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Cellulose–hemicellulose interactions at elevated temperatures increase cellulose recalcitrance to biological conversion†

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It has been previously shown that cellulose-lignin droplets' strong interactions, resulting from lignin coalescence and redisposition on cellulose surface during thermochemical pretreatments, increase cellulose recalcitrance to biological conversion, especially at commercially viable low enzyme loadings. However, information on the impact of cellulose–hemicellulose interactions on cellulose recalcitrance following relevant pretreatment conditions are scarce. Here, to investigate the effects of plausible hemicellulose precipitation and re-association with cellulose on cellulose conversion, different pretreatments were applied to pure Avicel® PH101 cellulose alone and Avicel mixed with model hemicellulose compounds followed by enzymatic hydrolysis of resulting solids at both low and high enzyme loadings. Solids produced by pretreatment of Avicel mixed with hemicelluloses (AMH) were found to contain about 2 to 14.6% of exogenous, precipitated hemicelluloses and showed a remarkably much lower digestibility (up to 60%) than their respective controls. However, the exogenous hemicellulosic residues that associated with Avicel following high temperature pretreatments resulted in greater losses in cellulose conversion than those formed at low temperatures, suggesting that temperature plays a strong role in the strength of cellulose–hemicellulose association. Molecular dynamics simulations of hemicellulosic xylan and cellulose were found to further support this temperature effect as the xylan–cellulose interactions were found to substantially increase at elevated temperatures. Furthermore, exogenous, precipitated hemicelluloses in pretreated AMH solids resulted in a larger drop in cellulose conversion than the delignified lignocellulosic biomass containing comparably much higher natural hemicellulose amounts. Increased cellulase loadings or supplementation of cellulase with xylanases enhanced cellulose conversion for most pretreated AMH solids; however, this approach was less effective for solids containing mannan polysaccharides, suggesting stronger association of cellulose with (hetero) mannans or lack of enzymes in the mixture required to hydrolyze such polysaccharides.

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Background

To overcome lignocellulosic biomass natural recalcitrance to biological conversion, thermochemical pretreatments, such as those employing dilute sulfuric acid, are often applied.[1] Although high severity dilute sulfuric acid pretreatment improves cellulose digestion, it suffers from hemicellulosic sugar losses.[2] Pretreatments applying only water (hydrothermal) and ammonia are attractive alternatives;[3] however, the solids produced by hydrothermal (*e.g.*, steam explosion without catalyst), ammonia (soaking in aqueous ammonia-SAA; ammonia fiber expansion- AFEX), and low severity dilute sulfuric acid pretreatments often contain a significant amount of residual hemicelluloses and are less enzymatically digestible than the pretreated biomass obtained from dilute acid and other low pH options, such as SO_2 pretreatment, applied at more severe conditions.[4–6] The protective sheathing provided by residual hemicelluloses is often considered responsible for low cellulose digestion for solids prepared by such pretreatments.[7,8]

Based on recent studies, it appears that above a certain pretreatment temperature/severity hemicelluloses (or their shorter fragments) diffuse and move from the plants secondary cell wall to the compound middle lamella and/or to the cell corner and (subsequently) dissolve.[9–11] The dissolved fragments of hemicellulose can adsorb back on cellulose upon/during cooling. Nevertheless, lignin is also believed to relocate upon thermochemical pretreatments,[12] and thus, xylan migration may be a result of lignin relocation since the two polymers can be covalently bonded.[13,14] However, this phenomenon has never been studied in detail. Further, not discussed much in the pretreatment literature, hemicelluloses softening temperature (glass transition temperature, T_g) is reported to be in the temperature range of typically applied for biomass pretreatments, 140 °C to 220 °C. Further, the hemicellulose T_g is greatly influenced by the moisture content and branching, with side groups reported to decrease T_g.[15–18] The reductions in hemicellulose molecular weight that takes place during pretreatment would also reduce the T_g, and increase the mobility of the polymers. Similar to what has been observed with lignin, hemicelluloses are expected to relocate at temperatures close to their softening point, and after relocation may re-associate with carbohydrates and other polymers upon/during cooling.[11]

Hemicellulosic oligomers solubilized during pretreatments can also precipitate out of solution due to reductions in temperature[19] and pH neutralization[20] and may reassociate with cellulose. The likelihood of oligomer precipitation and agglomeration is dependent on oligomer molecular weight, with higher DP (degree of polymerization) oligomers more likely to precipitate than lower DP.[19] Association with side-chains and other polymers can also influence precipitation, as the presence of uronic acids and lignin has been shown to limit agglomeration.[20] The strength of readsorption of the hemicelluloses onto cellulose is dependent on a number of factors including the type of hemicellulose and the presence of side-chains. Previous studies have observed that xylans tend to adsorb less strongly to cellulose than mannans,[21,22] and the presence and pattern of side chain substitutions on the hemicellulosic backbone reduces strength of adsorption.[23,24]

These relocalized and precipitated hemicelluloses may associate with cellulose more strongly than the non-mobilized hemicelluloses naturally present in biomass and may require higher cellulase loadings and/or supplementation of cellulase with higher loadings of hemicellulases and other accessory enzymes to increase cellulose conversion.[25,26]

Overall, very little information has been developed to determine the impact of relocalized hemicelluloses resulting from precipitation and/or migration on cellulose recalcitrance. In this vein, we recently showed that when dilute acid pretreatment was applied to a mixture of Avicel and beechwood xylan at 140 °C for 30 min in 1 wt% acid, the digestibility of the resulting pretreated solids containing miniscule amount of xylan (<2 wt%) was significantly reduced (>20% relative drop) compared to the control pretreated Avicel alone.[2] Furthermore, cellulase supplementation with xylanase enhanced glucose yields, confirming that residual xylan, probably resulting from precipitation and reassociation with cellulose, caused the drop in cellulose conversion. Thus, based on this finding and indirect evidence from the literature, we hypothesize that relocalized and precipitated hemicelluloses make cellulose more recalcitrant to biological conversion than native/non-mobilized hemicelluloses.

In this study, to understand the impact of relocalized hemicelluloses on cellulose recalcitrance, hydrothermal, low severity dilute acid, and ammonia pretreatments were applied to Avicel cellulose alone and Avicel mixed with various hemicelluloses (beechwood xylan, birch wood xylan, guar gum galactomannan, or konjac glucomannan). These model compounds were used in this study to avoid obfuscation of conclusions that may result due to the complexity of real biomass. The resulting pretreated solids were enzymatically hydrolyzed at low to high enzyme loadings. For comparison, cellulose physically mixed with hemicelluloses and their oligomers and lignin free holocelluloses containing non-mobilized hemicelluloses were also enzymatically hydrolyzed. Atomistic molecular dynamics computer simulations provide an interpretation of the mechanism of the drop in cellulose conversion.

