyl removal enhanced cellulose accessibility and cellulase effectiveness for corn stover hydrolysis with purified CBHI (Kumar and Wyman, 2009a, in press-c). In addition, Selig et al. (2008) in a recent study reported enhanced hydrolysis due to synergism between acetyl xylan esterase and cellobiohydrolase (CBHI). Based on our findings reported elsewhere (Kumar and Wyman, 2009c, in press-c) and other literature reports (Kormelink and Voragen, 1992; Mitchell et al., 1990; Silveira et al., 1999; Suh and Choi, 1996; Tenkanen et al., 1996) that xylooligomers strongly inhibit cellulase activity, we believe that acetylated xylooligomers released during hydrolysis/ pretreatment

Table 3
Yields of major fractions after pretreatment and solids composition for untreated and pretreated corn stover and poplar solids.

Pretreatment		Corn stover						Poplar					
		Yield of component left in pretreated solids, %			Composition of pretreated solids, %			Yield of component left in pretreated solids, %			Composition of pretreated solids, %		
		G	X	L	G	X	L	G	X	L	G	X	L
Untreated		–	–	–	34.4	22.8	18.0	–	–	–	43.8	14.8	29.1
AFEX		100.0	100.0	100.0	34.4	22.8	18.0	100.0	100.0	100.0	46.6	15.0	nd
ARP		98.6	48.1	29.5	61.9	17.9	8.8	93.2	68.2	60.8	57.5	13.5	24.8
Controlled pH		94.1	nd	nd	52.7	16.2	25.2	98.0	42.1	nd	58.8	7.0	32.2
Dilute acid	Sunds system	93.4	27.2	82.0	59.3	9.3	22.5	87.9	8.3	nd	57.3	2.1	46.1
	Parr reactor	94.0	nd	nd	64.4	2.9	26.4						
Flowthrough		–	–	–	–	–	–	80.2	5.7	62	65.6	1.6	33.7
Lime		97.1	nd	nd	56.7	26.4	14.6	98.1	96.2	50.1	53.1	16.8	18.0
SO_2		96.9	nd	nd	56.9	11.6	23.8	96.9	9.3	nd	55.1	2.5	nd

G-glucan, defined in terms of $C_6H_{10}O_5$.
X-xylan, defined in terms of $C_5H_8O_4$.
L-lignin.

Table 4
Acetyl contents for untreated and pretreated corn stover and poplar solids and fraction removed by pretreatment.

Pretreatment		Corn stover		Poplar	
		Acetyl, %	% Removed*	Acetyl, %	% Removed
Untreated		2.511 ± 0.064	NA	3.360 ± 0.208	NA
AFEX		1.694 ± 0.005	32.5	0.772 ± 0.026	77.0
ARP		0.300 ± 0.001	88.1	0.518 ± 0.003	84.6
Controlled pH		1.141 ± 0.055	54.6	0.940 ± 0.000	72.0
DA	Sunds	1.130 ± 0.003	55.0	0.330 ± 0.001	90.2
	Parr	0.202 ± 0.004	91.9	nd	nd
Lime		0.20 ± 0.001	92.0	0.171 ± 0.003	94.9
SO_2-steam explosion		1.154 ± 0.011	54.0	0.582 ± 0.001	82.7

* Amount removed during pretreatment.

may be stronger inhibitors of enzymes than pure xylooligomers, although this hypothesis needs experimental corroboration, (Kabel et al., 2007). The acetyl contents of unpretreated and pretreated solids and the percentage removed during pretreatment reported in Table 4 show that AFEX pretreatment of corn stover and controlled pH pretreatment of poplar removed the least amount of the acetyl portion, whereas lime removed the most for both substrates. Removal of acetyl groups was higher from poplar than from corn stover solids, probably due to greater severity and increased xylan removal. Although acetyl removal increased with severity/ xylan removal for a given pretreatment, no such general relationship was found for all pretreatments. Thus, in addition to time and temperature, the choice of reagents affects acetyl removal.

3.3. Biomass crystallinity

Among several features, crystallinity is believed to significantly affect enzymatic saccharification of glucan (Converse, 1993; Mansfield et al., 1999; Zhang and Lynd, 2004). For real biomass, however, it is very difficult to determine true cellulose crystallinity because conventional X-ray methods measure the crystallinity of the entire material including the hemicellulose and lignin in addition to amorphous cellulose (Kim and Holtzapple, 2006; Zhu et al., 2008). However, thermochemical pretreatments can change cellulose crystalline structure by disrupting inter/intra hydrogen bonding of cellulose chains (Mosier et al., 2005b), and X-ray measurements of crystallinity index (CrI) are still the best option to estimate their impacts on biomass crystallinity. As shown in Fig. 1 and summarized in Table 5 for untreated and pretreated poplar solids, low pH pretreatments significantly enhanced biomass crystallinity, while all high pH pretreatments had less effect and even reduced biomass crystallinity in some instances. This result suggests that amorphous cellulose breaks down more at lower than at higher pH and possibly that higher pH decrystallizes some of the cellulose.

Table 5
Crystallinity index (CrI) values as measured by X-ray method for untreated and pretreated corn stover and poplar solids prepared by leading pretreatment technologies.

Pretreatment	Corn stover	Poplar
Untreated	50.3	49.9
AFEX	36.3	47.9
ARP	25.9	49.5
Controlled pH	44.5	54.0
DA	52.5	50.6
FT	–	60.1
Lime	56.2	54.5
SO_2	–	56.5

The CrI values for corn stover were taken from Laureano-Perez et al. (2005).

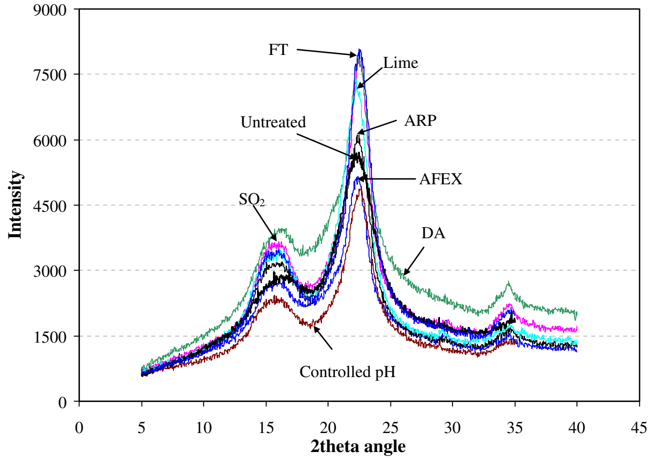

Fig. 1. XRD patterns of untreated and poplar solids pretreated by leading technologies.

