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Quantitative predictions of bioconversion of aspen by dilute acid and SPORL pretreatments using a unified combined hydrolysis factor (*CHF*)☆

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ABSTRACT

A combined hydrolysis factor (*CHF*) was developed to predict xylan hydrolysis during pretreatments of native aspen (*Populus tremuloides*) wood chips. A natural extension of previously developed kinetic models allowed us to account for the effect of catalysts by dilute acid and two sulfite pretreatments at different pH values. When xylan is modeled as two fractions with different hydrolysis rates, previously identified as fast and slow xylan, the model closely matches the experimental data. Extent of xylan hydrolysis is strongly correlated with pretreatment solids yield, energy consumption for size reduction, and substrate enzymatic digestibility (SED). Composition of the pretreatment hydrolysate was less correlated with extent of hydrolysis due to carbohydrate decomposition reactions. Substrate cellulose enzymatic conversion and enzymatic hydrolysis glucose yield can be predicted to approximately 10% accuracy using *CHF* alone.

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1. Introduction

Pretreatment and fractionation of lignocelluloses are essential for optimal utilization of plant biomass in biorefineries [1,2]. The chemical reactions taking place during pretreatment and fractionation of lignocelluloses are very complex, and typically involve hydrolysis, carbohydrate degradation, delignification and lignin condensation. The relative rates of these reactions depend on concentration of added chemicals and reaction temperatures. There is a large amount of data available for the rates of xylan hydrolysis under a wide range of conditions for many wood species [3].

Developing predictive reaction models that predict response of lignocelluloses is critical for process control and optimization of commercial biomass fractionation processes. Simple kinetics expressions are inadequate [4]. Severity factors, which are often based on kinetic arguments, have been used to combine reaction variables, such as temperature and time, into a single parameter. For example, H-factor [5,6] is used for process optimization and control throughout the chemical pulping industry. The combined severity factor (CSF) is an extension of this concept to include the effect of catalyst on biomass pretreatment [4,7,8]. Abatzoglou et al. [4] developed a general approach that used basic reaction kinetics to formalize the concept of combined severity factor. Good predictions were obtained at low severities, but the predictions were less satisfactory at high severities, for example, with xylan dissolution greater than 70%. Unfortunately, it is these high severity conditions that are of most interest for obtaining efficient biomass fractionation and enzymatic cellulose hydrolysis.

The first objective of the present study is to develop a unified combined hydrolysis factor (*CHF*) to include Sulfite Pretreatment to Overcome Recalcitrance of Lignocelluloses (SPORL) conditions [9,10] that accurately predicts xylan dissolution. SPORL processes have been shown to be very effective for pretreatment of woody biomass before bioconversion [11–14]. Native aspen (*Populus tremuloides*) wood chips were pretreated with dilute acid (DA) and SPORL. Two variants of SPORL pretreatments were used (1) sodium bisulfite and sulfuric acid (SPORL-acid) and (2) sodium bisulfite alone (SPORL high pH). Comparing these two SPORL pretreatments processes allows us to explore catalysis by various sulfur species, i.e., at low pH HSO_3^- and SO_2 will dominate, while in at high pH SO_3^- and HSO_3^- dominates [15,16]. In acid-catalyzed pretreatments, for which delignification was insignificant, xylan removal from plant cell wall was critical to facilitate enzymatic cellulose saccharification. Furthermore, the amount of xylan removal was directly correlated to cellulose hydrolysis efficiency [17,18].

☆ This work is conducted at the USDA Forest Service, Forest Products Laboratory while Zhu (W) was a visiting student form South China University of Technology and on Official government time of Zhu (J.Y.), Houtman, and Gleisner.

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Therefore, the second objective is to develop good correlations for prediction of key bioconversion process variables, such as, energy for size reduction, solid substrate enzymatic digestibility (SED), and sugar recovery, which are important for commercial scale economics.

2. Mathematical reaction model

The acid hydrolysis of lignocelluloses can be characterized by a phenomenological 2nd order rate expression, 1st order in catalyst and 1st order in xylan:

$$\frac{dX_R}{dt} = -k(T, C, \ldots)CX_R \quad (1)$$

where X_R is fraction of xylan retained in the solids or residual xylan, T is temperature, C is the catalyst concentration, and k is a rate constant expression. Many researchers have developed expressions for k [4,19]. Springer [19] observed that the expression shown in Eq. (2) fit the acid hydrolysis of aspen well.

$$\mathrm{Log}_{10}\frac{K_x}{C_H} = 15.083 - \frac{6171.3}{T} + 0.22219C_H \quad (2)$$

In this expression, K_x is the pseudo 1st order rate constant for the loss of xylan; T is absolute temperature; and C_H is the molarity of the acid. It can be shown that a similar functional form was used as a starting point by Abatzoglou et al. [4]. Building on these previous publications, we will use the expression shown in Eq. (3) for k.

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

In this expression, C_A and C_B are the initial concentrations of the acid and bisulfite respectively; α, β and γ are adjustable parameters, E is the apparent activation energy, R has a value of 8.314 J/mole K, and T is absolute temperature (K). For isothermal conditions and a specific reaction time (t) Eq. (3) leads to the following definition of a severity factor, which we will call combined hydrolysis factor (*CHF*), to distinguish it from *CSF* used in the literature [7].