Materials and methods

Substrates, reagents, and enzymes

Avicel® PH 101 cellulose (>98% purity, Lot No. BCBD6923 V, Fluka), α-cellulose (Lot No. 050 M0140 V), beechwood xylan (BWX, >70% purity, Lot No. BCBS8393 V), birch wood xylan (BIWX, 75.5% purity, Lot No. BCBS8393 V), and guar gum galactomannan (GalM; Lot No. 041 M0058 V) were purchased from Sigma-Aldrich (St Louis, MO, US). Konjac roots derived glucomannan (GluM) powder, a product of Konjac Foods, Sunnyvale, CA, was purchased through Amazon.com. Oligomers were prepared by hydrothermal pretreatment of

10 wt% BWX solids at 200 °C for 15 min.[27] Dacotah switchgrass (*P. virgatum*; $\frac{1}{4}$ inch) and poplar ($\frac{1}{4}$ inch) were generously provided by Ceres, Inc. (Thousand Oaks, CA) and Dr Venkatesh Balan originally at GLBRC, Michigan State University, MI, respectively. Corn stover ($\frac{1}{4}$ inch) was kindly provided by National Renewable Energy Laboratory (NREL) in Golden, Colorado. Pine sawdust (PSD) was obtained from Dr Chan Park's laboratory at the Center for Environmental Research and Technology (CE-CERT), University of California, Riverside. Accellerase®1500 cellulase (Protein content- 82 ± 5 mg ml^{-1}, Batch No.1681198062) and Multifect® Xylanase (Protein content- 42 ± 5 mg ml^{-1}, Lot No. 301-04021-015) were generously provided by DuPont™ Industrial Biosciences (formerly Genencor International), Palo Alto, CA. The enzymes protein content was determined by applying the standard BCA assay and using bovine serum albumin as a standard.[28] Sulfuric acid (72 wt%; Ricca), un-stabilized sodium chlorite (80% purity, Acros Organics, Lot No. B0130453), and ammonium hydroxide (28–30 wt%, Acros Organics) were purchased from Fisher Scientific. Other reagent grade chemicals, unless stated otherwise, were purchased either from Fisher Scientific (Pittsburgh, PA) or Sigma-Aldrich (St Louis, MO).

Pretreatments

Hydrothermal and low severity dilute sulfuric acid pretreatments were performed in a 1L high pressure Hastelloy Parr reactor (Parr Instruments, Moline, IL, USA) with a total working mass of 800 g. More information on the reactor and its operation is provided in detail in our previous publications.[29,30] The Avicel cellulose loading was 5 wt% (40 g dry basis), and xylan or other hemicelluloses were added to cellulose at a weight ratio of 0.5 (20 g) or 0.25 (10 g), respectively, representative of the typical ratio for natural lignocellulosic biomass.[31,32] The heating time of between 3 and 4 min to reach the desired reaction temperature was not included in the stated reaction time. The severity factor for hydrothermal and the combined severity factor for dilute acid pretreatments were employed to estimate the overall effects of time, temperature, and acid concentration on the extent of pretreatment[33,34] and are defined as follows:

$$\text{Severity factor (SF)} = \log R_o;\ R_o = t \times \exp\left[(T - 100)/14.75\right];$$

$$\text{Combined severity factor (CSF)} = \text{SF} - \text{pH}$$

where t is the reaction time in min, T is the pretreatment temperature in °C, and pH is the theoretical pH of pretreatment hydrolysate calculated based on initial acid concentration. Hydrothermal pretreatments were run at 180 °C for 30 min (SF- 3.83) and 200 °C for 15 min (SF- 4.12), and dilute acid pretreatments were conducted at 140 °C in 1 wt% sulfuric acid for 30 min (CSF-1.94) and at 160 °C in 0.5 wt% sulfuric acid for 10 min (CSF-1.78). Soaking in aqueous ammonia (SAA) pretreatments were applied to Avicel alone and mixed with BWX (cellulose to BWX wt ratio = 2) in high pressure 500 mL Pyrex® bottles (Fisher Scientific, Pittsburgh, PA) at 70 °C for 24 h in 15 wt% NH_4OH (liquid to solids ratio = 10; solids loading ~9.1 wt%). The bottles were intermittently mixed by shaking by hand.

Following pretreatment, the Parr reactor and Pyrex bottles were cooled to below 50 °C in a room temperature water bath and by air cooling, respectively, and the slurries were immediately transferred to 500 ml centrifuge bottles (Catalog No. 14-375-359, Fisher Scientific, Pittsburgh, PA). The bottles were centrifuged at 10 000 rpm for 10 min in a Beckman floor centrifuge (Model No. J2-21, Beckman Coulter, Inc., Brea, CA) to separate solids from liquid followed by washing repeatedly with room temperature deionized (DI) water until the pH of supernatant pH was close to neutral. The washed solids were collected from the centrifuge bottles and stored in Ziplock® bags at 4 °C for further experiments.

Ammonia Fiber Expansion (AFEX) pretreatments of Avicel alone and mixed with BWX (cellulose to BWX wt ratio = 2) and Avicel mixed with GluM (cellulose to GluM wt ratio = 4) were performed at Michigan State University, MI. The AFEX™§ reaction conditions were at the ammonia: water ratio for conventional AFEX pretreatment of corn stover (1 : 0.6). These pretreatments were conducted at 60 °C in a 300 ml 316 stainless steel Parr reactor in 25 g batches, each with a 1.5 : 1 g NH3 : g dry sample ratio and 0.9 : 1 g H_2O : g dry sample ratio for a 15 min residence time after addition of ammonia to the vessel. Each sample was mixed with water prior to loading in the reactor. Ammonia was loaded in a separate pressure vessel at the necessary weight and then dispensed to the reactor. External heat was applied as necessary to reach the set point, typically within 5–9 min of ammonia addition, and maintained over the course of the process. At the end of the reaction, ammonia was vented from the reactor, and the pretreated sample was removed from the vessel and left in the fume hood overnight to allow residual ammonia to evaporate. Two batches of pretreatments were conducted for each sample and combined prior to further experimentation. Upon receipt at UCR, the pretreated solids were thoroughly washed with room temperature DI water and stored at 4 °C.

Holocelluloses preparation

Holocelluloses were prepared by delignifying the lignocellulosic biomass samples by applying the standard sodium chlorite–acetic acid (SC/AA) method at 70 °C for 6 h.[35] The initial water to dry solids weight ratio was 32: 1, and sodium chlorite (0.6 g g^{-1} dry solids) and glacial acetic acid (0.6 ml g^{-1} dry solids) were added every 2 h with intermittent mixing. Solids were analyzed for moisture content and composition according to the NREL standard laboratory analytical procedures (LAPs).[36,37]

Oligomers determination

The amount of oligomers in liquid samples was determined by applying acid hydrolysis at 121 °C for 1 h in 4 wt% sulfuric

§ AFEX™ is a registered trademark of MBI International, Lansing, Michigan.

acid in accordance with the NREL standard protocol.[36] Oligomers concentration was calculated as:

$$\text{Oligomers concentration, g L}^{-1} = C_A - C_B$$

where C_A is the concentration of a sugar (g L^{-1}) in samples after acid hydrolysis adjusted for loss during acid hydrolysis and C_B is the concentration of a sugar (g L^{-1}) before acid hydrolysis.

Determination of solids composition

Compositional analysis was performed following the NREL standard two-step method.[36] Prior to analysis, untreated samples, washed solids collected after pretreatment, and delignified solids were dried at 55 °C for several days. The dried solids were then milled to pass through a 20 mesh (0.841 mm) screen using a Thomas Wiley® mini mill (Model No. 3383-L20, Thomas Scientific, Swedesboro, NJ). The dry solids (~300 mg) were subjected to primary hydrolysis at 30 °C for 1 h in 3 ml of 72 wt% sulfuric acid and then to secondary hydrolysis at 121 °C for 1 h in 4 wt% acid.

Enzymatic hydrolysis

Enzymatic hydrolysis was carried out using Accellerase®1500 cellulase at loadings of 5 and 15 mg protein per g glucan. Slurries of solids containing a glucan concentration of 10 g L^{-1} in 50 mM sodium citrate buffer (pH 5.0 ± 0.1) were incubated in triplicate at 50 °C and 150 rpm (Multitron shakers, Model AJ125; Infors-HT, Laurel, MD, USA) for up to 120 h, unless otherwise stated.[38] Hydrolysis was also performed with cellulase (15 mg protein per g glucan) supplemented with Multifect® Xylanase at 7.5 mg protein per g glucan (MXy1) or 30 mg protein per g glucan (MXy2), and cellulase at 60 mg protein per g glucan supplemented with xylanase at 60 mg protein per g glucan. Substrate blanks without enzymes and enzyme blanks without substrate were also run in parallel to account for non-hydrolytic sugar release.

