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Bioconversion of Woody Biomass to Biofuel and Lignin Co-Product Using Sulfite Pretreatment to Overcome the Recalcitrance of Lignocelluloses (SPORL)

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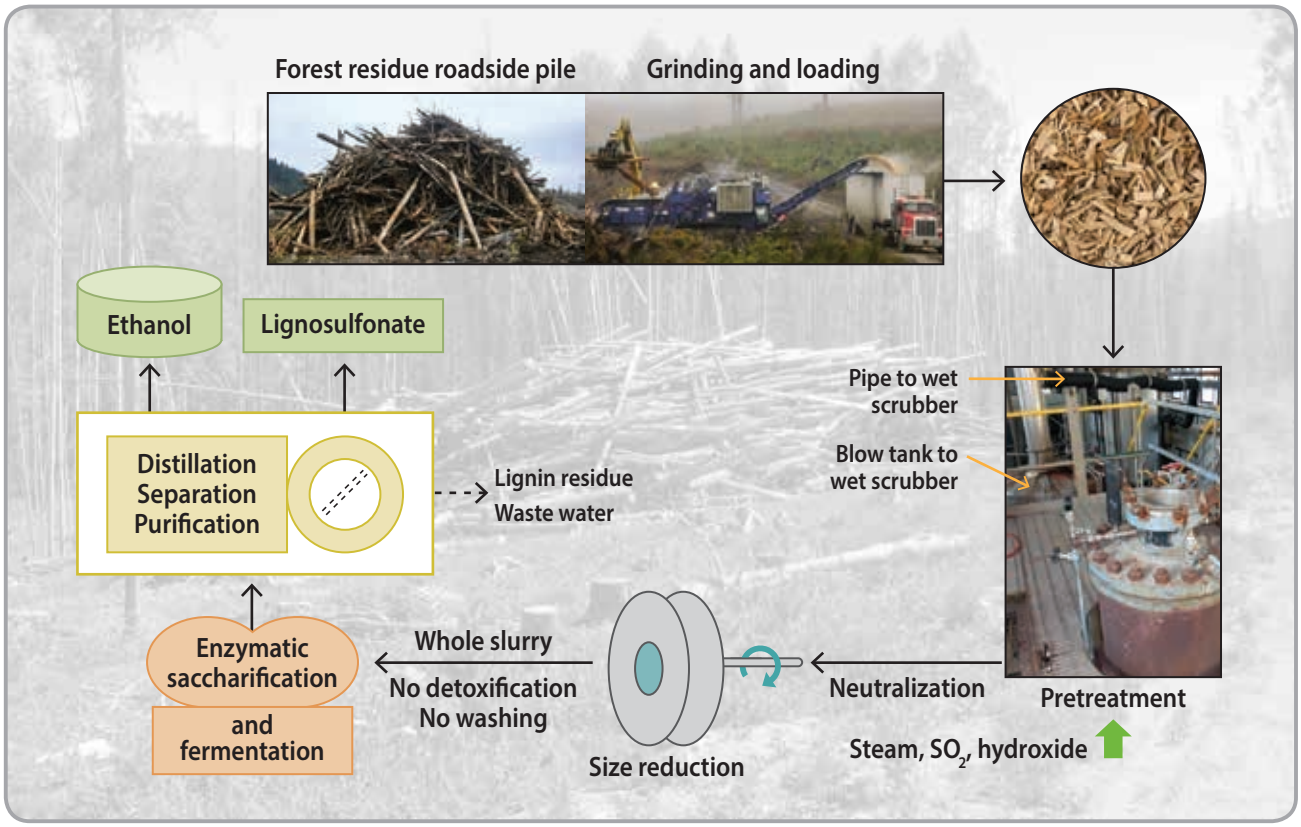

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Abstract

Sulfite pretreatment to overcome the recalcitrance of lignocelluloses (SPORL) promises to provide efficient bioconversion of woody biomass into bioethanol and lignin co-products. Results from several laboratory and pilot-scale studies are presented to demonstrate SPORL performance, with comparisons to competing technologies. Excellent ethanol yields of up to approximately 80% theoretical, based on glucan, mannan, and xylan content of wood, were achieved at titers over 40 g/L. This high productivity was accomplished using low cellulase loadings, 26 mL/kg wood, and without detoxification, solid–liquor separation, or supplementation of nutrients in fermentation. Lignin sulfonation from SPORL likely contributes to this high efficiency by reducing nonproductive binding of cellulase through electrostatic repulsion and reduced hydrophobic interactions between cellulase and lignin. Finally, a reaction-kinetics-based severity factor—the combined hydrolysis factor (CHF)—was developed to facilitate process scale-up and for determining optimum operating conditions for balancing sugar yield against sugar degradation.