3.4. Cellulase adsorption capacity of pretreated solids and enzyme extracted lignin

Cellulase adsorption on cellulose is essential to hydrolysis, and cellulase accessibility to glucan chains is considered to be one of the main factors affecting enzymatic saccharification (Chen and Grethlein, 1988; Esteghlalian et al., 2001; Jeoh et al., 2007; Kumar and Wyman, in press-b, 2008). However, enzyme adsorption onto solids and further their effectiveness can be affected by substrate and enzymes features and physical parameters, as reviewed elsewhere (Kumar, 2008). For example, sugars not only inhibit cellulase catalytic action but adversely affect its adsorption (Kumar and Wyman, 2008), and xylooligomers released during hydrolysis/pretreatment appear to inhibit cellulase and xylanase strongly (Kumar and Wyman, 2009c, in press-c; Suh and Choi, 1996). Furthermore, unproductive binding of enzymes to lignin makes less enzyme available for saccharification (Kumar and Wyman, 2009b, in press-d). Thus, cellulase adsorption capacities for pretreated solids and enzymatic lignin solids prepared by complete carbohydrate removal with enzymes were determined, with detailed descriptions of lignin preparations and determination of cellulase adsorption capacities given elsewhere (Kumar and Wyman, in press-a, 2009a). In brief, lime pretreated corn stover solids and flowthrough pretreated poplar solids had the highest cellulase adsorption capacities, while AFEX pretreated poplar solids and corn stover solids from dilute acid pretreatment with the Sunds reactor had the lowest. It is important to note that AFEX lignin preparations for both poplar and corn stover had the lowest adsorption capacity, suggesting why AFEX can make such efficient use of enzymes even though it removes little lignin. However, lignin prepared by SO_2 and lime for corn stover and poplar, respectively, had the highest cellulase adsorption capacities.

3.5. Copper number

Given the relatively high amount of CBHI protein typically in cellulase mixtures (about 60%), estimation of the number of reducing ends that are preferred active sites for CBHI would seem to provide a rough idea of substrate reactivity, but its role is still ambiguous (Kongruang et al., 2004; Kongruang and Penner, 2004). In this study, we estimated biomass reducing/chain ends using the copper number by Braidy's method as commonly employed in the pulp and paper industry to qualitatively measure the strength of products after bleaching (Hiller and Pacsu, 1946; Montonna and Winding, 1943; Rohrling et al., 2002). Unfortunately, other reducing components and chemicals present in real biomass can interfere with the accuracy of this method, making it quite approximate. For both corn stover and poplar, the copper numbers in Table 6 for AFEX pretreatment were not much different than before pretreatment, consistent with the fact that AFEX leaves all biomass components intact. However, because copper numbers decreased after ARP and lime pretreatment, it appears that these pretreatments remove copper reducing components, consistent with their removing a large portion of biomass and especially lignin and hemicellulose (Chang et al., 1997; Gupta et al., 2008; Kim and Holtzapple, 2005; Kim and Lee, 2005; Kim et al., 2006). Furthermore, solids resulting from low pH pretreatments and Avicel glucan (Zhang and Lynd, 2005) had much higher copper numbers than high pH pretreatments, suggesting larger numbers of

Table 6
Copper numbers inversely indicative of degree of polymerization for pure Avicel glucan, untreated corn stover, and poplar solids, and their pretreated solids resulting from leading pretreatments.

Pretreatment	Corn stover	Poplar
Avicel	6.24 ± 0.07	
Unpretreated	2.29	2.8
AFEX	2.16	2.78
ARP	1.78	2.08
Controlled pH	5.29	nd
Dilute acid	3.84	5.38
Lime	1.14	1.16
SO_2	5.46	5.98

reducing ends were available per gram of biomass. This result implies that greater proportions of endoglucanase to CBHI may be desirable for solids pretreated at high pH than for those from low pH.

3.6. Cellulose degree of polymerization (DPv)

Although the role of glucan chain length is not definitively known, it is believed to affect cellulose hydrolysis (Knappert et al., 1980; Kumar, 2008; Okazaki et al., 1981; Puri, 1984; Treimanis et al., 1998; Zhang and Lynd, 2006). Glucan chain length as represented by degree of polymerization (DP) can be determined in several ways, reviewed elsewhere (Pala et al., 2007; Zhang and Lynd, 2005). However, methods such as using BCA reagents reported by Zhang and Lynd (2005) and commonly used gel permeation (GPC)/size exclusion chromatography (Kleman-Leyer et al., 1996; Pala et al., 2007) are not appropriate for complex lignocellulosic biomass. Thus, to understand the effect of leading pretreatments on cellulose chain length, viscosity average degree of polymerization numbers (DPv), though less precise than number average degree of polymerization (DPn), were determined for corn stover and delignified poplar solids and compared with DPv values for pure glucan substrates such as Avicel, bacterial cellulose (BC), cotton, and filter paper (FP). The relative values of these DPv numbers are more meaningful for lignocellulosic biomass than the absolute numbers and are valuable for comparing effects of leading pretreatments on cellulose chain length.

As shown in Fig. 2a, DPv numbers for pure glucan substrates such as Avicel (~330), RAC (330), bacterial cellulose (~1670), filter paper (~1500), and dewaxed cotton linters (~1950) were in good agreement with those reported in the literature (Väljamäe et al., 1999; Zhang and Lynd, 2004, 2006). In addition, glucan chain lengths in the solids prepared by all pretreatments except AFEX were reduced significantly relative to the untreated corn stover chain length of ~7000. However, low pH pretreatments by dilute sulfuric acid and SO_2 depolymerized the glucan chain to the greatest extent (~65–70% reduction). Unfortunately, no data is available in the literature for raw lignocellulosic biomass for comparison to the results reported here. Dilute acid and steam explosion pretreatments are often reported to reduce cellulose DP to LODP (level off degree of polymerization) values, a bit lower than the values reported here of 2580 for DA-Sunds pretreated corn stover (Heitz et al., 1987). However, DPv for dilute acid corn stover pulp (holocellulose; ~1250) was in agreement with that reported for wood pulp by Zhang and Lynd (2004) and DPv values reported for delignified poplar solids prepared by DA pretreatment were close to the LODP values reported by Heitz et al. (1987). Pretreatment severity, compensation for the effect of hemicellulose, and DPv determination for holocellulose/α-cellulose[5] as opposed to the whole biomass used here may account for these differences (Heitz et al., 1987; Martinez et al., 1997).