Sugar release during enzymatic hydrolysis was followed by periodically withdrawing about 1 ml of a thoroughly mixed sample into a 2 ml micro-centrifuge tube (Eppendorf PCR clean microcentrifuge tubes, Catalog No. 05-402-95, Fisher Scientific, Pittsburgh, PA), mixing that sample with about 30 μl of 10 wt% sulfuric acid, and then centrifuging the combination at 14 600 rpm for 5 min in an Eppendorf centrifuge (Model No. 5424, Fisher Scientific, Pittsburgh, PA). The sulfuric acid was added to stop hydrolysis, avoid a acid negative HPLC peak by the Aminex® HPX 87-H column, and smooth integration of chromatograms. Then, about 480 μl of the clarified supernatant from the centrifuge was transferred into a 500 μl polypropylene snap ring vial (Vendor No. 98842; Grace Davison, Deerfield, IL) and run on HPLC along with sugar standards. Cellulose conversion for enzymatic reactions was calculated as:

$$\text{Cellulose conversion}(\%) = 100 \times [0.90 \times (\text{Glucose, g L}^{-1} + 1.053 \times \text{Cellobiose, g L}^{-1})]/\text{Initial cellulose, g L}^{-1}$$

where 0.90 and 1.053 are the mass conversion factors based on the stoichiometry for conversion of glucose to cellulose (162/180) and cellobiose to glucose (360/342), respectively.

The relative drop in cellulose conversion was calculated as:

$$\text{Relative drop in conversion}, \% = 100 \times [1 - (P/C)]$$

where C is the percent cellulose conversion for the untreated or pretreated control and P is the percent cellulose conversion for pretreated solids of AMH or untreated Avicel physically mixed with exogenous hemicellulose.

Analysis

Liquid samples were analyzed on a Waters Alliance high performance liquid chromatography (HPLC, Model e2695) equipped with an auto sampler (Waters 2695) and a refractive index (RI, Model No. 2414, Waters Co., Milford, MA) detector. The sugars were separated on a Bio-Rad Aminex® HPX-87H (Polystyrene-divinylbenzene sulfonic acid resin packing; 300 × 7.8 mm; Catalog No. 125-0140) column fitted with a micro-guard cation cartridge (Catalog No. 125-0129; 30 × 4.6 mm; Bio-Rad Laboratories, Hercules, CA). The column was heated to 65 °C, with 5 mM sulfuric acid at a flow rate of 0.6 ml min^{-1} as the carrier solvent. Since xylose, galactose, and mannose (XGM) are not separated well by an HPX-87H column, the concentrations of these individual sugars were determined by running samples on a Bio-Rad Aminex® HPX-87P (Catalog No. 125-0098; 30 × 7.8 mm; Bio-Rad Laboratories, Hercules, CA). Prior to running on the HPX-87P column, samples collected for compositional analysis were neutralized to about pH 5.0 with $CaCO_3$. The column was heated to 80 °C with double DI water at a flow rate of 0.6 ml min^{-1} as the carrier solvent. The chromatograms were integrated, and data was imported to Microsoft Excel files using Empower® 2 software (Waters Co., Milford, MA).

X-ray diffraction

X-ray diffraction (XRD) was performed on samples placed in a zero background holder using PANalytical Empyrean instrument (PANalytical Inc., Westborough, MA) using CuKα radiation at 40 kV and 40 mA from 5 to 40° and 0.0131° step size, 2666 points, counting time 48.195 s. Crystallinity index (CrI%) was calculated using Segal *et al.* peak height method.[39] XRD graphs were made using OriginPro v. 8.6 (OriginLab Corp., Northampton, MA) after smoothing by Savitzky–Golay filter (20 point window, 2nd order polynomial) using its in-built function.

Computational methodology

Models. All-atom molecular-dynamics simulations of a free unbranched xylan chain (degree of polymerization 30) and a cellulose fiber (degree of polymerization 20), Fig. 1, were performed using the CHARMM[40] force-field utilizing the GROMACS 5.1 simulation package[41] at 303 K and 445 K. The model of the free xylan chain was built using the molecule builder tool within Schrodinger's Maestro[42] software package while the cellulose fiber was used from previous simulation

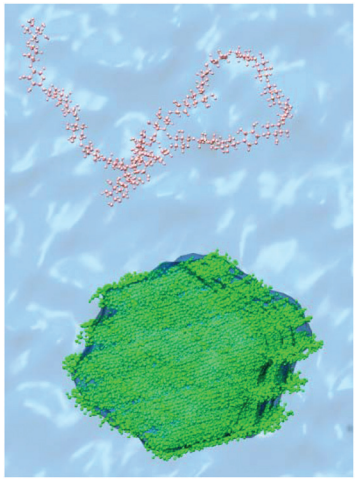

Fig. 1 Initial configuration of model system as viewed along celluose fiber axis. Green is cellulose, brown is hemicellulose.

studies.[43] Solvation with TIP3P[44] water and general system setup was performed using the CHARMM-GUI online interface.[45,46]

Molecular dynamics simulations. The simulations were performed in a three-step process: initial energy minimization, solvent relaxation, and pressure relaxation, and finally a production run in the canonical ensemble (NVT). 50 000 steps of energy minimization were performed using the steepest-descent algorithm as implemented in GROMACS 5.1 to a tolerance of 50 kJ mol^{-1} nm^{-1}. Solvent/pressure relaxation was performed for 2.5 nanoseconds, with both hemicellulose and cellulose restrained, at the target temperature 303 K and 445 K and pressures of ~1 bar. Pressures and temperature were controlled in the relaxation simulations, using the Berendsen thermostat and barostat.[47]

The system was subject to a temperature cycle: simulated at 303 K (to mimic the conditions before pretreatment) for 100 ns, then heated to 445 K (to mimic the pretreatment conditions) and simulated to 100 ns, then cooled back to 303 K (to mimic the cooling phase of the pretreatment). Five production realizations per temperature (303 K, 445 K and 303 K) were then performed using an integration time step of 2 fs and saving coordinates every 10 picoseconds for analysis. During the production simulations, the temperature was fixed with the V-Rescale thermostat[48] and bonds were constrained using the constrained using the LINCS[49,50] and SETTLE[51] algorithms. Energy minimization and solvent/pressure relaxation simulations were performed using the EDISON supercomputer at the National Energy Research Scientific Computing Center located at the Lawrence Berkeley National Laboratory and production simulations were performed using the TITAN supercomputer located at the Oak Ridge National Laboratory.

Analysis. Simulation analysis was performed using GROMACS analysis tools. The radial T distribution function of the solvent relative to the cellulose fiber surface at each temperature was calculated using the gmx rdf utility, and the gmx mindist utility was used to calculate the number of xylan–cellulose contacts (defined as pairs of atoms less than 0.3 nm apart) between hemicellulose and cellulose. Xylan–cellulose hydrogen-bonds were calculated using the gmx hbond utility with hydrogen-bond cutoffs of 0.3 nm and 20°.