Keywords: Sulfite (SPORL) pretreatment, woody biomass, biofuel/bioethanol, enzymatic saccharification/hydrolysis, nonproductive cellulase binding, fermentation, reaction kinetics/severity, process scale-up, lignosulfonate, lignin co-products

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Contents

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1. Introduction

In the past two decades, concerns over climate change, energy security, and sustainable economic development renewed our nation's interest in using renewable natural resources for producing green energy and biochemical and bioproducts using lignocellulosic biomass through the biorefinery concept. The United States has rich forestland with excellent productivity. Efficient utilization of forest biomass is critical to healthy forest management to protect watersheds and preserve ecosystems. Because of its availability and potential of being sustainably produced (Perlack and Stokes 2011) in large quantities in many regions of our country, woody biomass is an important biorefinery feedstock. Woody biomass also has the advantage over herbaceous biomass because of its relatively high density for easing transportation and flexible harvesting schedule for eliminating long-term storage (Zhu and Pan 2010).

Efficient and scalable pretreatment and fractionation of a variety of lignocellulosic feedstock are key to commercial success of bioconversion of lignocellulosic biomass to biofuels, biochemicals, and bioproducts. Unfortunately, when dealing with woody biomass such as harvest forest residues, especially those from softwood species, few pretreatments can effectively overcome its strong recalcitrance to microbial deconstruction for efficient enzymatic saccharification and subsequent bioconversion (Zhu and Pan 2010). Sulfite pretreatment to overcome the recalcitrance of lignocelluloses (SPORL) (Zhu and others 2009, 2015) has demonstrated good performance for efficient bioconversion of woody biomass at a pilot scale (Zhu and others 2015; Zhou and others 2015a, b). The advantage of SPORL lies in several facts: (1) It is commercially scalable because it was developed based on sulfite pulping. (2) The dissolved lignin is a lignin co-product (that is, lignosulfonate) and can be directly marketed without further processing. (3) Lignin sulfonation reduces nonproductive binding of cellulase to lignin, and lignosulfonate acts as a surfactant to enhance enzymatic saccharification (Wang and others 2013a,b; Zhou and others 2013a); therefore, washing of pretreated solids is not only unnecessary but also not preferred, which can simplify process integration and significantly reduce water usage. (4) Low sugar degradation into fermentation inhibitors allows direct fermentation of the pretreated whole slurry without detoxification and without compromising sugar yield even at lower temperatures (Zhou and others 2014; Gu and others 2016). In view of these advantages, the SPORL process may provide a pathway for bioconversion of low-grade woody biomass such as short-rotation poplars and forest harvesting residues.

2. The SPORL Process

The SPORL process was developed based on sulfite pulping with the understanding that the goal of pretreatment is to produce a readily enzymatically digestible solid substrate, different from the goal of chemical pulping, which is to achieve maximal delignification while protecting the strength of cellulosic fibers. Therefore, the SPORL process conditions purposely deviate from those of wood pulping, particularly at elevated temperatures and acidic conditions, as shown on the *T*–pH diagram in Figure 1. This is to increase carbohydrate degradation (including dissolution of hemicelluloses) because hemicellulose removal is critical to increasing cellulose accessibility to cellulase and subsequent depolymerization of cellulose (Leu and Zhu 2013). SPORL has four characteristics: (1) it dissolves a substantial amount of hemicelluloses (Zhu and others 2012; Leu and others 2013; Zhou and others 2013b; Zhang and others 2015); (2) it depolymerizes cellulose; (3) it partially delignifies biomass through lignin sulfonation and therefore the dissolved lignin is highly sulfonated (Zhou and others 2013b, 2014); and (4) the remaining solid lignin is also sulfonated (Lou and others 2013). The solid substrate from SPORL is not suitable to be used as wood fibers because of its low strength due to substantial degradation of polysaccharides and insufficient delignification, but it is readily digestible by enzymes at low dosages for sugar production (Leu and others 2013; Zhu and others 2011a).