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

Furthermore, many researchers [20–23] have observed that hardwood xylan appears to exist as two different components; one is hydrolyzed more slowly than the other. This observation is consistent with the two functions of hemicelluloses in wood cell walls [24,25]. Following the suggestion of Harris et al. [3], we assume that the fast and slow xylan have the same fundament chemistry. Therefore the rate of xylan dissolution can be expressed as

$$\frac{dX_f}{dt} = -kCX_f \quad (5a)$$

$$X_f(t=0) = (1-\theta)X_R \quad (5b)$$

$$\frac{dX_s}{dt} = -fkCX_s \quad (6a)$$

$$X_S(t=0) = \theta X_R \quad (6b)$$

where X_f and X_s are fractions of fast and slow residual xylan in the solids, respectively, with $X_R = X_f + X_s$ and X_R ($t=0$) = 1. k in Eq. (5a) is the rate constant for fast xylan hydrolysis and f in Eq. (6a) is the ratio of the rate constants between the slow and fast xylan hydrolysis reactions. θ is the initial fraction of slow reacting xylan. Integrating Eqs. 5 and 6 we obtain the following expression for X_R.

$$X_R = (1-\theta)e^{-CHF} + \theta e^{-fCHF} \quad (7)$$

Eq. (7) is an analytical solution for xylan dissolution based on kinetics but expressed in terms of a combined hydrolysis factor, *CHF*. By fitting the xylan content measured in the pretreated substrate using Eq. (7) to this newly defined *CHF*, we can obtain the parameters E, α, β, γ, for *CHF* calculations along with the initial fraction of slow reacting xylan, θ, and the ratio of the rate of constant between slow and fast xylan, f.

The rational of taking this *CHF* approach rather than the combined severity factor *CSF* as defined in the literature [7] are three: (1) the *CSF* cannot fit the xylan dissolution data well under severe hydrolysis conditions [4], which are of most interest for lignocellulose bioconversion. (2) The *CSF* is linearized around a particular temperature, which can result in inaccuracies when conditions stray far from the reference temperature. (3) CSF only accounts for acid catalysis, and thus cannot be applied to pretreatments such as SPORL.

3. Materials and methods

3.1. Materials

Fresh aspen (*P. tremuloides*) wood logs were obtained from northern Wisconsin, USA. The logs were hand-debarked and then chipped at the U.S.D.A. Forest Products Laboratory using a pilot chipper. The wood chips were then screened to remove the majority of material greater than 38 mm and less than 6 mm in length. The thickness of the accepted chips ranged from 1 to 5 mm. The chips were kept frozen until use. The chemical composition of the aspen wood chips is listed in Table S1 (in the Supplemental file).

Celluclast 1.5 L and Novozyme 188 (β-glucosidase) were generously provided by Novozymes North America (Franklinton, NC). Sodium acetate, sulfuric acid, and sodium bisulfite were all ACS reagent grade and used as received from Sigma–Aldrich (St. Louis, MO).

3.2. SPORL and dilute acid (DA) substrate production

All substrates were produced by following the schematic flow diagram shown in Fig. 1. Sub-processes connected with dashed lines were not carried out in this study. Single pretreatment experiments used 150 g oven dry (od) wood chips. The pretreatment liquid to wood ratio (L/W) was 3 (v/w) for all conditions. The wood chips and pretreatment solution were placed in sealed stainless steel 1-L pressure vessels (manufactured in-house). Three 1-L vessels were mounted inside of a 23-L wood pulping digester in an autoclave configuration as described elsewhere [9,26]. The 1-L pressure vessels were heated externally using steam. The digester was rotated end-for-end at 2 rpm for mixing. Pretreatments were conducted at 160, 170, and 180 °C. The temperature ramping time to these temperatures were approximately 5–10 min. Pretreatment duration was varied from 0 to 30 min with an increment of 10 min. Sulfuric acid charges of 0, 0.55, 1.10, and 1.65% (w/w) and sodium bisulfite charges of 1.5, 3.0, and 4.5% on od wood were used in SPORL pretreatments. Sulfuric acid charges were 1.1% for pretreatments conducted at 160 and 180 °C. Acid charges of 0, 0.55, 1.1, 2.2, 3.85, 4.5% were used at 170 °C. These experiments were designed so that comparisons of sugar and ethanol productions between SPORL and DA can be made under the same conditions except for the sodium bisulfite applied in all SPORL runs (Table S1).

The pretreated wood chips, which remained intact, were separated from the pretreatment hydrolysate using a screen. The pretreatment hydrolysates were analyzed for chemical composition. A disk refiner, equipped with plates of pattern D2-B505 (Andritz Sprout-Bauer Atmospheric Refiner, Springfield, OH), was used to accomplish the size reduction. The plate gap was set at 0.76 mm. Water was added during size reduction, which resulted in a solids discharge consistency of 10%. The energy consumption for this step was recorded as described elsewhere [27,28]. The size-reduced solids were directly dewatered by vacuum pressing using a canvas bag to a solids content of about 30%, without a separate washing step. The yield of solid (substrate) in the form of fibers or fiber bundles was then determined from the weight and moisture content of the collected substrate. The moisture content was determined gravimetrically by drying a portion of the collected solids in an oven at 105 °C overnight.

3.3. Enzymatic hydrolysis

Enzymatic hydrolysis experiments were conducted to measure the enzymatic hydrolysis glucose yield (EHGY) in terms of g/kg untreated wood using the SPORL and DA substrates. Enzymatic hydrolysis was conducted using commercial enzymes at 2% substrate solids (w/v) in 50-mL of sodium acetate buffer (pH 4.8, concentration 50 mM) on a shaker/incubator (Thermo Fisher Scientific, Model 4450, Waltham, MA) at 50 °C and 200 rpm. Enzyme loading was Celluclast 1.5 L at 10 FPU/g glucan and Novozyme 188 (β-glucosidase) at 15 CBU/g glucan. Hydrolysate was sampled periodically for glucose concentration analysis. Each data point is the average of two replicates. The average relative standard deviation was approximately 2%.

Fig. 1. Schematic experimental flow diagram showing pretreatment, substrate production, and enzymatic cellulose saccharification. (1) Digester; (2) screen; (3) disk mill; (4) vacuuming canvas bag; (5) shaking incubator.

