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Using low temperature to balance enzymatic saccharification and furan formation during SPORL pretreatment of Douglas-fir☆

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ABSTRACT

Comparing analytical results for Sulfite Pretreatment to Overcome the Recalcitrance of Lignocelluloses (SPORL) of Douglas-fir (*Pseudotsuga menziesii*) at two different temperatures shows that the apparent activation energy of sugar degradation is higher than that of hemicellulose hydrolysis, approximately 161 kJ/mole versus 100 kJ/mole. Thus, one can balance the production of degradation products against hemicellulose hydrolysis and therefore the enzymatic saccharification efficiency of the resultant substrate by changing pretreatment temperature and duration. Specifically, pretreatment at 165 °C for 75 min significantly reduced furan formation compared with the pretreatment at 180 °C for 30 min while maintaining the same pretreatment severity and therefore the same substrate enzymatic digestibility (*SED*). Obtaining high *SED* with Douglas-fir is also limited by lignin content. Fortunately, the bisulfite in SPORL provides delignification activity. By combining kinetic models for hemicelluloses hydrolysis, sugar degradation, and delignification, the performance of pretreatment can be optimized with respect to temperature, duration, acid, and bisulfite loading. The kinetic approach taken in this study is effective to design viable low temperature pretreatment processes for effective bioconversion of lignocelluloses.
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1. Introduction

Thermochemical pretreatment of lignocellulosic biomass is a prerequisite step for efficient fractionation and high value utilization of lignocelluloses through biorefineries [1,2]. Pretreatment opens the lignocellulose cell wall structure which improves cellulose accessibility to cellulase enzymes [3,4]. However, several promising and commonly practiced acidic pretreatments, such as dilute acid [5], Sulfite Pretreatment to Overcome the Recalcitrance of Lignocelluloses (SPORL) [6,7], Organosolv [8], SO_2-catalyzed steam explosion [9], can also degrade sugars to undesirable inhibitive compounds such as furans, which affects downstream processing through fermentation. Most pretreatment optimizations focus on achieving high cellulose enzymatic saccharification at the expense of increased sugar degradation [8,10]. One approach to address degradation product inhibition in fermentation is using low solids to dilute the concentration of inhibitors [11,12]. Unfortunately, low solids processing is not economical for commercialization. More recent efforts in reducing the formation of fermentation inhibitors included using a relatively low pretreatment severity along with a deacetylation step [13]. These efforts reduced cellulose saccharification efficiency and introduced more unit operations, i.e., additional biomass size reduction and xylanase treatment [13], which increase capital and operating costs.

The objective of the present study is to balance sugar production and furan formation, in the pretreatment of Douglas-fir using SPORL [6]. SPORL was chosen for the study because it has been shown to be effective for softwood bioconversion [12,14]. The approach taken is based on the difference in reaction kinetics between acid hydrolysis of hemicelluloses and degradation of sugars to furans. Specifically, we hypothesize that sugar degradation has higher activation energy than that of the hydrolysis of hemicelluloses, and thus lower temperatures may allow complete hemicellulose removal without extensive sugar degradation compared with a pretreatment of the same reaction severity at a higher temperature. Our previous study developed a combined hydrolysis factor (*CHF*), which combines temperature, chemical concentrations, and pretreatment duration into a single measure of pretreatment severity [15]. *CHF* is similar to the traditional severity factors, such as the H-factor used in the pulp and paper industry [16] and the combined severity factor (CSF) for hydrolysis of xylan of lignocelluloses [17,18], but is applicable to reactions involve more than one catalyst such as SPORL. *CHF* accurately predicted xylan dissolution of aspen wood by dilute acid and SPORL pretreatments [15]. Furthermore, *CHF* also correlated to pretreated substrate enzymatic saccharification well because hemicellulose dissolution is

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the dominant factor dictates cellulose saccharification of aspen with low lignin content [4,10,19]. Therefore, *CHF* can be used to design a low temperature pretreatment to reduce sugar degradation while achieve good enzymatic cellulose saccharification. This *CHF* approach is applicable to the widely studied dilute acid pretreatment as well as acidic pretreatments with more than one catalyst.

Softwoods have much higher lignin and mannan contents than hardwood such as aspen used in our previous study [15]. Delignification becomes important to enzymatic saccharification of softwoods; however hemicellulose removal remains a critical factor [4]. Because, sulfite loading is a dominant factor to delignification at acidic conditions, we can demonstrate the approach of using *CHF* to design a low temperature SPORL pretreatment of softwoods to balance enzymatic saccharification and furan formation. We will conduct paired pretreatments of Douglas-fir at two temperatures with identical chemical such as sulfite loadings and pretreatment severity CHF. We will examine furan production at these two temperatures at constant *CHF* or hemicellulose removal to validate the hypothesis. We will also present data showing the impact of sulfite on delignification and the effect of hemicellulose removal and delignification on enzymatic cellulose saccharification of Douglas-fir.