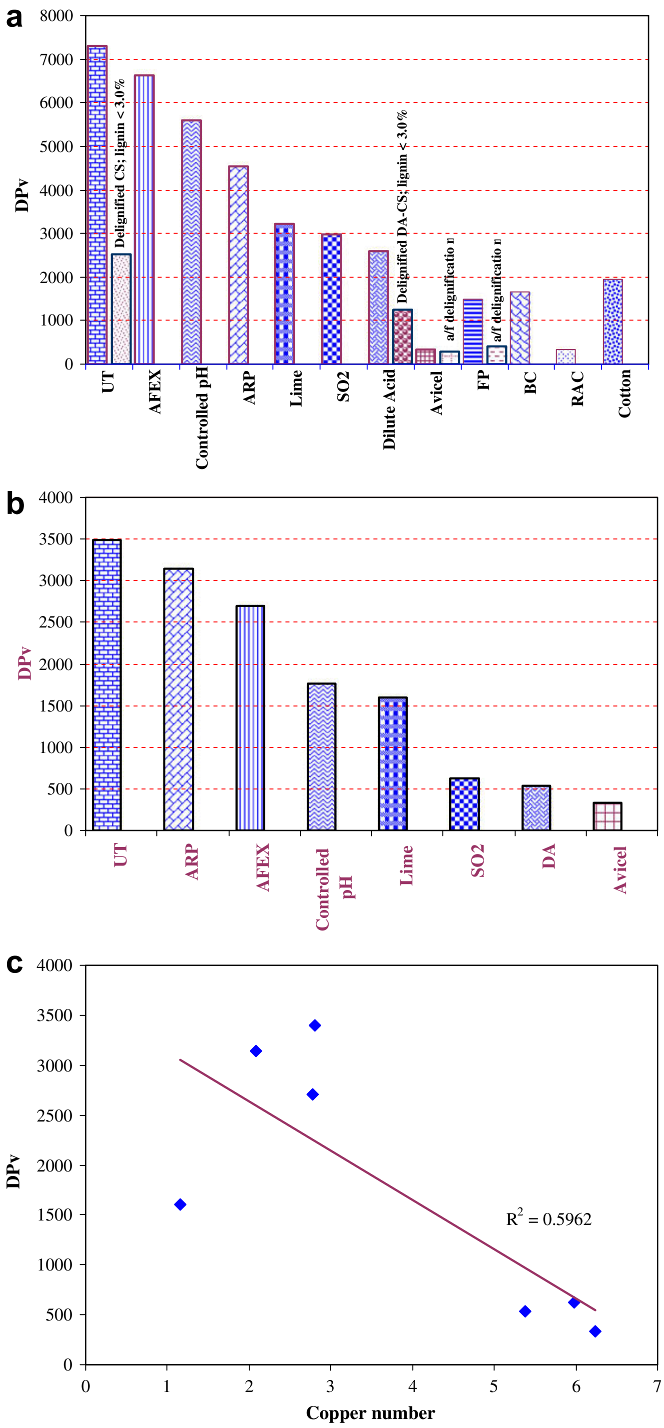

Fig. 2. Cellulose degree of polymerization (DPv) for (a) pure glucan substrates, untreated and delignified corn stover, and pretreated corn stover solids prepared by leading pretreatments and for, (b) delignified unpretreated and pretreated poplar solids and (c) relationship between DPv and copper number for Avicel and delignified poplar solids.

To further validate DPv numbers estimated for whole biomass, DPv was determined for untreated and dilute acid pretreated corn stover holocellulose. Although upon delignification the DPv ratio of untreated and dilute acid pretreated corn stover remained almost constant (~2.5), DPv dropped by about two thirds and by half for untreated corn stover and dilute acid pretreated corn stover,

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[5] Extracted from delignified substrate.

respectively, consistent with the reduction in filter paper DP (from ~1500 to 410), when it was taken through the chlorite delignification step, as shown in Fig. 2a. However, chlorite delignification had little effect on Avicel DP (~15% reduction), probably because it was already close to the LODP. Thus, it appears that chlorite delignification reduces glucan chain length, and DPv numbers reported for whole corn stover should roughly be equal to the actual cellulose chain length.

Viscosity average degree of polymerization for untreated and pretreated delignified poplar solids are shown in Fig. 2b. The untreated poplar glucan chain length (~3500) was greater than for untreated corn stover (~2520). Furthermore, ARP and AFEX pretreatments had a much lower impact on glucan DP than low pH pretreatments, assuming that chlorite delignification had an equal impact on glucan DP for all solids. By comparing DP for delignified dilute acid corn stover (severity- 2.53; DPv- 2520) and dilute acid poplar solids (severity-2.72; DPv- 535), it appears that pretreatment severity affects glucan chain length significantly. Consistent with the finding here, Heitz et al. showed that the holocellulose DP first remained unchanged and then dropped linearly as severity was increased (Heitz et al., 1987). Furthermore, a close to linear relationship (inverse) was observed between DPv and copper number (reducing ends), as shown in Fig. 2c for Avicel glucan and delignified poplar solids.

3.7. ESCA biomass surface characterization

Surface atomic compositions of carbon, nitrogen, and oxygen for the various untreated and pretreated milled poplar and corn stover materials are shown in Table 7. Biomass protein was the main source of nitrogen in untreated poplar and corn stover, and all of the pretreatments except AFEX lowered nitrogen contents due to degradation of protein during pretreatment and removal of the degraded protein by washing. On the other hand, concentrated ammonia in AFEX appears to modify the substrate to nitrogen containing derivatives. Three carbon peaks (C_1, C_2, and C_3) were obtained upon deconvolution of the ESCA carbon spectra, with their assignments given at the bottom of Table 7 (Ahmed et al., 1987; Hongzhang and Liying, 2007). Interestingly for poplar, we see a drop in C_1 and C_3 contributions while C_2 increases, but for corn stover, C_1 drops and C_2 and C_3 increase.

Oxygen to carbon (O/C) ratios for both untreated and pretreated biomass were calculated based on empirical carbon and oxygen compositions on the biomass surface. As reported elsewhere, O/C ratios for different biomass components are placed in the following order: cellulose (~0.83 based on formula) > hemicellulose > lignin (Chundawat et al., 2007; David, 1984). We can clearly see an increase in O/C ratio and decrease in C_1 contribution for all poplar and corn stover pretreatments compared to untreated samples. Furthermore, because lignin constitutes an aromatic structure with more carbon and less oxygen when compared to cellulose, this trend indicates lignin removal from the biomass surface and increased exposure of cellulose (Gustafsson et al., 2003). In addition, because lignin is more hydrophobic than cellulose, hydrophobicity as determined by contact angle measurement (Zhang et al., 2007), decreased with increasing oxygen to carbon ratio, as shown in Fig. 3 for Avicel and untreated and pretreated corn stover solids, (Chirkova et al., 2002).

For poplar, dilute acid and SO_2 pretreatments removed surface lignin more effectively than other pretreatments, with the expectation that this would expose more cellulose to enzymatic hydrolysis. Consistent with this finding, Negro et al. showed enhanced O/C ratios for *Pinus pinaster* wood and reduced hydrophobicity after steam explosion (Negro et al., 2003). For corn stover, AFEX and

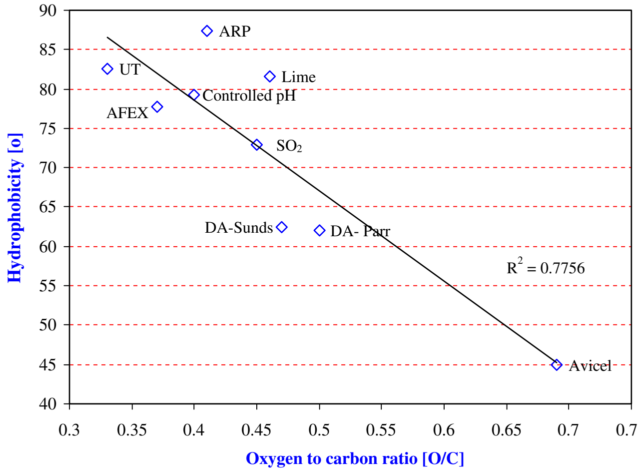

Fig. 3. Relationship between hydrophobicity and oxygen to carbon (O/C) ratio for Avicel and untreated and pretreated corn stover solids.