3.4. Analytical methods

The chemical compositions of the original and pretreated biomass were analyzed by the Analytical and Microscopy Laboratory of the Forest Products Laboratory [12,26]. Inhibitor concentrations in the pretreatment hydrolysates were measured using an HPLC equipped with an Econosphere™ C18 column (5-mm particle size, 250 mm × 4.6 mm, Alltech, Deerfield, IL) and a UV1000 ultraviolet detector (277 nm; Thermo Finnigan, San Jose, CA). Samples were run at ambient temperature and eluted at 0.8 mL/min with a linear gradient of 50–100% acidified methanol (containing 0.25% acetic acid) over 15 min. All analyses were carried out in duplicate. The average data were reported. For fast analysis, glucose in the enzymatic hydrolysate was measured in duplicate using a commercial glucose analyzer (YSI 2700S, YSI Inc., Yellow Springs, OH).

3.5. Definitions and calculations

The fraction of residual xylan in solid substrate, X_R, is determined from the measured substrate xylan content, C_{xylS}, and the solid substrate yield, S (Table S2 in the supplementary file).

$$X_R = \frac{S \cdot C_{xylS}}{100 Cxyl WD} \quad (8)$$

where C_{xylWD} = 16.4 is the xylan content of the untreated aspen wood (Table S2).

Enzymatic hydrolysis glucose yield, EHGY (g glucose/kg wood), is defined as the amount of glucose (g) produced from one kg of untreated wood after enzymatic hydrolysis. Xylose yield, Y_{xylose} (g xylose/kg wood), from pretreatment is determined from the product of measured xylose concentration (g/L) in the pretreatment hydrolysate and the pretreatment liquid to wood ratio (L/W = 3).

3.6. Statistical calculations

Parameters for non-linear regressions were obtained by minimizing the sum of the squared difference between the data and model, using the SOLVER add-in for Microsoft Excel. The spreadsheets are available from the authors upon request. The estimates of 95% confidence limits for the fitted parameters were obtained using the *F*-test for a nonlinear model described by Box et al. [29]. The determination of the adequacy of a particular model was done by using *F*-testing of nested regressions [29].

4. Results and discussion

4.1. Xylan hydrolysis and determination of combined severity factor (CHF)

As described above, pretreatments were conducted over a range of temperatures with various acid and bisulfite concentrations. Inspection of the xylan dissolution data reveals that we chose conditions that ranged from mild to severe, i.e., residual xylan varied from 0.9 to 0, but no trends can be observed using any single reaction variable (data not shown). Using the combined hydrolysis factor (*CHF*), as defined in Eq. (4), to transform the temperature, time and concentration to a single variable, the xylan dissolution data all lie on one curve when plotted versus *CHF* (Fig. 2). Nonlinear fitting of the xylan dissolution data, X_R, using Eq. (7) estimated parameters E, α, β, γ, for calculating *CHF* along with the initial fraction of slow reacting xylan, θ, and the ratio of the rate constant between slow and fast xylan, f.

Note, three experiments were conducted under high acid concentrations. The resulting *CHF* values were 63, 786 and 7925, which gave residual xylan values of 0.059, 0.010, and 0.0077, respectively. These data points reside in the high severity asymptote and were used for the fit but are not shown in Fig. 2, to allow better presentation of the rest of the data. The legend "DA" in Fig. 2 represents experiments labeled as *TxA2B0txx* (Table S1) with zero bisulfite charge, "SPORL high pH" represents experiments labeled as *TxA0B3txx* (Table S1) with zero acid dosage, "SPORL low pH" represents experiments labeled as *TxA2B3txx* (Table S1) with acid concentration of 0.2% and bisulfite charge 3% on wood, "DA acid variation" represents experiments labeled as *T7Ax* (Table S1), with varied acid dosage at 170 °C for 20 min holding time, or numbered as *T7-Ax*, and "Acid and bisulfite variation" represents experiments labeled as *T7AxBx* (Table S1), or numbered as *T7-Bx*, with varied acid and sulfite dosages at 170 °C for 20 min holding time. For convenience, we will use "DA" to refer to the runs labeled as "DA" and "DA

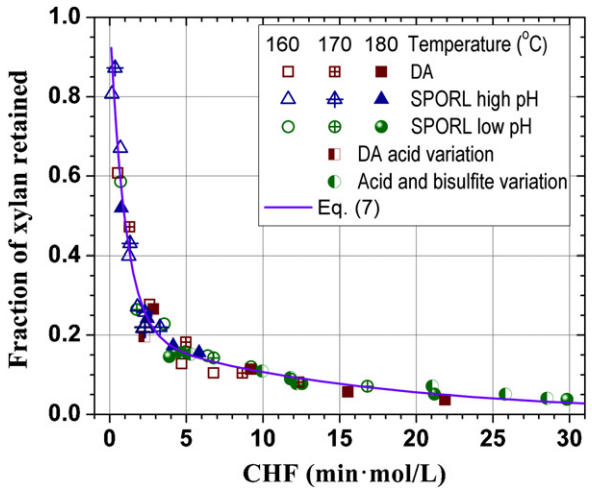

Fig. 2. Comparisons of experimentally measured fraction xylan retained, X_R, from three different pretreatment processes with predictions by model (Eq. (7)).

Table 1
Fitting parameters of xylan dissolution data using Eq. (7). The min and max values are the 95% confidence intervals.