2. Reaction model and determination of pretreatment temperature

2.1. Combined hydrolysis factor (CHF) to predict hemicellulose dissolution

To facilitate discussions, the previous proposed reaction model to describe xylan dissolution in acidic pretreatments [15] is presented below:

$$\frac{dX_f}{dt} = -kCX_f \tag{1a}$$

$$X_f(t=0) = (1-\theta)X_R \tag{1b}$$

$$\frac{dX_s}{dt} = -fkCX_s \tag{2a}$$

$$X_s(t=0) = \theta X_R \tag{2b}$$

where X_f and X_s are fractions of fast and slow residual xylan in solids, respectively, with $X_R = X_f + X_s$ and X_R ($t=0$) = 1. C is catalyst concentration. The rate constant k for fast xylan hydrolysis can be defined by Eq. (3) based on previous studies [17,20]. f in Eq. (2a) is the ratio of the rate constant between the slow and fast xylan hydrolysis reactions. θ is the initial fraction of slow reacting xylan.

$$k = e^{(\alpha-(E/RT)+\beta C_A+\gamma C_B)} \tag{3}$$

We also defined a combined hydrolysis factor (*CHF*) to predict xylan dissolution.

$$CHF = e^{(\alpha-(E/RT)+\beta C_A+\gamma C_B)}(C_A + C_B)t \tag{4}$$

where C_A and C_B are the molar concentrations of chemical A (acid) and chemical B (bisulfite) used in pretreatment, respectively; α, β and γ are adjustable parameters, E is the apparent activation energy, R is universal gas constant of 8.314 J/mole/K, and T is absolute temperature (K). Integrating Eqs. (1) and (2) produces a predictive equation for residual xylan content, X_R, in solids.

$$X_R = (1-\theta)e^{-CHF} + \theta e^{-fCHF} \tag{5}$$

In the case of Douglas-fir in this study, we expanded the definition of xylan to include the glucomannan found in this species of wood.

2.2. Prediction of carbohydrate degradation products

Sugar degradation to HMF and furfural has been observed to follow first order Arrhenius kinetics [21–23]. While many other products and reactions can occur in the temperature range of 165–180 °C, HMF and furfural can be used as indicator species neglecting further degradation of furans to acids. Previous empirical correlations suggested that the formation of furfural and HMF followed the same functionality with *CHF* [15]. We can lump all carbohydrate degradations into one pool to define a rate constant, k_d, for all sugar degradation reactions with an Arrhenius temperature dependence.

$$k_d = e^{(\alpha_d-(E_d/RT))} \tag{6}$$

In Eq. (6), α_d is an adjustable parameter, E_d is the apparent activation energy, R is universal gas constant of 8.314 J/mole/K, and T is absolute temperature (K). Defining D as the sum of the concentration of HMF and furfural, then Eq. (7) represents the rate of their formation. In this equation $(1-X_R)$ represents the sum of the concentrations of the carbohydrates released by hydrolysis.

$$\frac{dD}{dt} = k_d(1-X_R) \tag{7}$$

Substitute Eq. (5) into Eq. (7), the integrated rate expression becomes, with t defined as the time at temperature.

$$D = k_d \cdot t\left[1 - \frac{1-\theta}{CHF}(1-e^{-CHF}) - \frac{\theta}{f\cdot CHF}(1-e^{-f\cdot CHF})\right] \tag{8}$$

Evaluating paired pretreatments at two different temperatures, $T1 > T2$, with identical pretreatment severity *CHF* and chemical loadings, the ratio of sugar degradation products (HMF and furfural) formation can be obtained as follow:

$$\frac{D_{T1}}{D_{T2}} = \frac{k_d^{T_1}}{k_d^{T_2}} \cdot \frac{t^{T_1}}{t^{T_2}} = \exp\left[\frac{E-E_d}{R}\left(\frac{1}{T1}-\frac{1}{T2}\right)\right] \tag{9}$$

2.3. Pretreatment temperature determination

Excellent enzymatic saccharification efficiency and ethanol yield can be achieved for spruce, red pine, and lodgepole pine when SPORL pretreatment was conducted at $T1$ = 180 °C for approximately t^{T1} = 25–30 min [6,12,14,24]. The optimal bisulfite charges on wood in oven dry (od) weight were found between 8% and 10% with initial pH approximately 2, or sulfuric acid concentration in the initial pretreatment liquor of approximately 0.4% (v/v) or 0.075 mmol/L using liquor to wood ratio (L/W) of 3:1. Since Douglas-fir has higher lignin content than lodgepole pine and may be more recalcitrant, we will conduct most pretreatments at 180 °C for 30 min as control in this study.