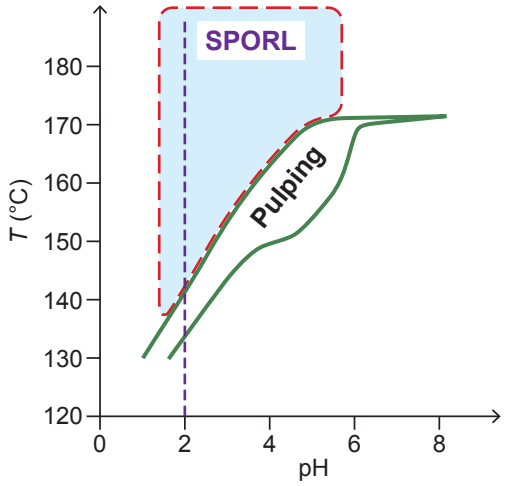

Figure 1—SPORL (sulfite pretreatment to overcome the recalcitrance of lignocelluloses) operating conditions compared with sulfite pulping in the temperature–pH diagram (from Zhu and others (2015)).

2.1 Laboratory Practice of SPORL

Laboratory study of SPORL can be conducted in wood pulping digesters (Fig. 2) using bisulfite such as $NaHSO_3$ and an acid such as H_2SO_4 to adjust pH to approximately 2 to prepare pretreatment liquor. When wood chips are used instead of fiberized materials, the pretreated materials require a size reduction step that can be accomplished by blowing the pretreated materials under pressure, as in steam explosion, or disk milling (Fig. 2), or a combination of the two. Separation of pretreatment spent liquor from pretreated solids is not necessary as SPORL produces a very small amount of inhibitors; furthermore, the dissolved lignin–lignosulfonate can enhance enzymatic saccharification (Wang and others 2013a,b; Zhou and others 2013a). The resultant SPORL whole slurry from size reduction can be directly fed to enzymatic hydrolysis and fermentation after neutralization without washing (Scheme I in Fig. 2). However, the washed water-insoluble solids (WIS) are often used to assess the effectiveness of pretreatment as measured by solid substrate enzymatic digestibility (SED) (Scheme II in Fig. 2). Overall, SPORL has several unique advantages over most competing processes, such as dilute acid (DA), alkaline, aqueous ammonia, ammonia fiber expansion (AFEX), and SO_2 steam explosion. Specifically, SPORL (1) is very effective on softwoods; (2) produces fewer inhibitors than DA and acid-catalyzed steam explosion and therefore detoxification is not necessary for high-titer biofuel production (Zhou and others 2014); (3) generates lignin sulfonation, which reduces nonproductive binding of cellulase to lignin through pH mediation (Lou and others 2013); (4) produces dissolved lignin–lignosulfonate, which is a surfactant that can be used to enhance enzymatic saccharification (Wang and others 2013a) and which can also be directly marketed as a co-product; and (5) does not require separation of pretreated solids from spent liquor, because of the above-mentioned effects of lignosulfonate and because using unwashed solids is preferred, which can substantially simplify process integration.

2.2 SPORL Operating Conditions

SPORL should be practiced, as in sulfite wood pulping (Bryce 1980), where sulfur dioxide (SO_2) is adsorbed into a hydroxide solution (Zhu and others 2015) to prepare the pretreatment solution. For softwoods, typical (minimal) chemical loadings of SO_2 and a base chemical, such as CaO, on wood are at approximately 6.5 and 1.8 wt%, respectively. This results in a pretreatment liquor with an initial pH slightly below 2.0 (measured at ambient temperature),