Results and discussion

Hemicelluloses precipitation/adsorption on cellulose

Table 1 summarizes the pretreatment conditions and corresponding compositions of untreated substrates, resulting pretreated solids, and delignified holocellulose solids. Pretreated AMH solids were determined to contain 2.1 to 14.6 wt% (corresponding to roughly 21 mg to 168 mg hemicellulose per g cellulose) hemicelluloses (mostly xylan except for pretreated solids of Avicel mixed with heteromannan) that otherwise was negligible in pretreated solids of Avicel alone, the control (data not shown). For Avicel cellulose mixed (ACM) with GluM or GalM, only hydrothermal pretreatment at 180 °C for 30 min and AFEX pretreatment were performed. The pretreated solids of ACM with GluM and GalM contained 87.0 wt% glucan and 14.6 wt% xylan, galactan, and mannan (XGM) and 93.4 wt% glucan and 5.2 wt% galactan plus mannan, respectively. Solids of ACM with BWX were also prepared at room temperature by mixing them (5 wt% cellulose loading, cellulose to BWX wt ratio = 2, and 800 g total reaction wt) in a Parr reactor for 30 min followed by centrifugation and washing several times with room temperature DI water. The washed solids were determined to contain 96.6 wt% glucan and 4.9 wt% xylan (~50 mg xylan per g cellulose). Solids of ACM mixed with BWX xylan prepared at SAA pretreatment conditions were determined to contain about 96.6 wt% glucan and 7.6 wt% xylan. As expected, dilute acid pretreated solids contained less hemicellulose than those from hydrothermal pretreatment, whereas the xylan content in SAA pretreated solids of Avicel mixed with BWX was close to that for hydrothermal pretreatment at 180 °C for 30 min. In general, all the solids produced by the various methods had fairly similar levels of precipitated hemicellulose following pretreatment and washing.

Previous studies of hemicelluloses adsorption/precipitation on cellulose (mostly pulp) were conducted at lower temperatures than the present work, however, showed that hemicellulose adsorption increased with temperature, loading, and ionic strength. For example, Han *et al.* performed birch wood xylan adsorption on eucalyptus pulp at 70 °C for 15 min and pH 9.0 and showed that at 3 wt% and 8 wt% xylan addition, pulp adsorbed 0.87 mg xylan per g pulp and 1.15 mg xylan per g pulp, respectively.[52] Whereas, Kohnke *et al.* investigated birch wood glucuronoxylan adsorption on softwood Kraft pulp at temperature of 80 °C–120 °C for 180 min in 0.1–0.5 M NaCl and reported that higher ionic strength and temperature resulted in greater adsorption (up to 62 mg g^{-1} pulp at a loading of 160 mg g^{-1} pulp).[53] In another study, Köhnke *et al.* concluded that the initial xylan concentration and temperature were the main parameters affecting xylan adsorption for

Table 1 Substrates, pretreatments and conditions applied, and composition of untreated substrates and pretreated solids resulting from hydrothermal, dilute acid, soaking in aqueous ammonia (SAA), and ammonia fiber expansion (AFEX) pretreatments, and delignified holocellulose solids

			Composition, wt%, dry basis			
Substrate	Pretreatment	Pretreatment conditions	Glucan	XGM	Ara	K-lignin
Avicel cellulose	None	NA	100.0	0.0	0.0	Neg.
α-Cellulose			81.6 ± 1.7	18.7 ± 0.5	0.3 ± 0.1	Neg.
Beechwood xylan (BWX)			2.0 ± 0.1	70.6 ± 1.1	0.0	Neg.
Glucomannan (GluM)			40.3 ± 0.4	57.0 ± 0.4	0.0	Neg.
Galactomannan (GalM)			5.2 ± 1.0	86.4 ± 2.1	2.4 ± 0.5	1.5 ± 0.6
Corn stover holocellulose€			51.6 ± 0.4	25.4 ± 0.1	3.8 ± 0.1	2.3 ± 0.3
Switchgrass holocellulose			47.0 ± 1.1	22.5 ± 0.6	2.0	2.5 ± 0.1
Poplar holocellulose			55.7 ± 0.3	17.1 ± 0.1	0.0	4.0 ± 0.1
Pine sawdust holocellulose			65.2 ± 2.2	22.3 ± 0.8	1.1 ± 0.1	1.3 ± 0.5
ACM with BWX at 2 : 1 wt ratio	Hydrothermal	Room temp-30 min	96.6 ± 0.4	4.90 ± 0.1	0.0	Neg.
		70 °C – 24 h; liquid to solid ratio- 10	96.6 ± 0.4	7.62 ± 0.3	0.0	Neg.
		180 °C – 30 min	90.8 ± 0.1	10.2 ± 0.1	0.0	Neg.
		200 °C – 15 min	95.0 ± 0.3	6.5 ± 0.1	0.0	Neg.
	Dilute acid	140 °C – 1 wt% acid- 30 min	98.5 ± 1.1	2.1 ± 0.5	0.0	Neg.
		160 °C – 0.5 wt% acid– 10 min	98.0 ± 2.2	3.5 ± 0.1	0.0	Neg.
	SAA	70 °C – 15% NH_4OH– 24 h; liquid to solid ratio- 10	94.9 ± 1.2	8.5 ± 0.1	0.0	Neg.
	AFEX	60 °C – 1.5 : 1 g NH_3 to g dry solids and 0.9 : 1 g H_2O : g dry sample – 15 min	92.4 ± 0.4	6.3 ± 0.1	0.0	Neg.
ACM with						
GluM′	Hydrothermal	180 °C – 30 min	86.5 ± 0.1	11.8 ± 0.1	0.0	Neg.
GalM			93.4 ± 0.1	5.2 ± 0.0	0.0	Neg.
GluM	AFEX	Same as above	87.0 ± 0.4	14.6 ± 0.2	0.0	Neg.

NA- not applicable; Neg.- negligible amount; XGM- xylan, galactan, plus mannan; Ara- arabinan; €- holocelluloses were prepared by sodium chlorite- acetic acid method at 70 °C for 6 h with fresh charges of sodium chlorite (0.6 g g^{-1} dry solids) and glacial acetic acid (0.6 ml g^{-1} dry solids) added every 2 h; ACM- Avicel cellulose mixed with; ′- Avicel cellulose to guar gum galactomannan or konjac glucomannan weight ratio was 4 : 1.

cotton linter, Lyocell, and softwood Kraft pulp treated at 60–120 °C and pH 7.[54] Assuming that all of the hemicelluloses that remain following pretreatment and washing are adsorbed to the cellulose, our values range from 21 mg XGM per g cellulose for low severity dilute acid pretreatment of ACM with BWX and up to 167 mg XGM per g cellulose for AFEX pretreatment of ACM with GluM. If we assume that the GluM adsorbed to the Avicel has the same proportion of glucose residues as the untreated material, then the estimated mg GluM adsorbed per g Avicel for hydrothermal pretreatment is 265 mg g^{-1} and for AFEX is 334 mg g^{-1}. This indicates greater adsorption of glucomannan to cellulose compared to galactomannan and xylan. These values are significantly higher than the previous studies, but the original hemicellulose loadings were also much lower in the former.

Effect of exogenous, precipitated hemicelluloses on cellulose conversion

Although the effects of hemicellulose adsorption/precipitation on pulp fiber properties, such as tensile strength, burst index, extent of hornification upon drying, have been investigated before,[52,55–57] their effect on cellulose biological conversion has not been investigated. Therefore, enzymatic hydrolysis of untreated and pretreated solids was performed at cellulase protein loadings of 5 and 15 mg g^{-1} glucan in pretreated solids. Fig. 2 shows cellulose conversion *vs.* hydrolysis time, and Table 2 summarizes the initial (4 h) and final (120 h) conversions and the relative drop in conversions compared to the control for both time points. Enzymatic hydrolysis data in Fig. 2 show that cellulose in pretreated solids for ACM with hemicelluloses (empty symbols) at both cellulase loadings was much less digestible than the Avicel control (filled symbols), and Table 2 shows that the relative drop in conversion ranged from ~11% to 64%.