	Min	Fitted value	Max	Suggested value	Unit
E	61,415	90,888	123,371	91,000	J/mole
α	17.33	25.64	34.81	25.6	none
β	25.66	34.51	44.27	34.5	L/mole
γ	−13.6602	−9.9414	−6.1233	−10.0	L/mole
θ	0.1160	0.2045	0.3327		none
f	0.0176	0.0650	0.1308		none

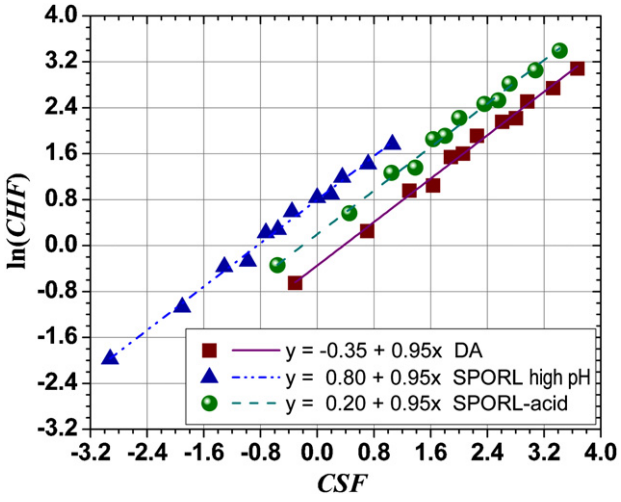

Fig. 3. Correlations between the combined hydrolysis factor (*CHF*) and the combined severity factor (Eq. (9)) for the reaction conditions of three different pretreatment processes conducted. The r^2 of the three correlations all >0.99.

acid variation", and "SPORL-acid" to the runs labeled as "SPORL low pH" and "Acid and bisulfite variation" in the following discussions.

Table 1 lists the parameter estimates and their corresponding confidence limits. The E value, 90,888 J/mole corresponds to 21.7 kcals/mole, 95% confidence interval of 14.7–29.5 kcals/mole. This value is lower than determined by Springer [19], 28.2 kcals/mole and Chum et al. [7], 27 kcals/mole, but our confidence interval contains these other values, so we cannot claim that the value we found in this study is different than previous work. The amount of slowly hydrolyzing xylan was 20.5%.

Since the combined severity factor (*CSF*), Eq. (9), has been used to correlate experimental data, we explored the relationship between *CHF* and *CSF* for the experimental conditions conducted. It was found that ln(*CHF*) is linearly correlated to *CSF* very well for each data set of dilute acid (no sulfite), SPORL high pH (no acid), and SPORL-acid as shown in Fig. 3. Furthermore, the estimated slopes are the same and the differences are only in the y intercepts. This is because the temperature dependences of kinetics are the same for both *CHF* and *CSF* and the differences in the y intercepts were contributed by the catalysts which are different.

$$CSF = \log\left[t \cdot \exp\left(\frac{T - T_{\text{ref}}}{\omega}\right)\right] - \text{pH}$$
$$= \log\left[t \cdot \exp\left(\frac{T - 100}{14.75}\right)\right] - \text{pH} \quad (9)$$

We suggest approximate values of $E = 91{,}000$ J/mole, $\alpha = 25.6$, $\beta = 34.5$, and $\gamma = -10.0$, as listed in Table 1 for calculating *CHF*. This is similar to use constant values of $T_{\text{ref}} = 100$ and $\omega = 14.75$ in calculating *CSF* using Eq. (9) without going through curve fitting (Fig. 2) using Eqs. (4) and (7).

4.2. Statistical analysis of correlations between xylan dissolution and other measurements

A linear correlation matrix was calculated among the xylan dissolution, pretreatment solids, xylose yield, enzymatic glucose yield, energy consumption for size reduction, substrate enzymatic digestibility (SED) that is defined as the percent of glucan in solid substrate converted to glucose enzymatically, Klason lignin content, and concentrations of acetic acid, furfural in the pretreatment hydrolysate (spent liquor). It was found that that residual xylan X_R is correlated strongly to solids yield, disk milling energy consumption, SED and EHGY as listed in Table 2.

While analysis of linear correlation coefficients provides a screening for the major relationships, more detailed analysis can provide insight into the other effects controlling behavior. We determined if the residual xylan captures the variance in the SED data using a nested linear regression analysis. Specifically, we conducted two linear regressions for SED one with only residual xylan as the dependent variable, and another adding acid concentration, bisulfite concentration, treatment time, and temperature as dependent variables. By calculating the residual sum of squared error (SSE_r) and dividing by the change in the number of degrees of freedom, we conducted an F-test to estimate the probability that the reduction in SSE caused by adding dependent variables was due to random variation. The calculated probability is $p = 0.448$ (Table 3). We also tested whether adding Klason lignin to the regression for SED and found that the probability the additional reduction in SSE was due to random variation was $p = 0.498$. Thus, for our pretreatments the major determinant of substrate enzymatic cellulose hydrolysis efficiency is xylan removal. Similar conclusions can be reached in the analysis of EHGY data as well, since this measurement is highly correlated with SED.

4.3. Correlation of pretreatment solids yield with CHF

The solids yields, S, vary with pretreatment type and conditions. In general, a higher temperature and longer pretreatment duration resulted in lower solids yield. Furthermore, the solids yield of a SPORL-acid pretreatment (with bisulfite) is lower than that of a DA pretreatment (no bisulfite) at the same acid charge and

Table 2
Linear correlation coefficients (R^2) between performance parameters.

	Residual xylan	Milling energy	SED	Solids yield	Xylose	Acetic acid	Furfural	EHGY	Klason lignin
Residual xylan	1.000								
Milling energy	0.886	1.000							
SED	0.789	0.756	1.000						
Solids yield	0.813	0.823	0.671	1.000					
Xylose	0.553	0.631	0.726	0.446	1.000				
Acetic acid	0.539	0.703	0.599	0.646	0.599	1.000			
Furfural	0.401	0.420	0.371	0.504	0.134	0.335	1.000		
EHGY	0.697	0.660	0.903	0.466	0.788	0.501	0.143	1.000	
Klason lignin	0.443	0.389	0.395	0.468	0.146	0.237	0.797	0.164	1.000

Table 3
Probability that the variance not explained by xylan hydrolysis is due to random variation.