Table 7
Relative amounts of nitrogen and carbons content and oxygen to carbon ratios for untreated and poplar and corn stover solids resulting from leading pretreatments.

Substrate	Pretreatment	Nitrogen, %	C_1, %	Reduction in C_1, %	C_2, %	Increase in C_2, %	C_3, %	Oxygen to carbon ratio, O/C	Increase in O/C ratio, %
Corn stover	Untreated	1.8	62.0	–	29.9	–	8.0	0.33	–
	AFEX	2.0	55.6	10.3	35.7	19.4	8.8	0.37	12.1
	ARP	0.9	43.1	30.5	38.5	28.8	8.4	0.41	24.2
	Controlled pH	1.1	52.9	14.7	38.6	29.1	8.4	0.40	21.2
	Dilute acid-Sunds	0.8	47.3	23.7	43.8	46.5	9.0	0.47	42.4
	Dilute acid-Parr	0.7	45.1	27.3	45.7	52.8	8.3	0.50	51.5
	Lime	0.6	48.6	21.6	42.2	41.1	9.2	0.46	39.4
	SO_2	0.9	49.9	19.5	41.9	40.1	8.2	0.45	36.4
Poplar	Untreated	0.7	61.9	–	29.8	–	8.3	0.30	–
	AFEX	2.5	58.1	6.1	35.8	20.1	6.1	0.36	20.0
	ARP	1.0	59.4	4.0	34.2	14.8	6.4	0.33	10.0
	Controlled pH	0.5	59.4	4.0	34.2	14.8	6.4	0.35	16.7
	Dilute acid	–	53.8	13.1	39.3	31.9	7.0	0.39	30.0
	Flowthrough	0.5	55.1	11.0	37.6	26.2	7.2	0.35	16.7
	Lime	–	61.0	1.5	32.3	8.4	6.7	0.33	10.0
	SO_2	0.3	49.1	20.7	43	44.3	7.9	0.43	43.3
	Avicel	0.55	18.7		65.4		15.9	0.69	

C1 corresponds to class of carbon that corresponds to carbon atoms bonded to carbon or hydrogen (C–C).
C2 corresponds to class of carbon atoms bonded to single non-carbonyl oxygen (C–O).
C3 corresponds to class of carbon atoms bonded to a carbonyl or two non-carbonyls (C=O or O–C–O).

dilute acid pretreatment with the Parr reactor showed the lowest (12%) and highest (51.5%) increases in O/C ratios, respectively (Fig. 3 and Table 7), while other pretreatments increased O/C ratios moderately. Low pH pretreatments increased the O/C ratio for poplar more than for corn stover and also more than high pH pretreatments, suggesting that the effect of these leading pretreatments varied with substrate. Moreover, the fact that low pH, high pH, and neutral pH pretreatments remove different components could account for the variation in O/C ratios.

3.8. FT-IR analysis

Normalized FT-IR spectra at a band position of 1510 (Chundawat et al., 2007) are presented in Figs. 4 and 5 for pretreated corn stover solids and poplar solids, respectively, and relative changes in chemical compositions of the solids from leading pretreatments along with assignment of band positions are shown in Table 8 and Table 9 for corn stover and poplar solids, respectively. The peak near 3348 cm^{-1} for the latter represents OH stretching, and lime pretreatment for corn stover and flowthrough for poplar had the greatest reduction in intensity of this peak, indicating that hydrogen bonds of cellulose were disrupted (He et al., 2008). The band position at 2900 cm^{-1} is attributed to C–H stretching, and lime pretreatment for corn stover and flowthrough for poplar again produced the greatest degree of change for each substrate, indicating that the methyl and methylene portions of cellulose were ruptured. ARP and lime for poplar and corn stover, respectively, reduced the 1745 cm^{-1} intensity associated with carbonyl C=O stretching the most, indicating more cleavage of lignin side chains (He et al., 2008; Windeisen et al., 2007). Lime and AFEX pretreatments for corn stover and ARP and AFEX pretreatments for poplar had the highest reduction in the 1720 cm^{-1} band attributed to hemicellulose acetyl and uronic ester groups or linkages in lignin and/or ester hemicellulose feurilic and p-coumaric acid carboxylic groups (Sun et al., 2005). Consistent with the literature, SO_2 catalyzed steam explosion for poplar solids and lime pretreatment for corn stover had the greatest reduction in the 1595 cm^{-1} band position assigned to aromatic skeletal vibration, and reduction or shift in this position is attributed to condensation reactions and/or splitting of lignin aliphatic side chains (Sun et al., 2005). Lime and dilute acid pretreatments for poplar and lime and AFEX/DA-Parr pretreatments for corn stover reduced the intensity of the 1245 cm^{-1} band attributed to the cleavage and/or alterations of acetyl groups the most (Sun et al., 2005; Windeisen et al., 2007). Although dependent on conditions, pretreatments can alter the ratio of amorphous to crystalline cellulose associated with the ratio of intensities at the bands 900 cm^{-1} and 1098 cm^{-1} (Laureano-Perez et al., 2005) as well as probably the crystalline cellulose polymorphs (Iα/Iβ) ratio measured by comparing the intensity of 750 cm^{-1} and 710 cm^{-1} bands (Boisset et al., 1999; Tokoh et al., 2002; Wada and Okano, 2001). However, such changes in cellulose crystallinity for low pH pretreatments that remove biomass components are difficult to determine using X-ray crystallography.