It is interesting to note that although 4.9 wt% xylan (51 mg g^{-1} cellulose) in the solids of ACM with BWX prepared at room temperature had an insignificant impact on cellulose conversion (only 7.7% and 1.8% relative drop in 120 h conversion at 5 and 15 mg, respectively, (Table 1)), dilute acid (160 °C for 10 min in 0.5 wt% acid) pretreated solids of ACM with xylan containing only 3.5 wt% xylan (36.2 mg g^{-1} cellulose) realized a much larger drop of 33.3% and 22.7% in cellulose 120 h conversion at 5 and 15 mg cellulase protein loading, respectively, than for their respective controls. Similar trends were observed for solids produced by dilute acid pretreatment of ACM with BWX at 140 °C for 30 min in 1 wt% sulfuric acid. These solids contained ~2 wt% precipitated xylan (20 mg xylan per g cellulose) but suffered 27.4% and 31.0% relative drops in 120 h cellulose conversions at 5 and 15 mg cellulase protein loadings, respectively. This data suggest that temperature and/or pH played a role in stronger xylan association with cellulose resulting in its enhanced recalcitrance as the solids of ACM

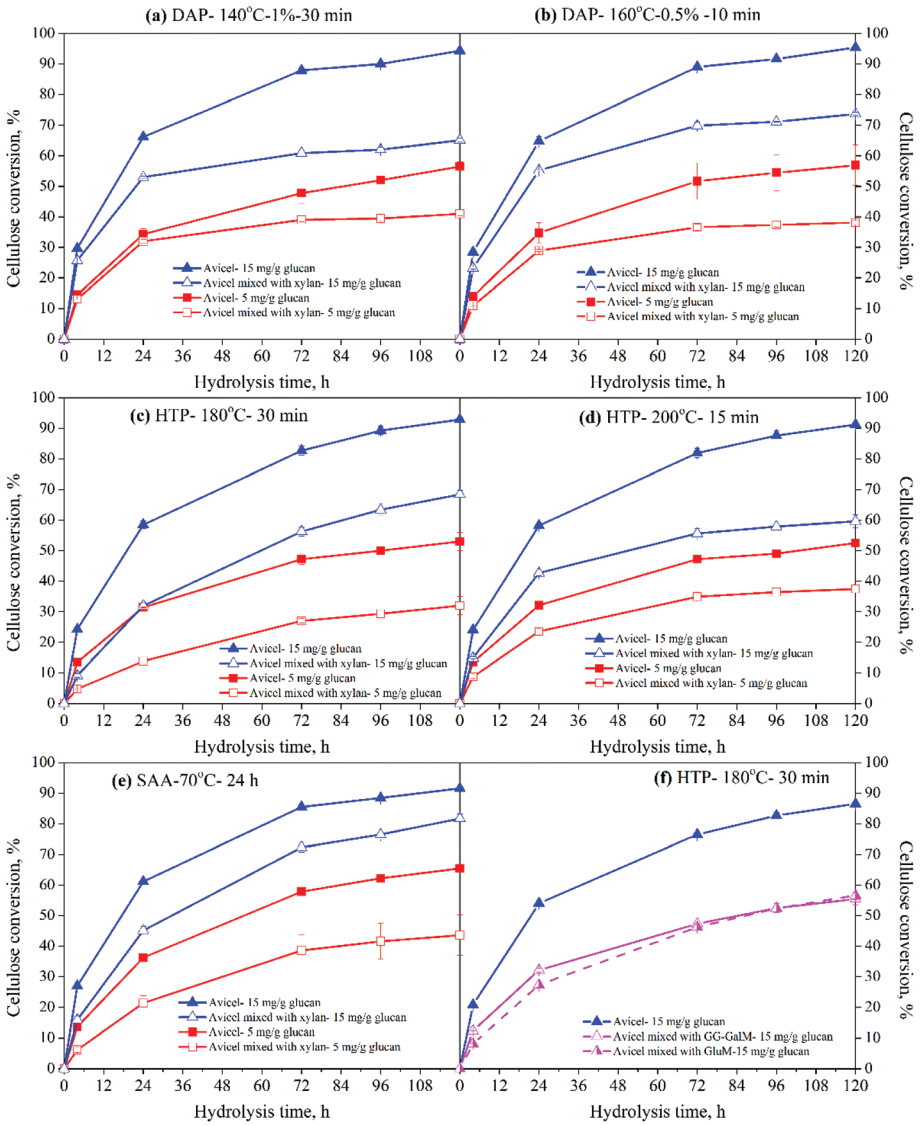

Fig. 2 Cellulose conversion *vs.* hydrolysis time (h) for enzymatic hydrolysis of pretreated solids of Avicel cellulose alone (control; solid symbols) or Avicel cellulose mixed with beechwood xylan (or glucomannan (GluM) or galactomannan (GalM); empty symbols) prepared by (a) dilute acid pretreatment (DAP) at 140 °C in 1 wt% sulfuric acid for 30 min, (b) DAP at 160 °C in 0.5 wt% sulfuric acid for 10 min, (c) hydrothermal pretreatment (HTP) at 180 °C for 30 min, (d) HTP at 200 °C for 30 min, (e) soaking in aquoes ammonia (SAA) at 70 °C for 24 h, and (f) HTP at 180 °C for 30 min at Accellerase® 1500 cellulase protein loadings of 5 (square) and 15 (triangle) mg g^{-1} glucan in pretreated solids, 50 °C and 150 rpm. **Note**- Experiemental conditions were as shown in Table 1 and discussed in Materials and methods.

with xylan prepared at room temperature showed negligible drop in conversion. Another interesting observation was that for dilute acid pretreated solids, at both conditions shown in Fig. 2a, b and Table 2, the drop in the first 4 h conversions was not significant. However, we saw a wide gap in cellulose conversions as hydrolysis progressed further. On the other hand, hydrothermally pretreated solids of ACM xylan containing higher residual xylan than dilute acid, Table 2, showed the opposite trend in that the drop in conversion at the early stages of hydrolysis was highest (up to ~65% at 5 mg cellulase protein loading) but dropped with progress in hydrolysis (Fig. 2c and d). However, the final sugar yields from solids of ACM with xylan were still lower than from the control. Although the larger drop for hydrothermally pretreated solids than for the dilute acid pretreated solids could be due to more xylan in the former, differences in xylan location on the cellulose surface due to different pretreatment chemistries and/or the association strength with cellulose for these two pretreatments may produce different conversion patterns.

SAA pretreated solids of Avicel mixed with BWX realized a greater conversion drop of 33.3 and 10.7% at 5 and 15 mg cellulase protein loadings, respectively, than from the control at similar protein loadings. However, surprisingly, washed AFEX solids of ACM with BWX prepared at 60 °C realized negligible loss in cellulose conversions (data not shown). Although further investigation is needed, the higher digestibility of

Table 2 Summary of 4 h and 120 h cellulose enzymatic conversions (%) and % relative drop in cellulose conversion over control