Parameter	*p* value
SED	0.448
Refine energy	0.002
Solids yield	<0.000
Xylose	0.542
Acetic acid	0.014
Furfural	<0.000
EHGY	0.263
Klason lignin	<0.000

temperature for the same pretreatment duration (Table S2). The SPORL pretreatment at high pH (without acid) produced a higher solid yield than the SPORL-acid (with acid) and DA pretreatment at the same temperature for the same pretreatment duration. This is due to the reduced xylan removal at high pH levels (low *CHF*). Solids loss was mainly due to removals of hemicelluloses (xylan for aspen) and lignin and dissolution of glucan. In acid based pretreatments, such as the three pretreatments examined in the present study, lignin removal was less than 30% (not shown) while hemicellulose removal was more than 60% for most runs (Fig. 2). Glucan dissolution is minimal. Linear regression analysis suggests a good correlation between solids yield and residual xylan with $r^2 = 0.813$ (Table 2). By calculation we determined that the xylan loss accounts for approximately 40% of the total mass loss. Since residue xylan is an exponential function of *CHF*, we can fit the solids yield data using an exponential correlation with *CHF* as shown in Fig. 4 to provide quantitative prediction.

$$S = S_0 + b \cdot \exp\left(\frac{-CHF}{2}\right) = 62 + 27\exp\left(\frac{-CHF}{2}\right) \quad (10)$$

The results in Fig. 4 show a spread up to 15% in solids yields for *CHF* below 15 under different conditions. The spread is due to the spread of lignin removal that is not correlated to *CHF* (not shown).

4.4. Correlation between disk milling energy of pretreated wood chips and CHF

Pretreatment can significantly reduce energy consumption for wood chip size reduction [27]. However, the amount of energy reduction depends on pretreatment process and the pretreatment severity [27]. The energy savings comes from two factors: (1) the reduced amount of solid material due to pretreatment solids loss as discussed in the previous section and (2) the loosening of wood structure due to the removal of wood components, such as hemicelluloses and lignin. Statistical analysis in Section 4.2 indicated that disk milling energy correlates to residual xylan very well with $r^2 = 0.89$ (Table 2). On the other hand, disk milling energy was less correlated to lignin removal with $r^2 = 0.43$ (Table 2). This is consistent with the data in our previous study using four poplar wood samples [17]. As a result, we can expect that the energy consumption for size reduction through disk milling can be predicted using residual xylan content. For simplicity, we can fit the disk milling energy data with *CHF* alone in Fig. 5, which results in the following expression,

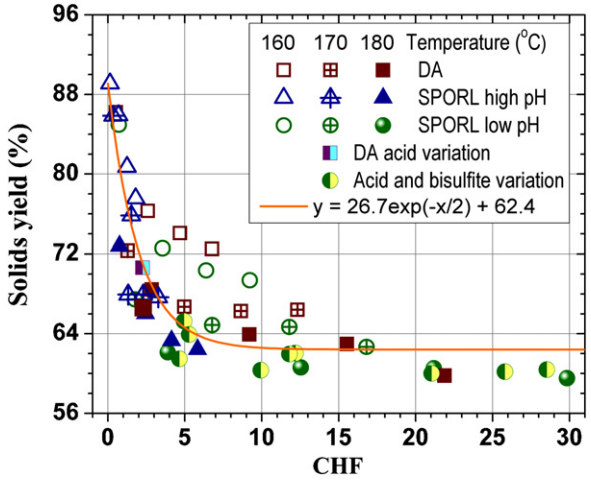

Fig. 4. Correlation between substrate solids yield from three different pretreatment processes and the combined hydrolysis factor (*CHF*). The solid line is Eq. (10).

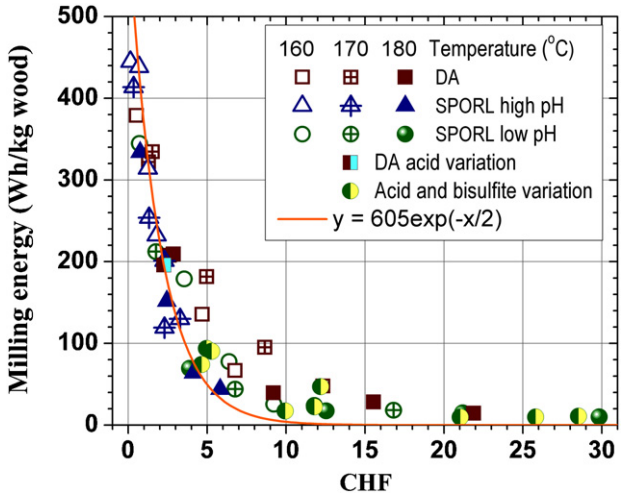

Fig. 5. Experimentally measured energy consumption for size reduction of pretreated wood chips with comparison to predictions by a model based on xylan hydrolysis. The solid line is Eq. (11).

$$E = E_0 \cdot \exp\left(\frac{-CHF}{2}\right) = 605\exp\left(\frac{-CHF}{2}\right) \quad (11)$$

where E_0 is the milling energy for unpretreated (*CHF* = 0) wood chips under the identical milling conditions.