In typical wood pulp mill operations, wood chip loading time is on the order of a couple of hours and using a pretreatment duration similar to chip loading time should not cause capacity problem. Therefore, we chose $T2$ = 165 °C for t^{T2} = 75 min as the comparison treatment, which give a *CHF* similar to control under the same chemical loadings, to compare the effect of pretreatment temperature on furan formation and enzymatic saccharification at constant CHF (Eq. (4)) with similar hemicellulose dissolution [15]. Specifically, $T2$ or t^{T2} is determined using the following expression,

$$\exp\left(\frac{-E}{RT1}\right)\cdot t^{T1} = \exp\left(-\frac{E}{RT2}\right)\cdot t^{T2} \tag{10}$$

Table 1
List of SPORL pretreatment conditions conducted at 165 °C and 180 °C with liquid to wood ratio *L*/*W* = 3:1, along with pretreatment severity: combined hydrolysis factors (*CHF*).

Pair no.	Sulfuric acid (% v/v)	Sodium bisulfite (% w/w)	Initial liquor pH	*T* = 165 °C		*T* = 180 °C	
				Duration *t* (min)	*CHF*	Duration *t* (min)	*CHF*
I	0.1	8	2.53	75	6.48	30	6.44
II	0.4	12	1.97	75	9.04	30	8.98
III	0.25	8	2.07	75	12.38	30	12.30
IV	0.4	10	1.89	75	14.77	30	14.66
V	0.4	8	1.80	50	15.65	20	15.54
VI[a]	0.4	8	1.80	75	23.47	30	23.31
VII	0.4	8	1.80	100	31.30	40	31.08
VIII	0.4	6	1.67	75	35.90	30	35.65
IX	0.6	8	1.63	75	54.46	30	54.08
	0.4	14	4.10			30	5.39
	0	8	4.15			30	4.16
	0.4	4	1.55			30	51.37

[a] Triplicate runs were conducted for this pair of pretreatments at 165 °C and 180 °C.

3. Materials and methods

3.1. Raw material

Douglas-fir (*Pseudotsuga menziesii*), an evergreen conifer species native to western North America, wood chips were collected by Weyerhaeuser Company from a pulp mill in Washington State. Wood chips were first air dried before shipping to the Forest Products Laboratory, Madison, WI. The moisture content of the raw material received was 7.62%.

3.2. Enzymes and chemicals

A commercial enzyme cocktail of CTec2 was generously provided by Novozymes North America (Franklinton, NC). The average activity of the CTec2 was 150 filter paper unit (FPU)/mL determined by a literature method [25]. Sodium acetate, sulfuric acid, and sodium bisulfite were acquired from Sigma-Aldrich (St. Louis, MO), all chemicals were of ACS reagent grade.

3.3. SPORL pretreatment and substrate production

All pretreatments were conducted in a 23 L laboratory rotating pulping digester heated by a steam jacket as described previously [24,26]. Three 1 L bomb reactors were mounted inside the digester in an autoclave configuration. Wood chips of 150 g in oven dry (od) weight were placed in each 1 L reactor along with dilute acidic sulfite solution with solution to wood ratio *L*/*W* of 3:1 (v/w). Pretreatments were conducted under a range of conditions. The sulfuric acid and sodium bisulfite charge on od wood were varied from 0.1% to 0.6% (v/v) equivalent to 0.019–0.113 mmol/L or 0.55–3.3% (w/w) and 4–14% (w/w), respectively. The pretreatment time was varied from 50 to 100 min at pretreatment temperature 165 °C, and from 20 to 40 min at 180 °C. Detailed pretreatment designs are listed in Table 1. Two pretreatments respectively conducted at 165 °C and 180 °C under the same acid and sulfite loading consist of one pair. After each pretreatment, the solids remained wood chips and was separated from the spent liquor using a screen. The spent liquor was collected and stored at 4 °C until analysis.

The pretreated solids was disk milled using an 8-inch manual driven disk refiner (Andritz Sprout–Bauer Refiner, Springfield, Ohio) as described previously [7]. Refining was conducted under atmospheric conditions with a disk plate gap of 1.0 mm. Water was added to facilitate disk milling to result in a discharge solid consistency of approximately 10% (w/w). The material collected was directly dewatered through pressing using a canvas bag to a solids content of approximately 30% without separate washing. The yield of solid substrate in the form of fibers or fiber bundles was determined from the weight and moisture content of the collected solids.