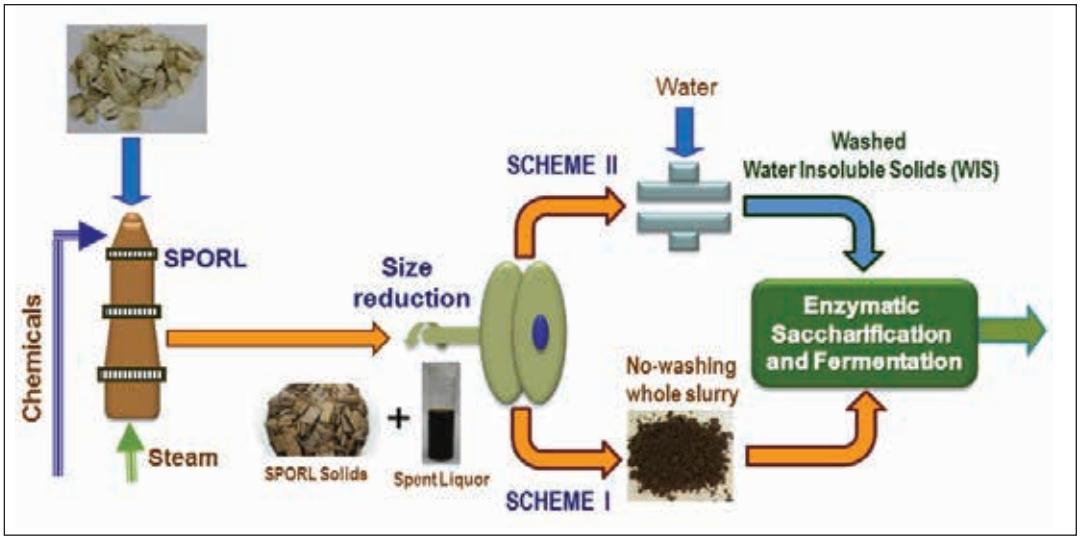

Figure 2—Schematic flow diagram of wood chip pretreatment using SPORL along with two schemes of enzymatic saccharification and fermentation at laboratory bench scale: (I) using washed water-insoluble solids (WIS) only; (II) using the pretreated whole slurry without solids and liquor separation or solids washing.

Table 1—Typical pretreatment conditions of SPORL for different feedstock

Reaction conditions	Softwoods	Hardwoods or herbaceous biomass
Temperature (°C)	140–180	130–170
Time (min)	240–30	240–30
Liquor to wood (L/kg)	2–5	2–6
Catalyst choice I (wt% wood)		
A: H_2SO_4	~2.0	~1.0
B: Bisulfite (e.g., $NaHSO_3$)	8–12	3–5
Catalyst choice II (wt% wood)		
A: SO_2	6.5–20	3.0–10
B: Hydroxide (e.g. CaO)	1.0–2.0	0.5–1.0

which gives excellent pretreatment (Zhu and others 2015). This level of SO_2 loading is only approximately 30% of the amount used in wood pulping. With SPORL, the typical liquor to wood ratio (v/w) is between 3 and 4 and pretreatment temperature can be varied between 140 and 180 °C with corresponding pretreatment times of 30–240 min using this minimal SO_2 loading. The details for determining pretreatment duration using a severity factor at a given temperature are presented Section 4.5. Increased SO_2 loading can significantly reduce pretreatment duration, as found in our recent study (Gu and others 2016).

For easing liquor preparation in laboratory studies, sodium (or magnesium, calcium, ammonia, for example) bisulfite HSO_3^{-1} (Zhu and others 2009; Wang and others 2009, 2015) or sulfite SO_3^{-2} (Shuai and others 2010), or a combination of the two, can be used with the addition of an acid to adjust pH to approximately 2.0, as discussed in Section 6. The term sulfite and bisulfite are used interchangeably in SPORL because the amount of the active reagents bisulfite (HSO_3^{-1}), sulfite (SO_3^{-2}), and free sulfur dioxide (SO_2) present in the pretreatment liquor depends on the pH of the liquor (Ingruber 1985). Most reported SPORL studies used sodium bisulfite ($NaHSO_3$) with sulfuric acid (H_2SO_4) at loadings on wood between 3 and 12 wt% and 1.1 and 2.2 wt%, respectively, depending on the feedstock (Zhu and others 2009, 2012; Leu and others 2013; Zhou and others 2013b; Zhang and others 2014, 2015; Cheng and others 2015). The ranges of typical pretreatment conditions are listed in Table 1.