4.5. Correlation of substrate enzymatic digestibility (SED) with CHF

Removal xylan is effective in improving SED by acid based pretreatment due to improved cellulose accessibility. Published data indicated that SED is proportional to substrate xylan content [17,18]. Again we can fit the SED data with *CHF* alone to simplify the empirical expression as shown in Fig. 6b using Eq. (12).

$$\mathrm{SED} = \mathrm{SED}_0 - h \cdot \exp\left(\frac{-CHF}{4}\right) = 93 - 72\exp\left(\frac{-CHF}{4}\right) \quad (12)$$

where SED_0 is the fitting obtained SEDs at 100% removal of xylan, *h* (=72%) is related to the slope between SED and xylan removal (Fig. 6a). For prediction purposes, complete cellulose conversion can be assumed, therefore $\mathrm{SED}_0 \approx 100$.

The fitting results in Fig. 6b show a spread of approximately ±10% in SED from the predicted values. This deviation is due to contributions to SED by random variation (Discussion on nested statistical analysis in Section 4.1) and factors other than xylan removal as shown in Fig. 6a. Eq. (12) provided a very simple and quick estimation of SED by using this unified *CHF* without conducting enzymatic hydrolysis to ±10% accuracy. This is particularly useful for pretreatment scale up to minimize optimization experiments.

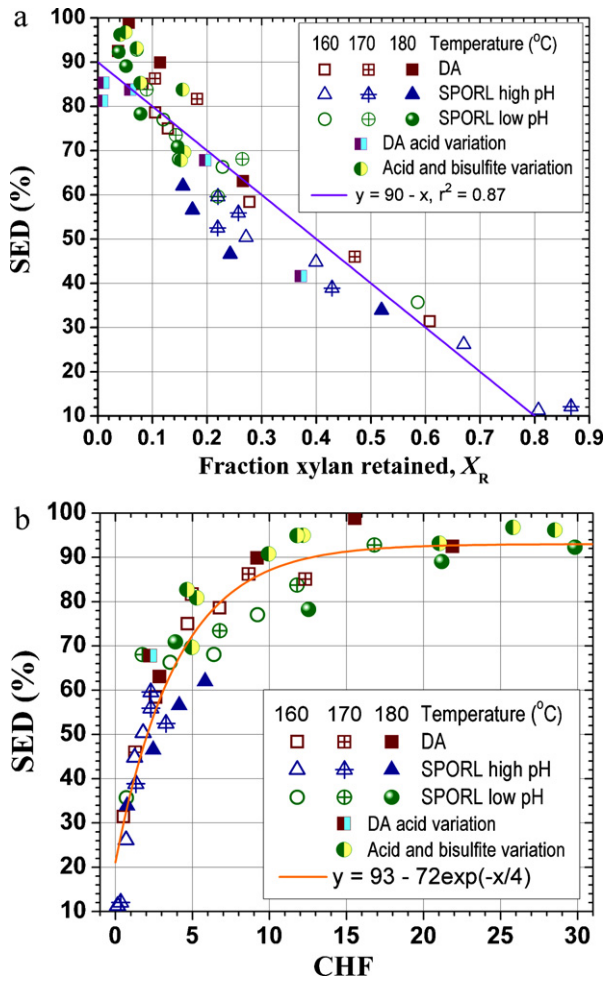

Fig. 6. (a) Correlations between measured substrate cellulose enzymatic digestibility (SED) and fraction xylan retained in solid substrate, X_R. (b) Comparisons between predicted substrate cellulose enzymatic digestibilities (SEDs) based on xylan hydrolysis and experimental data. The solid line is Eq. (12).

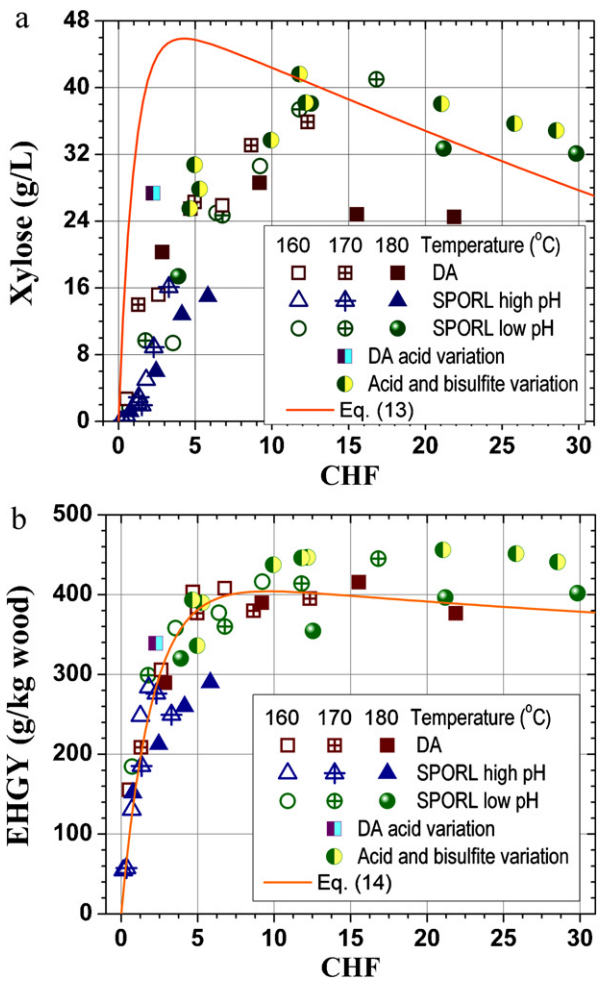

Fig. 7. (a) Xylose concentrations in the pretreatment hydrolysates from three different pretreatment processes and comparisons with estimations based on xylan hydrolysis and furfural productions. (b) Enzymatic hydrolysis glucose yields (EHGYs) from three different pretreatment processes and comparisons with estimations based on xylan hydrolysis and HMF productions.