3.4. Chemical composition analysis

The Douglas-fir solid substrates, both untreated and pretreated, were ground using a Wiley mill (Model #2, Arthur Thomas Co, Philadelphia, PA) to pass a 40 mesh (~1 mm) screen. The resulting materials were hydrolyzed using sulfuric acid in two stages as described previously [26]. The hydrolysate was then analyzed for carbohydrates using an HPLC (Dionex IC-3000, Dionex Corporation, Sunnyvale, CA) with pulsed amperometric detection (HPAEC-PAD) by the Analytical Chemistry and Microscopy Lab of the Forest Products Laboratory. A Dionex Carbo Pac PA-1 Analytical column (4 mm × 250 mm) with a 4 mm × 20 mm PA-1 guard column was used. The Klason lignin content was measured gravimetrically after washing and drying the solid residues from the acid hydrolysis. The pretreatment spent liquor was also analyzed for sugar and fermentation inhibitors using the same HPLC system as previously described [26]. For rapid analysis, glucose in the enzymatic hydrolysate was measured using a commercial glucose analyzer (YSI 2700S, YSI Inc., Yellow Springs, OH). The reported data were the averages of replicate measurements.

3.5. Enzymatic hydrolysis

All hydrolysis experiments were conducted using a commercial cellulase cocktail CTec2 at 2% substrate solids (w/v) in 50 mL flasks on a shaker (Thermo Fisher Scientific, Model 4450, Waltham,

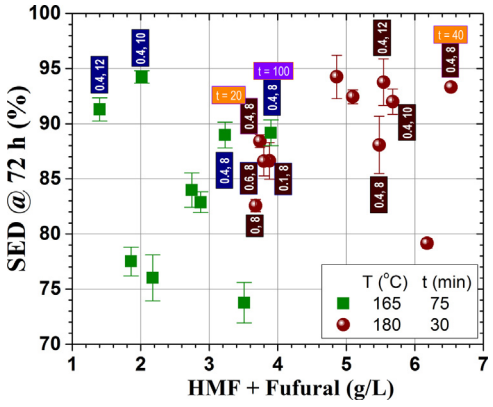

Fig. 1. Comparisons of substrate cellulose enzymatic digestibility (*SED*) and furan (HMF + furfural) production from SPORL pretreatments at 165 °C and 180 °C.

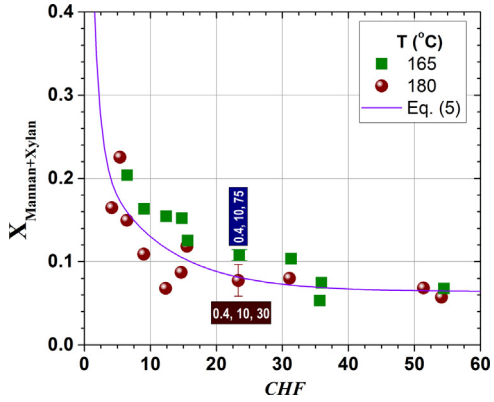

Fig. 2. Xylan and Mannan remaining in the pretreated solids for various levels of pretreatment represented by the combined hydrolysis factor (*CHF*).

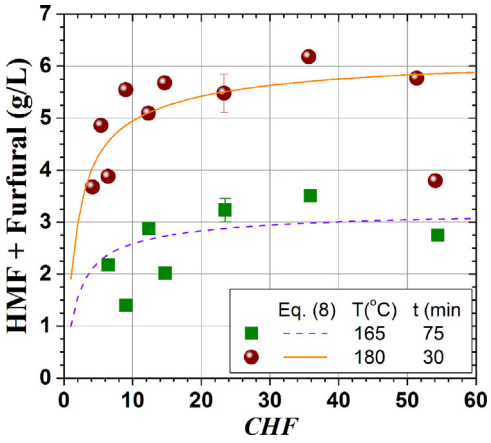

Fig. 3. Correlation between the HMF and furfural concentration in the pretreatment hydrolysate with the combined hydrolysis factor (*CHF*) in comparisons with model predictions.

MA) at 50 °C and 200 rpm. A sodium acetate buffer solution of pH 5.5 at concentration 50 mM was used to conduct enzymatic hydrolysis at an elevated pH, higher than pH 4.8–5.0 used in most literature studies. Our previous studies discovered significant enhancement of enzymatic saccharification of lignocelluloses when hydrolysis of lignocellulose is conducted at elevated pH of 5.5 due to the reduction of nonspecific binding of cellulase to lignin [27–29]. The cellulase loading was 0.1 mL (15 FPU)/g glucan. Duplicate hydrolysis runs were conducted. Hydrolysate samples were taken periodically for glucose determination. The average results from duplicate runs were reported. The substrate enzymatic digestibility (*SED*), defined as the percentage glucan in the substrate enzymatically saccharified to glucose, was determined to represent enzymatic saccharification efficiency.