3. Reaction Kinetics for Process Optimization and Scale-up

Rather than using conventional statistical factorial design for SPORL process optimization, a kinetic-based reaction model and parameters were developed for SPORL process optimization and scale-up design. This is because (1) statistical factorial design assumes only one set of optimal condition in a range studied (that is, monotonic process behavior), which is not always true, especially for a process like SPORL that involves several process variables and (2) maximal sugar yield does not necessarily translate to maximal biofuel yield due to process inhibition in downstream processing such as fermentation. Therefore, process optimization allowing balancing sugar yield with inhibitor formation is desirable. This section describes user-friendly reaction kinetics models for process optimization and design.

3.1 Pretreatment Severity—Combined Hydrolysis Factor (CHF)

The chemical reactions taking place during pretreatment of lignocelluloses are very complex, involving hydrolysis of polysaccharides, degradation of depolymerized carbohydrates, delignification, and lignin condensation. Developing predictive reaction models can help process control and optimization in commercial biomass conversion. For wood pulping, removing lignin and preserving carbohydrates are critically important. Prior reaction kinetic studies were therefore primarily focused on delignification (Lourenço and others 2011; Santos and others 1997; Miranda and Pereira 2002). For bioconversion of lignocelluloses, delignification is relatively less important than dissolution of hemicelluloses (Leu and Zhu 2013). Therefore, modeling hemicellulose dissolution is the key to predicting substrate enzymatic digestibility SED. Effective models have been developed to predict acid-catalyzed hydrolysis of xylan (Springer 1966; Saeman 1945; Morinelly and others 2009; Lu and Mosier 2008; Jacobsen and Wyman 2000; Zhao and others 2012). Although these models are useful, it is desirable to develop simple reaction-kinetic-based factors, such as reaction severity factor, that combine all reaction conditions into one single parameter, for process scale-up design and bioconversion predictions. The *H*-factor, an integration of reaction temperature over time or thermal energy input, has been widely used for scaling wood pulping in the pulp and paper industry (Brasch and Free 1965; Vroom 1957). The combined severity factor (CSF) (Abatzoglou and others 1992; Chum and others 1990; Larson and others 1999) was developed based on *H*-factor after modification to include the effect of a catalyst (Eq. (1)) (that is, acid) on lignocellulose reaction:

$$\mathrm{CSF} = \log\left[t \cdot \exp\left(\frac{T - T_{\mathrm{ref}}}{\omega}\right)\right] - \mathrm{pH} = \log\left[t \cdot \exp\left(\frac{T - 100}{14.75}\right)\right] - \mathrm{pH} \quad (1)$$

Predictions of hemicellulose dissolution using CSF were good at low severities (that is, hemicellulose dissolution below approximately 70%); however, predictions were poor at hemicellulose dissolution above 70% under higher severities (Abatzoglou and others 1992) that are of most interest for bioconversion of lignocelluloses. The poor prediction

at high severities is primarily due to the inhomogeneity of hemicelluloses. Biphasic models are often used to improve the prediction of hemicellulose dissolution and xylose yield (Zhao and others 2012; Zhang and others 2012a).

SPORL has two catalysts, SO_2 and a base, and therefore a CSF that simply uses pH to account for the effect of acid is not applicable. Here we will derive a combined hydrolysis factor (CHF) that can accurately predict hemicellulose dissolution by pretreatments using more than one catalyst. The hydrolysis of lignocelluloses can be characterized by a phenomenological second-order rate expression, first order in catalyst and first order in xylan:

$$\frac{dX_R}{dt} = -k(T, C, ...)CX_R \tag{2}$$

where X_R is the fraction of xylan retained in the solids or residual xylan, T is temperature, C is catalyst concentration, and k is rate constant as developed by several researchers (Abatzoglou and others 1992; Springer and others 1963). The following expression was found to fit acid hydrolysis of aspen well (Springer and others 1963):

$$\mathrm{Log}_{10}\frac{K_x}{C_H} = 15.083 - \frac{6171.3}{T} + 0.22219C_H \tag{3}$$

where K_x is the pseudo first-order rate constant for dissolution of xylan, T is temperature in kelvins, and C_H is acid molar concentration. Following Eq. (3), we can express the rate constant k for a reaction involving two catalysts such as SPORL as