4.6. Predicting sugar recovery using CHF

Xylose production depends on both xylan hydrolysis and xylose degradation. Nested statistical analysis in Section 4.1 suggests xylan dissolution dictates xylose recovery. We can estimate xylose concentration in the pretreatment hydrolysate by subtracting the amount of furfural produced from the amount of xylan hydrolyzed, i.e.

$$C^{\text{est}}_{\text{xylose}}(\text{g/L}) = \chi\left[10C_{xylWD}\frac{(1-X_R)}{0.88*(\text{L/W})} - A\cdot C_{\text{furfural}}\right] \quad (13)$$

where wood xylan content C_{xylWD} = 16.4%, liquor to wood ratio L/W = 3, 0.88 is the ratio of the molecular weight between xylan and xylose, A = 3.81 is to account for the ratio of molecular weight between xylose and furfural and the further degradation of furfural. χ = 0.9 is a fitting factor due to the incomplete conversion of xylooligomers to xylose. The large discrepancies between the data and Eq. (13) in the region of CHF < 8 are due to incomplete conversion of xylooligomers to xylose. The spread of furfural data shown in Fig. S1 also contributed to the spread of xylose data.

The predictions by Eq. (13) suggest that maximal xylose including oligomeric xylose recovery should be achieved at CHF = 4 (Fig. 7a) at xylan dissolution of 85% (Fig. 2) Xylose degradation dominates beyond CHF = 12 which resulted in the reduction in xylose concentration. At a given CHF < 4, the SPORL-acid pretreatment produced higher xylose concentration than the SPORL high pH pretreatment due to greater conversion of xylooligomers to xylose facilitated by the strong acid hydrolysis in the SPORL-acid process. The degradation of xylose in the SPORL-acid pretreatment was slightly less severe than that in DA at high CHF > 12 as shown in Fig. S1, which results in a higher xylose yields than the DA runs (Fig. 7a).

Enzymatic hydrolysis glucose yield (EHGY) primarily depends on SED and glucose degradation to HMF since glucan degradation in pretreatment is minimal. We can take the similar approach for xylose to use the following equation to estimate EHGY based on Eqs. (12) and (16),

$$\text{EHGY}^{\text{est}}(\text{g/kg wood}) = G(10C_{\text{glnWD}})\left[1-\exp\left(\frac{-CHF}{2}\right)\right] - B\cdot\frac{\text{L}}{\text{W}}\cdot C_{\text{HMF}} \quad (14)$$

Good fitting was obtained as shown in Fig. 7b with G = 0.93 to represent the average glucan yield from pretreatment. Wood glucan content C_{glnWD} = 43.78%, B = 1.52 is to account for the ratio of molecular weight between glucose and HMF and the further degradation of HMF.

4.7. Furan and acetic acid production

Furfural is a product of xylose decomposition and correlated to xylan. Therefore, furfural concentration in the pretreatment hydrolysate can be correlated to *CHF* by Eq. (15) as shown in Figure S1. However, the catalyst effects on the xylose degradation reaction to furfural are different from those on the xylan dissolution reaction, which explains the reduction in predictive power from Eq. (15). Similarly, the HMF data can also be expressed by *CHF* by Eq. (16) as shown in Fig. S1.

$$C_{\text{furfural}} = F_0 \cdot \left[1 - \exp\left(\frac{-CHF}{50}\right)\right] = 17.5\left[1 - \exp\left(\frac{-CHF}{50}\right)\right] \quad (15)$$

$$C_{\text{HMF}} = H_0 \cdot \left[1 - \exp\left(\frac{-CHF}{50}\right)\right] = 1.6\left[1 - \exp\left(\frac{-CHF}{50}\right)\right] \quad (16)$$

Attempts were also made to correlate acetic acid production with *CHF*. The results (not shown) indicate that acetic acid concentration increases with *CHF* and gradually leveled out. This is because acetyl groups are produced by xylan deacetylation, has reaction kinetics different from xylan hydrolysis.

5. Conclusions

This study unified xylan hydrolysis reaction variables, temperature, reaction time, and catalyst concentration, using a combined hydrolysis factor (*CHF*) for three different pretreatments of wood chips of native aspen (*P. tremuloides*). These pretreatments are dilute acid (DA) and Sulfite Pretreatment to Overcome Recalcitrance of Lignocelluloses (SPORL) with or without acid application. Excellent agreement between the predicted residual xylan content of the remaining solids and experimental data were obtained over the entire range of severities. The enzymatic digestibilities of the resultant solid substrates (SEDs) from the three different pretreatment processes were found to correlate to substrate residual xylan content. Evidence for delignification and solution phase decomposition of carbohydrates was found. Since these processes occur by processes other than xylan hydrolysis and less related to *CHF*. The significance of the work is that parameters such as SED can be predicted to approximately 10% accuracy using *CHF*, which can facilitate scale up pretreatment experiments.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.procbio.2012.02.012.