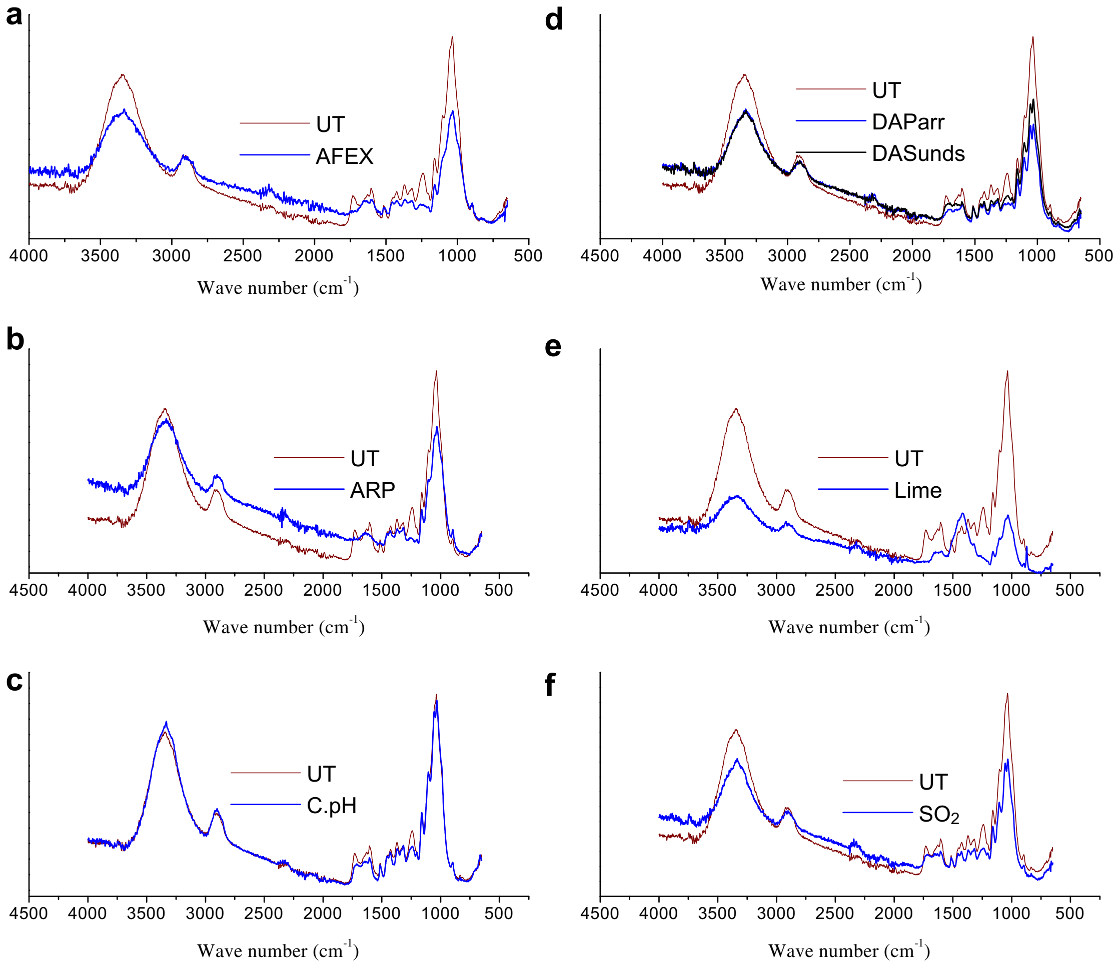

Fig. 4. Chemical changes in corn stover solids, as determined by FTIR-ATR, after (a) AFEX, (b) ARP, (c) controlled pH, (d) dilute acid with the Sunds and Parr, (e) lime, and (f) SO_2 pretreatments.

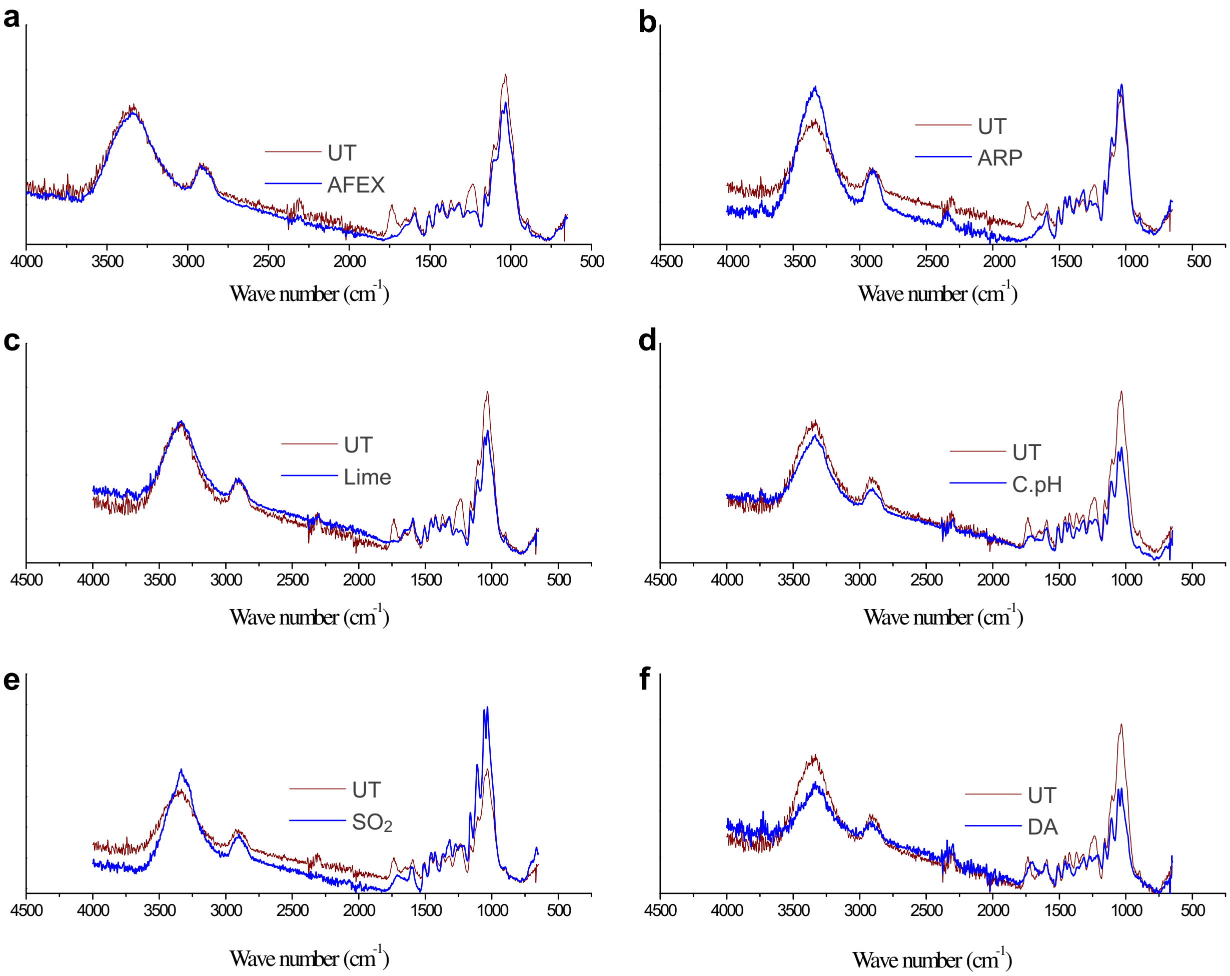

Fig. 5. Chemical changes in poplar solids, as determined by FTIR-ATR, after (a) AFEX (b) ARP (c) lime (d) controlled pH (e) SO_2 and (f) dilute acid pretreatments.

Table 8
Relative changes in corn stover solids after leading pretreatments[*].