4. Results and discussion

4.1. Enzymatic saccharification efficiency and furan formation at 165 °C and 180 °C

While the same range of *SED* values can be obtained at the two temperatures, the total furan concentration, i.e., HMF + furfural, in the pretreatment spent liquors (hemicellulosic sugar streams) is higher at the higher temperature (Fig. 1). Pretreatments at 180 °C produced approximately double the concentration of furans as those at 165 °C. Furthermore, under at least two pairs of identical chemical loadings, pretreatments conducted at 165 °C for 75 min produced similar *SED* (>90%) as those conducted at 180 °C for 30 min. The format of (0.4, 10, 75) is used to represent pretreatment chemical loadings of (acid concentration in v/v%, bisulfite charge on od wood in w/w%, pretreatment duration) or simply (0.4, 10) to represent pretreatment chemical loading throughout the manuscript as shown in Fig. 1.

4.2. Using CHF to predict hemicellulose hydrolysis of Douglas-fir

When compared to our previous work with aspen [15], the current study did not include any low *CHF* conditions. The amount of xylan + mannan remaining varied between 5% and 25% (Fig. 2), thus, we do not have sufficient data to completely refit the model. Since xylan and mannan are the major hemicelluloses and other minor hemicelluloses were undetectable in all pretreated solids (Table 2), the sum of xylan and mannan were used as hemicelluloses in data analysis. We can refit the amount of the slow xylan + mannan, as it is expected to be different for different wood species. The values of adjustable parameters $\alpha = 28.5$, $\beta = 17$, $\gamma = -10$ and activation energy $E = 100{,}000$ were based on xylan + mannan removal from a separate pretreatment study of lodgepole pine under a range of pretreatment conditions including low *CHF* conditions [30]. The best fit of the Douglas-fir results shows that the amount of "slow" hemicelluloses $\theta = 18.0\%$, the ratio of reaction rate between "slow" and "fast" hemicelluloses $f = 0.10$. While the data in Fig. 2 show some scatter about the model, a statistical test between paired (Table 1) measurements for 165 and 180 °C at constant *CHF*, did not show any effects at a 95% confidence level on hemicellulose remaining. All fitting parameters are listed in Table 3 to facilitate reading.

A further observation can be made when compared to our previous work [15], with aspen *CHF* values of 30 resulted in low values of residual xylan <1%, but with Douglas-fir, the xylan + mannan was never below 5% even at *CHF* value greater than 50.

4.3. Variation of sugar degradation with pretreatment temperature and severity CHF

In contrast to the hemicellulose dissolution, the formation of furans from sugar degradation did show a statistically significant difference between two temperatures. When going from 165 to 180 °C, the amount of HMF + furfural formed increased by as much as 100% as xylan + mannan hydrolysis stayed constant (Fig. 3). Different symbols were chosen for the data from each of the pretreatment temperature. A *t*-test of the data from paired pretreatments (Table 1) shows a significant difference between the two temperatures for all *CHF* values, $p < 0.001$. These data suggest that the activation energy of sugar degradation is higher than hydrolysis and that lower temperatures should be favored to reduce furans.

While only two temperatures were used for this study, we can estimate the activation energy E_d defined by Eq. (6). The best fit to the data was obtained with $\alpha_d = 41.0$ and $E_d = 160{,}930$ J/mole (Table 3), which is significantly higher that the value of $E = 100{,}000$ J/mole determined for hemicellulose hydrolysis reported previously [30]. HMF + furfural production can be predicted using Eq. (8) as shown in Fig. 3. Overall the predicted HMF + furfural production agrees with experimental data well except for a data point at 180 °C with severity *CHF* > 50 when furan degradation becomes important. The two pair pretreatments (V and VII in Table 1) conducted using duration time different from that for the rest of the pretreatment runs, i.e., $t^{T=180} = 30$ and $t^{T=165} = 75$, were excluded in Fig. 3 due to complication for plotting, however the predicted values agree with experiments well for these two pairs of pretreatment.

While the sugar degradation products showed a significant temperature effect, the production of acetic acid is not affected by temperature as shown Fig. 4. *T*-testing of the paired data indicated that there was no significant difference in acetic acid

Table 2
Chemical compositions of untreated wood and pretreated solid substrates along with pretreatment solids yields.