Pretreatment			Cellulose conversion, %			
			5 mg		15 mg	
Type	Conditions	Substrate	4 h	120 h	4 h	120 h
Dilute acid	140 °C – 1 wt% acid- 30 min	Avicel alone (control)	14.5	56.5	29.7	94.3
		Avicel cellulose mixed (ACM) with BWX¥	13.1 (9.7§)	41.0 (27.4)	25.7 (13.5)	65.1 (31.0)
	160 °C – 0.5 wt% acid- 10 min	Avicel alone (control)	13.9	56.9	28.4	95.4
		ACM with BWX	10.9 (21.6)	38.1 (33.0)	23.2 (18.3)	73.7 (22.7)
	Room temp. – 30 min	Avicel alone (control)	15.2	58.7	27.9	94.4
		ACM with BWX	12.2 (19.3)	54.2 (7.7)	26.7 (5.8)	92.6 (1.8)
Hydrothermal	70 °C – 24 h	Avicel alone (control)	15.2	58.7	27.9	94.4
		ACM with BWX	9.1 (40.5)	43.7 (25.5)	19.8 (29.2)	84.0 (11.1)
	180 °C – 30 min	Avicel alone (control)	13.5	53.0	24.2	92.8
		ACM with BWX	4.8 (64.4)	32.0 (39.6)	9.2 (62.0)	68.4 (26.3)
		ACM with GluM€	NA	8.1 (66.8)	56.6 (39.0)	
		ACM with GalM€	6.2 (54.1)	24.5 (53.8)	12.4 (55.4)	55.4 (40.3)
	200 °C – 15 min	Avicel alone (control)	13.5	52.5	24.0	91.2
		ACM with BWX	8.8 (34.8)	37.4 (28.8)	14.9 (37.9)	59.6 (34.6)
Soaking in aqueous ammonia	70 °C – 15% NH_4OH– 24 h	Avicel alone (control)	13.6	65.4	27.0	91.5
		ACM with BWX	6.2 (54.4)	43.6 (33.3)	15.9 (41.1)	81.7 (10.7)

§- Numbers in parenthesis are relative drop in cellulose conversion, %, over control as defined in materials and methods; NA- not available; BWX- beechwood xylan; GluM- glucomannan; GM- galactomannan; ¥- prior to pretreatment, Avicel was mixed with BWX at 2 : 1 weight ratio; €- prior to pretreatment, Avicel was mixed with GluM or GalM at 4 : 1 weight ratio.

AFEX treated Avicel (~75% at 5 mg in 120 h) compared to the untreated solids (51% at 5 mg in 120 h) and the XRD data shown in Fig. S1† suggest that the reduction in crystalline cellulose and minor cellulose III formation by AFEX pretreatment[58,59] may have resulted in weaker associations with hemicelluloses. It is unknown what effect cellulose III formation has on hemicelluloses adsorption/association, but given the changes in the hydrogen bonding patterns, it seems possible that it could result in weaker associations with hemicellulose compared to the other pretreatments that do not alter the crystalline structure of cellulose. However, the low temperature of 60 °C used for AFEX pretreatment may also have played a role in cellulose–hemicellulose interaction (strength). Solids of ACM with BWX prepared in water at SAA conditions (70 °C for 24 h), realized larger drop in cellulose conversion than solids prepared at room temperature (Table 1). However, this can be due to a little higher precipitated xylan in the solids of ACM with BWX prepared at 70 °C than at room temperature. Pretreatments on Avicel cellulose mixed with monomeric xylose were also performed. The resulting pretreated washed solids had no xylose and the pretreated solids digestibility was very much similar to their respective controls (data not shown) suggesting that xylose did not interact with cellulose as strongly as its polymer xylan.

On the other hand, although solids produced by hydrothermal pretreatment of ACM with GalM contained only 5.2 wt% XGM (54 mg XGM per g glucan), 120 h cellulose conversions showed the largest drop of 53.8 and 40.3% at 5 and 15 mg cellulase per g glucan, respectively, compared to the control. As shown in Table 2, similar drops in conversions were also observed for solids produced by hydrothermal pretreatment of ACM with GluM, suggesting stronger association of cellulose with (hetero) mannan than xylan, consistent with our previous statement based on the pretreated biomass composition and literature reports.[60] Solids from AFEX pretreatment of ACM with GluM, however, showed a yield loss of only ~12% and 1.4% at 5 and 15 mg cellulase loadings, respectively (data not shown). The insignificant drop in cellulose conversion for AFEX treated solids, than for the hydrothermally pretreated solids of ACM with GluM shown in Fig. 2f and Table 2, further suggest that temperature plays a vital role in cellulose–hemicellulose association (strength). In support of this hypothesis, the GluM was also found to have insignificant effect on cellulose conversion when it was physically mixed with cellulose before enzymes addition in enzymatic hydrolysis.[61]

Intrinsic *vs.* exogenous hemicelluloses

Further experiments were performed to compare the effects of exogenous (precipitated) *vs.* intrinsic hemicelluloses (xylan) on cellulose conversion. A model α-cellulose from Sigma presumably containing about 18.7 wt% intrinsic xylan (229 mg xylan per g cellulose) and no lignin, virtually lignin free (<3 wt% lignin dry basis) corn stover holocellulose containing 25.4 wt% structural XGM (*i.e.*, 492 mg g^{-1} glucan), switchgrass holocellulose containing 22.5 wt% XGM (*i.e.*, 478 mg g^{-1} glucan), poplar holocellulose containing 17.1 wt% XGM (307 mg g^{-1} glucan), and pine saw dust holocellulose containing 22.3 wt% XGM (342 mg g^{-1} glucan) were used as intrinsic hemicelluloses containing substrates. It was assumed that holocelluloses and the model compound α-cellulose had insignificant amount of relocated/exogenous hemicellulose. The enzymatic hydrolysis data in Fig. 3 reveal that the initial 4 h glucan conversions at cellulase protein loading of 15 mg for all holocelluloses (except for pine saw dust) were lower than for the model

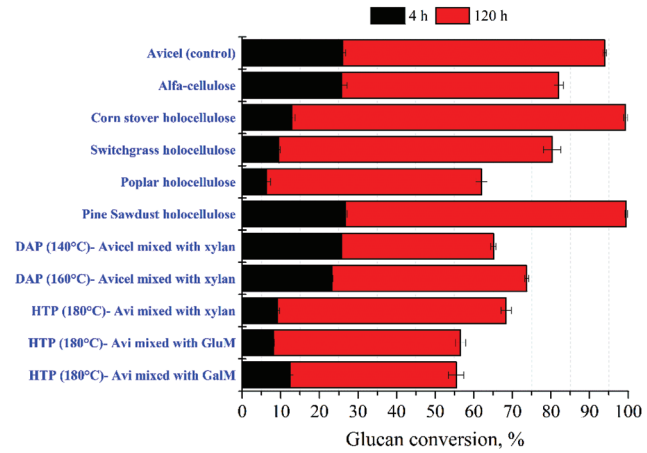

Fig. 3 Comparison of the effects of intrinsic hemicelluloses in α-cellulose and lignin-depleted holocelluloses, and precipitated hemicelluloses in pretreated solids of Avicel mixed with hemicelluloses prepared by hydrothermal (HTP), dilute acid (DAP), and soaking in aqueous ammonia (SAA) pretreatments on glucan 4 and 120 h conversions at a cellulase loading of 15 mg protein per g glucan. **Note**- Experiemntal conditions were as shown in Table 1 and described in Materials and methods.

compound α-cellulose and solids resulting from pretreatment of the Avicel cellulose control and ACM with BWX.