Band position	Assignment	Pretreatment							
		UT	AFEX	ARP	C. pH	DA-Sunds	DA-Parr	Lime	SO_2
3348	O–H stretching (indicates rupture of cellulose hydrogen bonds)		18.40	6.24	−2.80	18.28	17.37	42.68	15.87
2900	C–H stretching (related to rupture of methyl/methylene group of cellulose)		0.38	−12.37	−3.91	4.16	3.98	26.75	3.17
1745	Carbonyl bonds (associated with lignin side chain removal)		11.51	2.44	14.79	7.75	15.47	31.58	8.76
1720	Carboxylic acids/ester groups		15.38	8.37	10.94	6.52	15.41	35.82	7.69
1595	Aromatic ring stretch (associated with lignin removal)		9.64	11.63	13.58	14.28	16.53	29.27	13.79
1260	Ester absorbance (related to removal of uronic acid)		24.23	23.45	11.53	18.50	23.89	41.82	19.71
1245	C–O adsorption (resulting from acetyl groups cleavage)		29.48	29.19	14.99	21.66	29.11	47.32	23.33
1059	C–O stretch		29.58	21.96	−0.04	20.91	31.19	58.20	23.59
1098/900[a]	Amorphous to crystalline cellulose ratio	0.46	0.61	0.62	0.46	0.51	0.53	0.66	0.51
750/710	Ratio of crystalline cellulose polymorphs (Iα/Iβ)	0.88	0.93	0.93	0.86	0.91	0.88	0.89	0.89
900	Removal of amorphous cellulose		−0.63	−11.20	0.01	9.40	17.64	33.53	14.92

[*] %Relative change = 100*(intensity of UT solids − intensity of pretreated solids)/ intensity of UT solids; where positive number indicates reduction.
[a] The ratio of intensity at two band positions; C.pH – controlled pH.

3.9. Scanning electron microscopy

SEM images of poplar solids pretreated by leading pretreatments are shown in Fig. 6 at different magnifications. It appears that AFEX pretreatment did not disrupt microfibrils much and had a lower impact on particle size compared to other pretreatments (Fig. 6a). However, although not certain, some lignin droplets appear to be present on the surface of AFEX pretreated solids at a 3000 magnification, suggesting that some lignin melted during AFEX pretreatment and agglomerated on the surface. These results are consistent with reports by Chundawat et al. (2007) that carbon rich components (lignin) were found on the surface after AFEX pretreatment. SEM images for ARP pretreated solids reveal formation of some holes on the biomass surface and disruption of the biomass network consistent with hemicellulose and lignin removal during pretreatment, as shown in Fig. 6b (Kim and Lee, 2005). Controlled pH pretreatment seemed to disrupt the biomass structure to some extent, while dilute acid reduced fiber length and totally

Table 9
Relative chemical changes in poplar solids after leading pretreatments*.

Band position	Assignment	Pretreatment							
		UT	AFEX	ARP	C.pH	DA	FT	Lime	SO_2
3348	O–H stretching (indicates rupture of cellulose hydrogen bonds)		1.71	−23.10	6.57	4.98	12.61	−2.64	−15.94
2900	C–H stretching (related to rupture of methyl/methylene group of cellulose)		1.58	0.81	8.31	0.17	5.73	−1.53	3.94
1745	Carbonyl bonds (associated with lignin side chain removal)		32.76	48.34	21.67	11.45	12.79	20.82	38.58
1720	carboxylic acids or ester groups		27.29	43.15	12.96	4.90	−0.76	17.96	22.96
1595	Aromatic ring stretch (associated to lignin removal)		6.55	10.65	9.04	5.68	6.47	−3.22	13.36
1260	Ester absorbance (related to removal of uronic acid)		19.03	12.77	17.90	20.90	15.31	23.54	−1.70
1245	C–O adsorption (resulting from acetyl groups cleavage)		23.67	20.52	20.94	26.84	19.50	28.86	2.97
1059	C–O stretch		11.16	−11.37	22.31	36.98	23.32	15.86	−51.95
1098/900	Amorphous to crystalline cellulose ratio	0.51	0.52	0.43	0.50	0.57	0.62	0.58	0.38
750/710[A]	Ratio of crystalline cellulose polymorphs (Iα/Iβ)	0.88	0.87	0.81	0.85	0.93	0.91	0.88	0.74
900	Removal of amorphous cellulose		−1.94	16.45	2.53	−11.78	−20.57	−12.33	26.45

[*] %Relative change = 100*(intensity of UT solids – intensity of pretreated solids)/ intensity of UT solids; where positive number indicates reduction.
[A] The ratio of intensity at two band positions; C.pH – controlled pH.

disrupted the biomass structure, as shown in Fig. 6c and Fig. 6d, respectively. Flowthrough and lime pretreatments both removed a large proportion of hemicelluloses and lignin (Chang et al., 1997; Kaar and Holtzapple, 2000; Liu and Wyman, 2003, 2004, 2005), resulting in formation of many apertures of various sizes on the surface and reduced the fiber length, as shown in Fig. 6e and Fig. 6f. SEM images in Fig. 6g for SO_2 pretreated solids show that fibrils were shortened and resembled SEM images for pure cellulose reported in literature (Wang et al., 2006; Zhao et al., 2007).

4. Discussion

Table 10 summarizes the physical and chemical features characterized in this first-of-a-kind study of solids produced by application of leading pretreatments to shared sources of corn stover and poplar wood, the expected impacts on sugar release by enzymatic hydrolysis based on literature reports, and the impact of each pretreatment on these features. As noted, AFEX and lime pretreatments removed the least and most acetyl groups, respectively, from the xylan backbone for both substrates. Although acetyl removal did not appear to be related to xylan removal for all pretreatments, it is apparent that higher xylan removal would result into greater acetyl removal. Because acetyl side chains on the hemicellulose backbone may interfere with xylanase activity (Fernandes et al., 1999; Mitchell et al., 1990), its removal may reduce the amount of enzyme, and particularly accessory enzymes for debranching, required for high yields.

As expected, pretreatments at low pH removed a major portion of the hemicellulose, and pretreatments at high pH other than AFEX removed mostly lignin. However, the extent of removal of each of these components varied with substrate, pretreatment choice, and the harshness of the conditions employed. Although the majority of studies reported in the literature showed that removal of either xylan or lignin or both is necessary for effective cellulose saccharification, we believe that removal of either or both serves one purpose: “disruption of carbohydrate-lignin networking, which enhances enzymes adsorption, generally labeled as accessibility, and their effectiveness due to reduced inhibition by xylooligomers and unproductive binding with lignin.”

On the other hand, high pH pretreatments such as lime that removed lignin could enhance the biomass crystallinity index by leaving a larger fraction of crystalline cellulose. However, as shown in Table 10, both ammonia pretreatments reduced crystallinity, even though ARP removed a significant portion of amorphous xylan and lignin, consistent with reports in the literature (Gollapalli et al., 2002; Igarashi et al., 2007; Nechwatal and Nicolai, 2003; Wadsworth et al., 1979). Because lignin and xylan are both amorphous, their removal increased the crystallinity index, however, the drop in lignin and hemicellulose content improved enzyme access to cellulose and reduced non-productive binding of enzyme, enhancing cellulose digestion by enzymes despite an increase in crystallinity index (Kumar and Wyman, 2009c; Lynd et al., 1996; Wyman et al., 2005a). In another study, we found that lignin removal benefits enzymatic hydrolysis of xylan more than glucan, with the resulting xylan removal apparently exposing more glucan (Kumar and Wyman, in press-a, 2009a). In addition, reduction in cellulose crystallinity during pretreatment could enhance cellulose digestion due to greater enzyme adsorption. Consistent to this, AFEX removes very little hemicellulose or lignin, the drop in crystallinity index observed for AFEX pretreated corn stover solids may be mostly attributed to generation of more amorphous cellulose, resulting in greater cellulose digestion. This interpretation is reinforced by the much smaller change in crystallinity and much lower digestibility of AFEX pretreated poplar solids compared to corn stover, as reported elsewhere (Kumar and Wyman, 2009c, in press-d; Wyman et al., 2005a).