Substrate label		Klason lignin (%)	Arabinan (%)	Galactan (%)	Glucan (%)	Xylan (%)	Mannan (%)	Solid yield (w/w %)[b]
Untreated wood		29.3	1.02	2.83	43.04	7.54	9.61	
Pair No	*Pretreated solid substrates*[a]							
I	T165-t75-A1-B8	30.6			58.08	2.64	2.71	65.39
	T180-t30-A1-B8	30.1			59.38	2.27	1.85	62.31
II	T165-t75-A4-B12	24.9			63.23	2.03	2.55	61.16
	T180-t30-A4-B12	27.5			62.06	1.52	1.68	58.46
III	T165-t75-A2.5-B8	31.0			56.28	2.07	2.26	61.24
	T180-t30-A2.5-B8	34.1			51.62	0.70	1.07	65.70
IX	T165-t75-A4-B10	29.3			59.52	2.09	2.09	62.42
	T180-t30-A4-B10	33.5			57.18	0.98	1.41	62.51
V	T165-t50-A4-B8	34.0			57.99	1.57	1.86	62.76
	T180-t20-A4-B8	34.1			54.79	1.70	1.41	65.31
VI	T165-t75-A4-B8R1	34.0			55.24	1.53	1.59	61.07
	T180-t30-A4-B8R1	35.2			55.48	1.44	1.31	61.02
VI	T165-t75-A4-B8R2	34.2			56.98	1.12	1.61	62.79
	T180-t30-A4-B8R2	37.0			55.05	0.70	1.00	60.83
VI	T165-t75-A4-B8R3	34.9			57.03	1.48	1.55	63.41
	T180-t30-A4-B8R3	37.5			55.95	0.86	1.19	61.95
VII	T165-t100-A4-B8	34.2			57.10	1.36	1.40	64.34
	T180-t40-A4-B8	35.2			51.88	1.15	1.05	62.48
VIII	T165-t75-A4-B6	37.8			55.82	0.72	1.22	65.97
	T180-t30-A4-B6	40.0			54.13	0.63	0.92	59.05
IX	T165-t75-A6-B8	38.5			54.65	0.74	1.14	62.02
	T180-t30-A6-B8	39.8			54.24	0.88	0.77	59.68
	T180-t30-A4-B14	23.3			59.99	2.13	4.12	61.91
	T180-t30-A0-B8	30.8			59.48	2.29	2.16	63.53
	T180-t30-A4-B4	43.9			48.98	0.71	1.30	58.53

[a] Txx standards for pretreatment temperature in °C; txx is pretreatment duration in min; Axx is sulfuric acid loading in mL/L; Bxx is sodium bisulfite charge on wood (oven dry weight) in w/w%; Rxx is replicate number for the specified set of condition.
[b] Defined as percent of starting materials recovered as solids.

Table 3
List of fitting parameters.

Hemicellulose dissolution		Sugar degradation	
E	100,000 J/mole [30]	E_d	160, 930 J/mole
α	28.5 [30]	α_d	41.0
β	17.0 [30]		
γ	−10 [30]		
θ	0.18		
f	0.10		

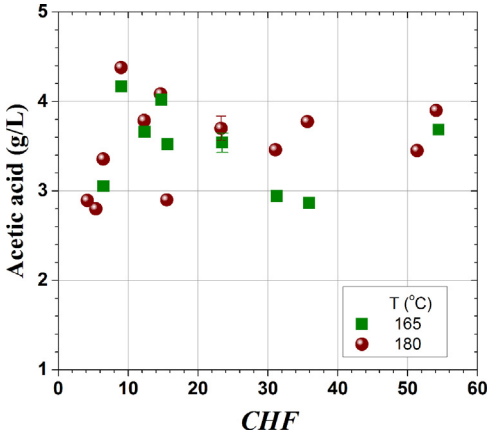

Fig. 4. Comparisons of acetic acid production as a function of pretreatment severity *CHF* from SPORL pretreatments at 165 °C and 180 °C.

concentration in the pretreatment hydrolysate. In fact for the range of *CHF*s studied, the variation in acetic acid concentration was small, this is because acetic acid is formed by the hydrolysis of acetyl groups on hemicelluloses and is not a degradation product.

4.4. Delignification

Sulfite has been used to solubilize lignin through sulfonation reactions to produce chemical pulps in the pulp and paper industry. Acid pretreatments are known to cause condensation reactions that make lignin more difficult to remove. While *CHF* can provide good predictions for hemicellulose dissolution (Fig. 2) and furan production (Fig. 3), it cannot provide good predictions of delignification. The general trend of reduced delignification as *CHF* increases suggests lignin condensation reactions took place. The addition of bisulfite, however, facilitated lignin removal, as one would expect. A very strong correlation between sodium bisulfite concentration and delignification was obtained for pretreatments at constant sulfuric acid loading of 2.21% (w/w) (Fig. 5). Furthermore, delignification was independent of pretreatment temperature (Fig. 5). This suggests that not only similar hemicellulose removal but also

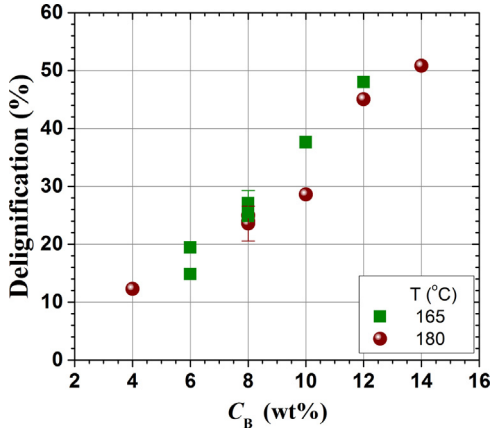

Fig. 5. Correlations of delignification with sodium bisulfite loading at constant sulfuric acid charge of 2.21%.

the same degree of delignification can be achieved at different temperatures as long as the pretreatment severities and the chemical loadings were the same, important to ensure similar substrate enzymatic saccharification as discussed below.