The final 120 h glucan conversion of the pretreated Avicel control was greater than that of the intrinsic xylan containing SWG and poplar holocelluloses and α-cellulose. This outcome can be attributed to limited cellulose accessibility and/or cellulase inhibition by oligomers released during enzymatic hydrolysis from these substrates.[4,8] Corn stover holocellulose was more digestible than the Avicel control. Because softwood is considered to be the most recalcitrant and contain more complex hemicelluloses, it was surprising that the glucan in PSD holocellulose (despite ~22.4% intrinsic XGM, 342 mg XGM per g glucan) was highly digestible. However, the final 120 h conversions for some of the solids resulting from pretreatment of ACM with hemicelluloses were nearly equal or much lower than results from hydrolysis of holocelluloses or α-cellulose that contained much higher amounts of intrinsic hemicelluloses (229–492 mg XGM per g cellulose). For example, as shown in Fig. 3, cellulose conversion from solids produced by dilute acid (140 °C–30 min- 1 wt% sulfuric acid; ~2.0 wt% precipitated, exogenous xylan) and hydrothermal (200 °C–15 min; 6.5 wt% xylan) pretreatment of ACM with xylan were equal to that for poplar holocellulose that contained about 17.2 wt% XGM and was the least digestible among intrinsic hemicelluloses containing substrates. Furthermore, holocellulose of PSD that contained about 22.3% XGM was more digestible than solids from pretreatment of ACM with GluM (11.8% XGM) or GalM (5.2% XGM). Overall, this data suggests that exogenous hemicelluloses that precipitate on solids during pretreatment can reduce cellulose accessibility and/or inhibit cellulases more than the intrinsic hemicelluloses.

Exogenous hemicelluloses added prior to pretreatment *vs.* prior to enzymatic hydrolysis

It is possible that the observed drop in cellulose conversions from the previous results was due to the mere physical presence of xylan or xylooligomers (XOs) in the media competitively inhibiting cellulases during hydrolysis as opposed to a pretreatment induced association limiting conversions. In order to evaluate this possibility, cellulose was physically mixed with BWX or XOs (at loadings of 2 or 5 g L^{-1}) before enzyme addition. Hydrolysis data in Fig. 4 show that the relative drop in cellulose conversion for all pretreated solids of ACM with xylan was equal or higher than at both concentrations of physically mixed xylan and the lowest concentration of XOs of 2 g L^{-1}. However, consistent with our earlier findings,[4,25] the drop in cellulose conversion was the highest when the physically mixed XOs concentration was 5 g L^{-1}. Since the slurry formed by enzymatic hydrolysis of pretreated solids of ACM with xylan at a glucan loading of 10 g L^{-1} contained less than 1.2 g L^{-1} of xylan, the drop in cellulose conversion for such solids could not be attributed to either cellulase inhibition by XOs released during enzymatic hydrolysis of these solids and/or just inhibition of cellulase action by physically present xylan, suggesting that it was mainly due to stronger association of xylan with cellulose. For ACM with GluM, we previously showed that GluM, when physically mixed with Avicel cellulose, did not have a significant impact on cellulose conversion up to a concentration of ~1.2.[61]

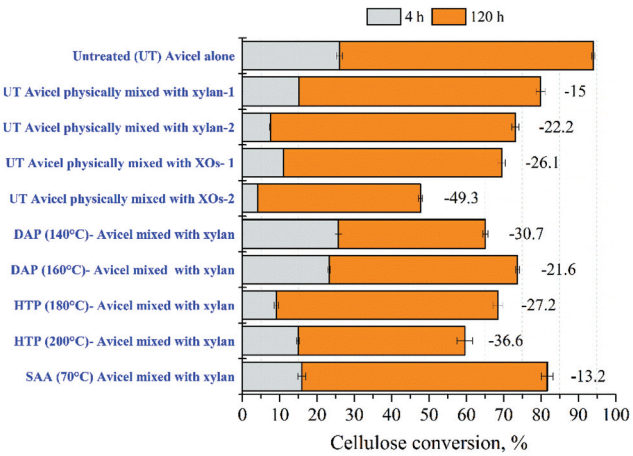

Fig. 4 Comparison of effects of precipiated xylan in dilute acid (DAP), hydrothermal (HTP), and soaking in aqueous ammonia (SAA) pretreated solids *vs.* addition of xylan or xylooligomers (XOs) to Avicel immediately prior to enzyme addition on cellulose 4 and 120 h conversions at cellulase loading of 15 mg protein per g glucan. **Note**- Xylan-1 or XOs-1: beechwood xylan (BWX) or XOs physically mixed with cellulose at a weight ratio of 0.2 : 1. Xylan-2 or XOs-2: beechwood xylan or XOs physically mixed with cellulose at a weight ratio of 0.5 : 1. HTP (180 °C or 200 °C)- hydrothermally pretreated at 180 °C or 200 °C. DAP (140 °C or 160 °C)- dilute acid pretreated at 140 °C or 160 °C. SAA (70 °C) represents soaking in aqueous ammonia at 70 °C for 24 h. Other conditions were as shown in Table 1 and in materials and methods. Numbers on the graphs are % relative drop in cellulose conversion in comparison to the control.

Molecular simulations

To obtain a molecular-level description of hemicellulose–cellulose interactions at room and near-pretreatment temperatures, atomistic molecular dynamics (MD) simulations were performed. The interactions between hemicellulose xylan and cellulose were quantified by calculating the number of intermolecular contacts between the two substrates. Data shown in Table 3 makes it clear that the hemicellulose analogue forms significantly more contacts with cellulose at 445 K (172 °C) than at 303 K (30 °C). Further analysis of hemicellulose–cellulose hydrogen-bonding (Fig. 5), indicates that on average hemicellulose and cellulose contain more inter-molecular hydrogen-bonds (*i.e.*, they form more xylan–cellulose hydrogen-bonds) at 445 K than at 303 K. In other words, hemicellulose binds more strongly to cellulose at pretreatment temperatures, consistent with the experimental findings in the previous sections.

Recent work[62,63] suggested that modifications to the solvation structure of cellulose may lead to cellulose–cellulose aggregation at high temperatures.[61,62] Therefore, to rationalize the above findings, and to test whether this mechanism may also be in effect for hemicellulose–cellulose aggregation at near-higher-pretreatment temperatures, we examined the hydration of cellulose at 303 and 445 K. The radial distribution function, which probes the density of water relative to distance from the cellulose surface, was calculated, and showed that the solvation shell of cellulose at 445 K is different when compared to 303 K (Fig. 6). At 445 K, the first peak (near 0.45 nm) is greatly reduced in height and broader than at 303 K, implying that the cellulose solvation shell is depleted and less ordered at 445 K. Indeed, the first peak at 445 K, with only a minor decay, suggested that water binding to the cellulose surface is almost lost. As noted above, previous studies of cellulose solvation[64] during hydrothermal pretreatments indicated that the loss of order in the cellulose solvation shell promoted cellulose–cellulose aggregation. It was found that, compared to room temperature, cellulose aggregation at pretreatment temperatures is mainly due to a more significant decrease in water–cellulose interaction energy that outweighs a relatively smaller decrease in water entropy at the surface. The resulting increase in hemicellulose–cellulose contacts observed here also seem to be correlated with the same mechanism. However, more detailed calculations would be necessary to confirm this.

To evaluate the effects of cooling on cellulose hemicellulose interaction, the simulations were cooled to 303 K, approximately the temperature at which bio-conversion occurs. We calculated the number of cellulose–xylan contacts and found that they do not diminish upon cooling (Table 3). This behavior is consistent with experiments that showed that xylan binds cellulose irreversibly.[65] We also note that non-reversible

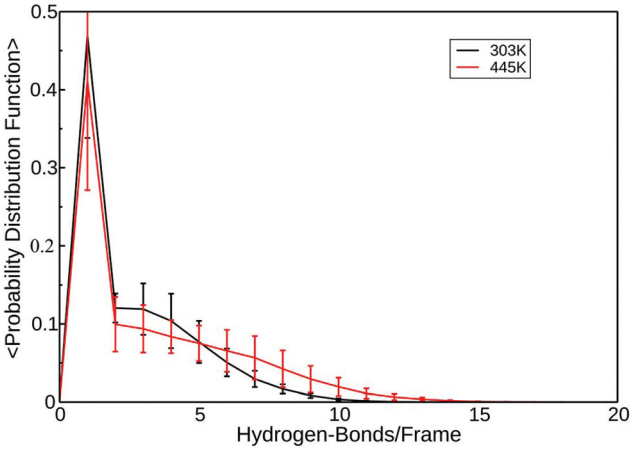

Fig. 5 Average cellulose–xylan hydrogen-bonds per frame distributions. Errors bars are equal to the standard error of the mean (over all trajectories).