Overall, the results from this study suggest that crystallinity index provides a composite picture of overall biomass crystallinity that includes changes in composition and is not an accurate representation of what effect pretreatments have on just cellulose crystallinity. In contrast to the crystallinity index data in Table 10, the ratios of amorphous to crystalline cellulose in Table 8 and Table 9 measured by FT-IR show that all pretreatments reduced cellulose crysallinity, with the extent dependent on the substrate type and pretreatment used. This data suggests that FT-IR may be a better tool for measuring changes in just cellulose crystallinity after pretreatment than crystallinity index measurements.

As summarized in Table 10, low pH pretreatments decreased cellulose DPv, and it appears that a reduction in cellulose chain length accompanies xylan removal for low pH systems. Yet data for lime and ARP pretreatments suggest that lignin removal had a less severe impact on cellulose chain length than xylan removal, supporting our hypothesis that lignin controls xylan accessibility which in turn controls cellulose accessibility. On the other hand, ammonia pretreatments had the least impact on this measure of cellulose chain length. Furthermore, chlorite delignification for holocellulose preparation reduced cellulose DP significantly, but the degree depended on the initial cellulose DP. Overall, chain length reduction, crystallinity increase, and xylan/lignin removal appear more or less interdependent for thermochemical pretreatments, and determining their individual impacts on enzymatic saccharification is not yet possible, as reported by others (Chang and Holtzapple, 2000; Jeoh et al., 2005; Ucar and Fengel, 1988; Yang and Wyman, 2004).

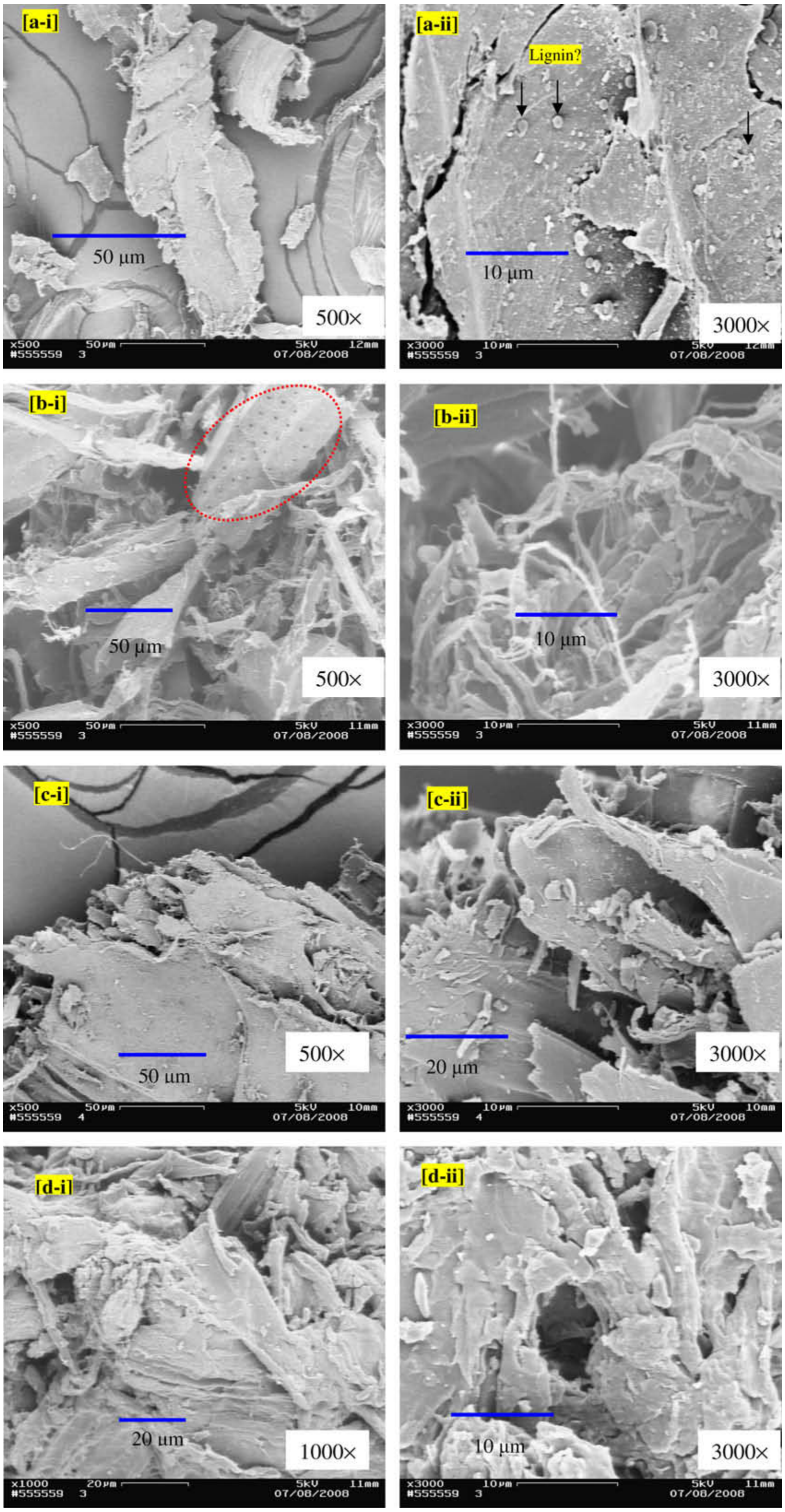

Fig. 6. SEM images at various magnifications for poplar solids prepared by leading pretreatments of [a] AFEX at 500× (i), 3000× (ii), [b] ARP at 500× (i), 3000× (ii), [c] controlled pH at 500× (i), 3000× (ii), [d] dilute acid at 1000× (i), 3000× (ii), [e] flowthrough at 500× (i), 1000× (ii), (iii) 2000× (iii), [f] lime at 500× (i), 3000× (ii), and [g] SO_2 at 200× (i), 500× (ii), and 2000× (iii).