4.5. Effect of hemicellulose and lignin removal on enzymatic saccharification

For aspen, *SED* correlate with xylan dissolution, or *CHF* [15], suggesting pretreatments with similar *CHF* produce substrates with similar *SED* independent of pretreatment temperature. Delignification becomes important for enzymatic saccharification of softwoods with higher lignin content [4]. Because *CHF* is a hemicellulose hydrolysis severity measure and is not a delignification severity measure, we use a simple bilinear model (Eq. (11)) to fit *SED* data with the fractions of remaining hemicelluloses and lignin in substrate, X_R and X_{Lignin}, to provide reasonable estimates. The amount of delignification varied from 10% to 50% (Fig. 5) for the SPORL pretreatments conducted in the present study and cannot be neglected.

$$SED = C + A \cdot X_R + B \cdot X_{Lignin} \quad (11)$$

where *SED*, X_R and X_{Lignin} are in fractions. Using the *SED* data obtained at CTec2 loading of 15 FPU/g cellulose and remaining xylan and mannan for X_R, we obtained $C = 1.70$, $A = -1.36$, $B = -0.96$. Reasonable predictions of *SED* were obtained to within approximately ±5% of the experimental data (Fig. 6).

4.6. Estimation of optimum temperature and time for SPORL

We reexamine the data presented in Fig. 1 to balance sugar yield and furan formation for pretreatment optimization. The sugar yields and furan concentration in the pretreatment spent liquor from 15 pretreatments were compared to determining the optimal pretreatment conditions (Table 4). Although different criteria can be applied to rank these pretreatments, we separately ranked the pretreatments conducted at 165 °C from those at 180 °C each in the order of total monomeric sugar yield. The results clearly show that pretreatments conducted at 165 °C not only produced higher total monomeric sugar yield, but also significantly lower furan formation than those conducted at 180 °C under the same chemical loadings. Both runs T165-t75-A4-B10 and T165-t75-A4-B12 achieved excellent sugar yield. The lower furan concentration of run T165-t75-A4-B12 is very attractive. The slightly lower sugar yield may

Table 4
Sugar yields and furan concentrations of comparative pretreatments at 165 °C and 180 °C.

Rank	Pretreatment label	*SED* (%)	EHGY[a]	Mannose yield[a]	Xylose yield[a]	G + M + X yield[b]	HMF (g/L)	HMF + Furfural (g/L)	Acetic acid (g/L)
2-T165	**T165-t75-A4-B12**	**91.3**	**39.23**	**5.97**	**1.43**	**48.91; 72.9**	**0.94**	**1.40**	**4.17**
1-T180	T180-t30-A4-B12	93.8	37.80	5.08	1.13	46.75; 69.7	3.99	5.54	4.38
	T165-t75-A2.5-B8	82.3	31.74	6.22	1.61	42.04; 62.7	1.93	2.87	3.66
2-T180	T180-t30-A2.5-B8	92.4	34.83	6.28	1.62	45.78; 68.3	3.59	5.09	3.79
1-T165	**T165-t75-A4-B10**	**94.2**	**38.91**	**6.92**	**1.87**	**50.52; 75.3**	**1.35**	**2.02**	**4.02**
	T180-t30-A4-B10	92.0	36.54	3.88	0.97	43.71; 65.2	3.88	5.68	4.08
	T165-t50-A4-B8	77.5	31.34	7.33	2.11	43.58; 65.0	1.20	1.86	3.52
	T180-t20-A4-B8	88.4	31.74	6.62	1.88	43.65; 65.1	2.59	3.74	2.90
4-T165	T165-t75-A4-B8Avg	89.0	34.83	7.02	2.02	47.10; 70.2	1.98	3.23	3.54
4-T180	T180-t30-A4-B8Avg	88.1	33.36	5.74	1.54	44.26; 66.0	3.64	5.48	3.70
3-T165	T165-t100-A4-B8	89.2	35.65	6.65	1.99	47.78; 71.3	2.51	3.90	2.94
	T180-t40-A4-B8	93.3	33.61	4.92	1.26	43.61; 65.0	4.22	6.53	3.46
	T165-t75-A6-B8	84.0	31.63	6.75	1.93	44.68; 66.6	1.41	2.75	3.68
	T180-t30-A6-B8	86.6	28.51	6.06	1.68	41.19; 61.4	2.10	3.80	3.90
3-T180	T180-t30-A4-B14	94.5	39.04	4.00	1.00	45.15; 67.9	3.61	4.86	2.80

[a] EHGY stands for enzymatic hydrolysis glucose yield. All yields are in wt% untreated wood. Oligomeric sugars are not included.
[b] G + M + X stands for total glucose (including both EHGY and pretreatment dissolved glucose), mannose, and xylose yield. The first number is in wt% untreated wood. The second number is in % of theoretical wood sugar.