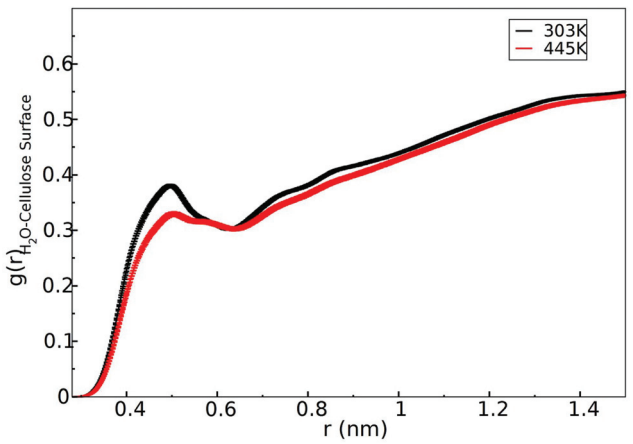

Fig. 6 Average radial distribution functions of water with respect to the cellulose surface. Standard error of the mean (over all trajectories) is equal to the width of the curves.

Table 3 Temperature dependence of the number of contacts between hemicellulose xylan and cellulose, as the system is subject to a temperature cycle. Data were calculated from four independent MD trajectories (averaged over the last 10 ns of each trajectory), and their ensemble average. Reported ranges are the standard error of the mean

Temperature	Trajectory	Contacts (last 10 ns)
303 K (after heating from 0 to 303 K)	0	194
	1	76
	2	166
	3	193
	4	141
	Ensemble avg.	154 ± 22
445 K (after heating from 303 K to 445 K)	0	288
	1	147
	2	344
	3	461
	4	270
	Ensemble avg.	302 ± 51
303 K (after cooling from 445 K to 303 K)	0	302
	1	301
	2	257
	3	268
	4	270
	Ensemble avg.	280 ± 21

binding upon pretreatment temperatures has also been observed in previous simulations of cellulose aggregation.[62]

Proposed mechanism for drop in cellulose conversion

The previously reported mechanisms for hemicellulose adsorption onto cellulose and the effects of parameters such as pH, hemicellulose types, temperature, and time have suggested that hemicellulose xylan coats the cellulose surface due to hydrogen bonding between the two polymers.[66–69] However, xylan aggregation in the solution and/or on the cellulose surface can also result in globules above certain concentrations.[55,70–73] Furthermore, xylans have also been reported to penetrate into the cellulose pores.[73] Heteromannans, specifically mannan and glucomannan, on the other hand, have been shown to form crystals with shish-kebab structure on the cellulose surface.[74,75]

The present study shows that the strong adsorption and association of hemicelluloses with cellulose slow conversion of cellulose mediated with cellulase at low as well as moderately high enzyme loadings. Fig. 2 and Fig. S2† show that the drop in cellulose conversion for pretreated solids containing precipitated hemicellulose varied with cellulose and hemicellulose types. Because pretreated solids were thoroughly washed with DI water before enzymatic digestion, enzymes inhibition/deactivation by toxic compounds such as furfural, 5-hydroxymethylfurfural *etc.*[76,77] can be ruled out to be the cause for drop in cellulose conversion. XRD analysis of samples in Fig. S3† revealed that the change in crystallinity and/or crystal dimensions of solids for ACM with hemicellulose did not cause the drop in cellulose conversion as the XRD spectra and the calculated crystallinity index (CrI) values for pretreated solids containing hemicellulose were similar to their respective controls. Furthermore, as shown in Table 1, solids were found to contain negligible K-lignin and/or pseudo-lignin, the negative impact of K-lignin (and/or pseudo-lignin)[2,78] can also be ruled out the ruling cause for the drop in conversion for ACM with hemicellulose.

However, since it is believed that cellobiohydrolases preferentially attack cellulose from the hydrophobic face, any barrier that prevents these enzymes from recognizing the cellulose hydrophobic face should therefore affect its hydrolysis negatively.[79] In fact, in a recent study, it was shown that xylan adsorption on the cellulose surface moves from the cellulose hydrophilic face to the hydrophobic face with increasing temperature.[67,80] Thus, based on the findings in the literature and this study, it can be hypothesized that at elevated temperatures hemicelluloses strongly associate with the cellulose surface, as schematically shown in Fig. 7, possibly on the hydrophobic face, through hydrogen bonding, and the negative impact on the access of cellulase to cellulose slowed hydrolysis. Indeed, the simulation work presented here provides strong support of this hypothesis as inter-molecular contacts and hydrogen bonds were found to be increased at 445 K. In several previous studies, supplementation of cellulase with xylanase (and other hemicellulases) enhanced cellulose accessibility and consequently cellulose conversions.[4,25] The similar observations for this study shown in Fig. S4† suggested that the limited accessibility of cellulose to cellulase was possibly the main cause for the drop in cellulose conversion for pretreated solids of cellulose mixed with hemicelluloses. Furthermore, the negligible drop in cellulose conversion at high enzyme loadings seen in Fig. S5† suggest the same mechanism. However, the retardation of cellobiohydrolase processivity and/or unproductive adsorption of enzymes by adsorbed hemicelluloses could also impact cellulase effectiveness, but further research is needed to determine their potential role.

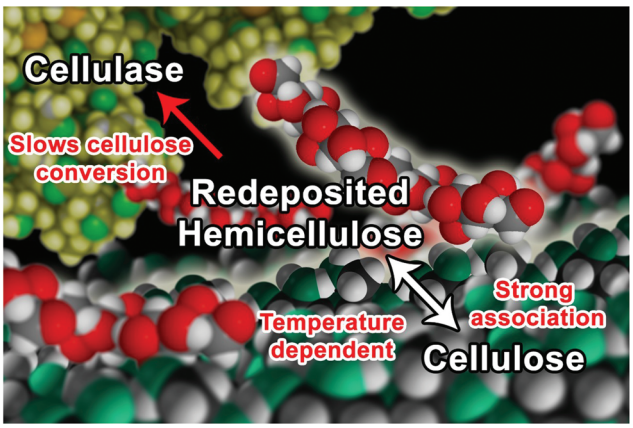

Fig. 7 Representative schematic of hemicellulose–cellulose interactions.

Conclusions

Strong cellulose–hemicellulose associations formed at elevated temperatures were shown to retard cellulose conversion mediated with fungal cellulase. The greater drop in cellulose conversion due to adsorbed hemicelluloses (otherwise termed exogenous hemicelluloses) onto the cellulose surface than that by intrinsic hemicelluloses in lignin-free holocelluloses suggested that relocalized and precipitated hemicelluloses can reduce cellulose access more and/or be more inhibitory to cellulase than the intrinsic hemicellulose present in the plant cell wall. Molecular dynamics simulations confirmed the increase in cellulose–hemicellulose associations at elevated temperatures and explain the increase by a loss of order in the cellulose solvation shell. Furthermore, cellulose and hemicellulose types and pretreatment chemistries affected the cellulose conversion kinetics and patterns. However, further work is required to clearly establish mechanisms and roles for these and other parameters such as cellulose and hemicellulose types and loadings in pretreatment and the effect of lignin on cellulose conversion due to non-structural hemicelluloses.

Conflicts of interest

There are no conflicts to declare.

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