ESCA analysis showed that all pretreatments effectively removed lignin from the biomass surface as measured by surface oxygen to carbon ratios, resulting in the expected increase in cellulose exposure and suggesting a reduction in surface hydrophobicity. However, the increase in O/C ratio is less for AFEX pretreatment of corn stover at moderate temperatures (90 °C) compared to other pretreatments at higher temperatures (150–185 °C). The data here also showed that water washing of

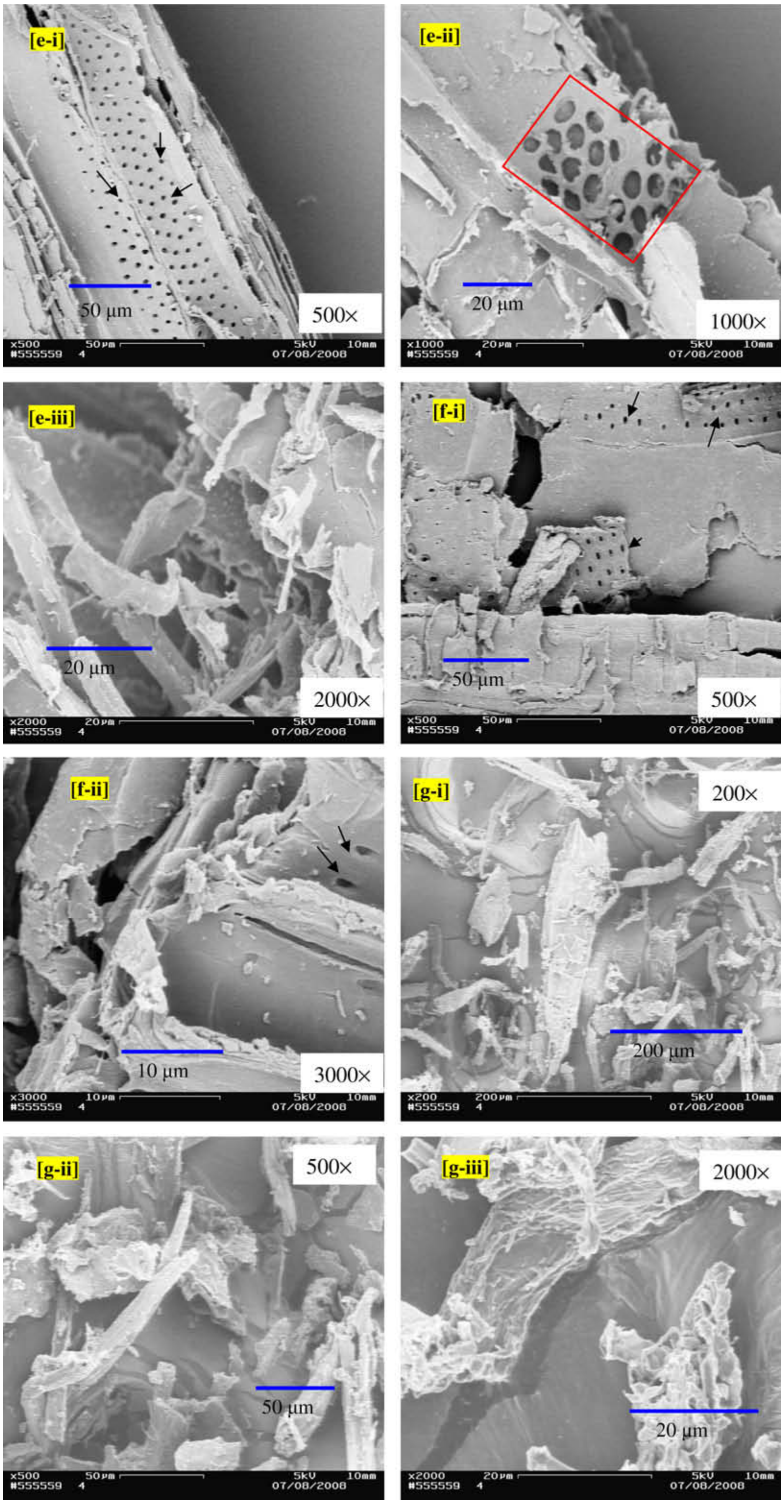

Fig. 6 (*continued*)

pretreated biomass removed most of the lignin as identified by more carbon and less oxygen compared to cellulose and other extractives, thereby exposing more cellulose (Gustafsson et al., 2003). On the other hand, for unwashed AFEX treated corn stover, a decrease in O/C ratio was shown elsewhere (Chundawat et al., 2007), due to deposition of soluble lignin components and other degradation products on the biomass surface.

Cellulase adsorption experiments reported elsewhere (Kumar and Wyman, in press-a, 2009a) showed that lime and flowthrough pretreatments had the highest surface area, and AFEX lignin preparations for both substrates had the lowest cellulase adsorption capacity, consistent with suggestions in the literature that ammonia alters lignin (Sewalt et al., 1996; Weimer et al., 1986). ARP pretreatment may not have the same impact as a result of using ammonia hydroxide instead of non-aqueous ammonia in AFEX or differences

Table 10
Summary of changes in important biomass features for corn stover and poplar solids following leading pretreatments.

Pretreatment	Acetyl groups removal, %		% Xylan removal		% Lignin removal[a]		% Change in biomass crystallinity		% Reduction in Cellulose DP		% Increase in surface O/C carbon ratio	
	Likely impact of the parameters on glucan/xylan hydrolysis,+ means a positive impact											
	+/+		+/NA		+/+		Inconclusive[b]		+/NA		+/+	
	CS	POP	CS	POP	CS	POP	CS	POP	CS	POP	CS	POP
SO_2	54	82.7	>60	91	ND		–	13.2	59.3	82.0	36.4	43.3 [H]
Dilute acid[c]	55–92	90.2	70–95	>90	5–18	ND	4.4	1.4	64.6 [H]	84.7 [H]	42–52 [H]	30
Flowthrough	–	–	>85	94.3 [H]	ND	38	–	20.4	–	ND	–	16.7
Controlled pH	54.6	72 [L]	–	57.9	ND	ND	−11.5	8.2	23.5	49.3	21.2	16.7
Lime	92 [H]	94.9 [H]	ND	3.8	70.5	49.9	11.7	9.2	56.0	54.0	39.4	10 [L]
ARP	88.1	84.6	52	31.8	ND	39.2	−48.5	−0.8	37.9	10.0 [L]	24.2	10
AFEX	32.5 [L]	77	0.0	0 [L]	0.0	0.0	−27.8	−4.0	9.2 [L]	22.5	12.1 [L]	20

% Change values of features are relative to untreated substrate; NA – not applicable; ND – not determined; H – highest; L – lowest.

[a] Similar to major changes in other biomass features, lignin removal and especially xylan removal depends on pretreatment severity.

[b] See discussions.

[c] For corn stover dilute acid pretreatment data for both type of reactor system, Sunds and Parr reator, were combined

in the amount of water used in these two pretreatments. Copper numbers results reported in this paper qualitatively suggested that all pretreatments except AFEX and lime increased the number of reducing ends, in agreement with the changes in cellulose DP measured here. In addition, FT-IR data showed that pretreatment alters the crystalline cellulose polymorphs ratio ($I\alpha/I\beta$), with the greatest enhancements by AFEX pretreatment of corn stover (~6%) and dilute acid pretreatment of poplar (~5.8%). The literature generally indicates that cellulase hydrolyzes crystalline cellulose $I\alpha$ (Hayashi et al., 1997a,b) with greater effectiveness (glucose production per amount of enzyme adsorbed) than for cellulose $I\beta$ (Igarashi et al., 2006a,b), although some studies report that both fractions ($I\alpha$ and $I\beta$) are depleted at the same rate (Boisset et al., 1999).

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