Table 5
Performance of the SPORL process at 2.21% sulfuric acid and 10% sodium bisulfite charges.

Time (min)	Pretreatment temperature (°C)						
	165	167.5	170	172.5	175	177.5	180
10	Low *SED*	Low *SED*	Low *SED*	Low *SED*	Low *SED*	Low *SED*	Low *SED*
15	Low *SED*	Low *SED*	Low *SED*	Low *SED*	Low *SED*	Low *SED*	Low *SED*
20	Low *SED*	Low *SED*	Low *SED*	Low *SED*	Low *SED*	Low *SED*	Inhibit
25	Low *SED*	Low *SED*	Low *SED*	Low *SED*	Low *SED*	Inhibit	Inhibit
30	Low *SED*	Low *SED*	Low *SED*	Low *SED*	Inhibit	Inhibit	Inhibit
35	Low *SED*	Low *SED*	Low *SED*	OK	Inhibit	Inhibit	Inhibit
40	Low *SED*	Low *SED*	OK	Inhibit	Inhibit	Inhibit	Inhibit
45	Low *SED*	Low *SED*	OK	Inhibit	Inhibit	Inhibit	Inhibit
50	Low *SED*	OK	Inhibit	Inhibit	Inhibit	Inhibit	Inhibit
55	Low *SED*	OK	Inhibit	Inhibit	Inhibit	Inhibit	Inhibit
60	OK	OK	Inhibit	Inhibit	Inhibit	Inhibit	Inhibit
65	OK	Inhibit	Inhibit	Inhibit	Inhibit	Inhibit	Inhibit
70	OK	Inhibit	Inhibit	Inhibit	Inhibit	Inhibit	Inhibit
75	OK	Inhibit	Inhibit	Inhibit	Inhibit	Inhibit	Inhibit
80	OK	Inhibit	Inhibit	Inhibit	Inhibit	Inhibit	Inhibit
85	Inhibit	Inhibit	Inhibit	Inhibit	Inhibit	Inhibit	Inhibit

Low *SED*: *SED* < 90%; inhibit: inhibitor level > 3 g/L; OK: *SED* ≥ 90% and inhibitor level ≤ 3 g/L.

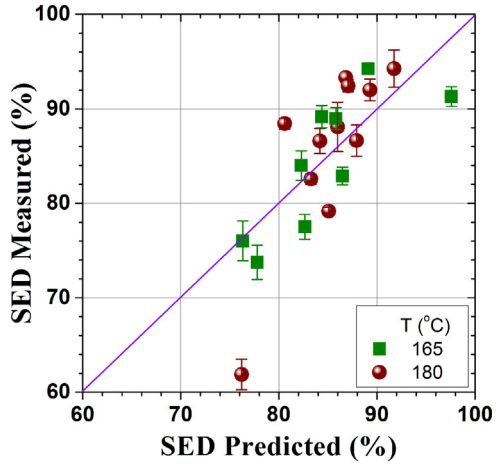

Fig. 6. Comparisons of model (Eq. (11)) predicted and measured substrate cellulose enzymatic digestibility (*SED*).

be due to oligomeric sugars that were unaccounted for by the data shown. The acetic acid concentrations are all below 4.5 g/L, not an issue for most organisms.

Using the predictive models for furan formation (Eq. (8)) and *SED* (Eq. (11), we can predict *SED* and inhibitor concentrations for any set of pretreatment temperature and duration under given sulfuric acid and sulfite loadings. The performance of a SPORL process at 2.21% sulfuric acid and 10% sodium bisulfite was given in Table 5 as an example. For a viable process, we need the *SED* to be greater than 90% and the inhibitor level to be below 3 g/L. For low temperatures and short times the hemicellulose removal is insufficient, but at higher temperatures and longer times the amount of inhibitors is too high for efficient fermentation, thus for intermediate times and temperatures a viable process is possible, as indicated with an "OK" in this Table 5.

5. Conclusions

SPORL pretreatments of Douglas-fir were conducted at two temperatures. The combined hydrolysis factor (*CHF*) was extended to Douglas-fir for predicting hemicellulose dissolution. Significantly higher furans were produced at the higher temperature when hemicellulose dissolution was held constant. A first order kinetic model was developed to predict sugar degradation to furans. The estimated activation energy of sugar degradation was higher than that of hemicellulose hydrolysis. The models were used to determine region of time and temperature parameter space in designing viable low temperature pretreatment processes with high sugar yield and low inhibitor production to facilitate effective bioconversion of lignocelluloses.

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