References

[1] Zhu JY, Pan XJ, Zalesny Jr RS. Pretreatment of woody biomass for biofuel production: energy efficiency, technologies and recalcitrance. Appl Microbiol Biotechnol 2010;87:847–57.
[2] Lynd LR. Overview and evaluation of fuel ethanol from cellulosic biomass: technology, economics, the environment, and policy. Ann Rev Energy Environ 1996;21:403–65.
[3] Harris JF, Baker AJ, Connor AH, Jeffries TW, Minor JL, Pettersen RC, Scott RW, Springer EL, Wegner TH, Zerbe JI. Two-stage, dilute sulfuric acid hydrolysis of wood: an investigation of fundamentals. General Technical Report, GTR-FPL-045. In. Madison, WI: USDA Foest Service, Forest Products Laboratory; 1985.
[4] Abatzoglou N, Chornet E, Belkacemi K, Overend RP. Phenomenological kinetics of complex systems: the development of a generalized severity parameter and its application to lignocellulosics fractionation. Chem Eng Sci 1992;47(5):1109–22.
[5] Brasch DJ, Free KW. Prehydrolysis-Kraft pulping of pinus radiata grown in New Zealand. TAPPI 1965;48(4):245–8.
[6] Vroom KE. A means of expressing cooking times and temperatures as a single variable. Pulp Paper Mag Can 1957;58:228–31.
[7] Chum HL, Johnson DK, Black SK, Overend RP. Pretreatment-catalyst effects of the combined severity parameter. Appl Biochem Biotechnol 1990;24/25:1–14.
[8] Larsson S, Palmqvist E, Hahn-Hagerdal B, Tengborg C, Stenberg K, Zacchi G, Nilvebrant N-O. The generation of fermentation inhibitors during dilute acid hydrolysis of softwood. Enzyme Microb Technol 1999;24:151–9.
[9] Zhu JY, Pan XJ, Wang GS, Gleisner R. Sulfite pretreatment (SPORL) for robust enzymatic saccharification of spruce and red pine. Bioresour Technol 2009;100(8):2411–8.
[10] Wang GS, Pan XJ, Zhu JY, Gleisner R. Sulfite pretreatment to overcome recalcitrance of lignocellulose (SPORL) for robust enzymatic saccharification of hardwoods. Biotechnol Prog 2009;25(4):1086–93.
[11] Tian S, Luo XL, Yang XS, Zhu JY. Robust cellulosic ethanol production from SPORL-pretreated lodgepolep pine using an adapted strain *S. cerevisiae* without detoxification. Bioresour Technol 2010;101:8678–85.
[12] Zhu JY, Zhu W, O.Bryan P, Dien BS, Tian S, Gleisner R, et al. Ethanol Production from SPORL-pretreated lodgepole pine: Preliminary evaluation of mass balance and process energy efficiency. Appl Microbiol Biotechnol 2010;86(5):1355–65.
[13] Tian S, Zhu W, Gleisner R, Pan XJ, Zhu JY. Comparisons of SPORL and dilute acid pretreatments for sugar and ethanol productions from aspen. Biotechnol Progr 2011;27(2):419–27.
[14] Zhu JY, Gleisner R, Scott CT, Luo XL, Tian S. High titer ethanol production from simultaneous enzymatic saccharification and fermentation of aspen at high solids: a comparison between SPORL and dilute acid pretreatments. Bioresour Technol 2011;102(19):8921–9.
[15] Bryce JRG. Sulfite pulping. In: Casey J, editor. Pulp and paper: chemistry and chemical technology, vol. I, 3rd ed. New York: John Wiley & Sons; 1980. p. 291–376.
[16] Ingruber O. Sulfite science, Part I. Sulfite pulping cooking liquor and the four bases. In: Ingruber O, Kocurek M, Wong A, editors. Sulfite science and technology, vol. 4, 3rd ed. Atlanta: The Joint Textbook Committee of the Paper Industry, TAPPI/CPPA; 1985. p. 3–23.
[17] Wang ZJ, Zhu JY, Gleisner R, Chen KF. Ethanol production form poplar wood the rough enzymatic saccharification and fermentation by dilute acid and SPORL pretreatments. Fuel 2012;95:606–14.
[18] Yang B, Wyman CE. Effect of xylan and lignin removal by batch and flowthrough pretreatment on the enzymatic digestibility of corn stover cellulose. Biotechnol Bioeng 2004;86(1):88–95.
[19] Springer EL. Hydrolysis of Aspenwood xylan with aqueous solutions of hydrochloric acid. TAPPI 1966;49(3):102–6.
[20] Kobayashi T, Sakai Y. Hydrolysis rate of pentosan of hardwood in dilute sulfuric acid. Bull Agric Chem Soc Jpn 1956;20:1–7.
[21] Maloney MT, Chapman TW, Baker AJ. Dilute acid hydrolysis of paper birch: kinetics studies of xylan and acetyl-group hydrolysis. Biotechnol Bioeng 1985;27(3):355–61.
[22] Montané D, Salvadó J, Torras C, Farriol X. High-temperature dilute-acid hydrolysis of olive stones for furfural production. Biomass Bioenergy 2002;22(4):295–304.
[23] Zhao X, Zhou Y, Liu D. Kinetic model for glycan hydrolysis and formation of monosaccharides during dilute acid hydrolysis of sugarcane bagasse. Bioresour Technol 2012;105:160–8.
[24] Akerholm M, Salmen L. Interactions between wood polymers studied by dynamic FT-IR spectroscopy. Polymer 2001;42(3):963–9.
[25] Olsson A-M, Bjurhager I, Gerber L, Sundberg B, Salmen L. Ultra-structural organization of cell wall polymers in normal and tension wood of aspen revealed by polarization FTIR micro spectroscopy. Planta 2011;233:1277–86.
[26] Luo X, Gleisner R, Tian S, Negron J, Horn E, Pan XJ, et al. Evaluation of mountain beetle infested lodgepole pine for cellulosic ethanol production by SPORL pretreatment. Ind Eng Chem Res 2010;49(17):8258–66.
[27] Zhu W, Zhu JY, Gleisner R, Pan XJ. On energy consumption for size-reduction and yield from subsequent enzymatic sacchrification of pretreated lodgepole pine. Bioresour Technol 2010;101(8):2782–92.
[28] Zhu JY, Wang GS, Pan XJ, Gleisner R. Specific surface to evaluate the efficiencies of milling and pretreatment of wood for enzymatic saccharification. Chem Eng Sci 2009;64(3):474–85.
[29] Box GEP, Hunter WG, Hunter JS. Statistics for experimenters: an introduction to design, data analysis, and model building. New York, NY: John Wiley & Sons; 1978.