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

where C_A and C_B are the initial molar concentrations of the two catalysts used in pretreatment, respectively, for example acid and bisulfite for SPORL studies conducted in laboratory; α, β, and γ are adjustable parameters; E is the apparent activation energy; and R is the universal gas constant of 8.314J/mole/K. Based on Equation (2), the CHF that combines temperature, chemical concentrations, and pretreatment duration into a single measure as a reaction severity can be expressed as (Zhu and others 2012)

$$\mathrm{CHF} = k(C_A + C_B)t = e^{(\alpha - E/RT + \beta C_A + \gamma C_B)}(C_A + C_B)t \tag{5}$$

CHF is very similar to CSF (Eq. (1)), but it is more precise in defining the effects of temperature, with the introduction of activation energy E, reaction chemical loadings, and concentration of each catalyst. CHF was compared with CSF for a set of DA pretreatments and two sets of SPORL pretreatments at low acid and pH ranges. As shown in Figure 3, excellent linear correlations were obtained.

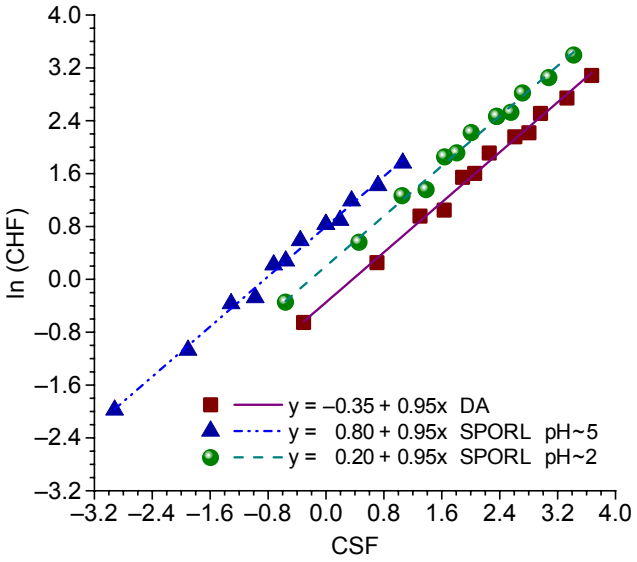

Figure 3—Correlations between combined hydrolysis factor (CHF) and the conventional combined severity factor (CSF) defined in Eq. (1) for a set of dilute acid (DA) and two sets of SPORL pretreatments of aspen (from Zhu and others (2012)).

3.2 Using CHF to Predict Hemicellulose Dissolution

To obtain accurate prediction of hemicellulose dissolution using CHF, two fractions of hemicelluloses with different reaction rates can be assumed to account for the inhomogeneity of hemicelluloses (Kobayashi and Sakai 1956; Maloney and others 1985; Montané and others 2002) similar to the biphasic concept (Zhao and others 2012; Zhang and others 2012a). Furthermore, the fast and slow hemicelluloses are assumed to have the same fundamental dissolution chemistry, and therefore the rate of dissolution can be expressed as

$$\frac{dX_f}{dt} = -kCX_f \tag{6a}$$

$$X_f(t=0) = (1-\theta)X_R \tag{6b}$$

$$\frac{dX_s}{dt} = -fkCX_S \tag{6c}$$

$$X_s(t=0) = \theta X_R \tag{6d}$$

where X_f and X_s are fractions of fast and slow residual hemicelluloses such as xylan in hardwood, respectively, with $X_R = X_f + X_s$ and $X_R\,(t=0) = 1$; k in Equation (6a) is the rate constant for fast xylan hydrolysis, and f 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 Equations (6a) and (6c) results in the following expression for X_R, the amount of xylan remaining in the solid:

$$X_R = (1-\theta)e^{-\mathrm{CHF}} + \theta e^{-f\,\mathrm{CHF}} \tag{7}$$

Equation (7) is an analytical solution for xylan dissolution based on kinetics but expressed in terms of pretreatment severity CHF. By fitting the remaining xylan content measured in the pretreated substrates using Equation (7) to CHF, 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 constant between slow and fast xylan, f, can be obtained.

3.3 Using CHF to Predict Sugar Degradation

The hemicelluloses or cellulose dissolved by acidic pretreatments can be degraded into furan and may be further degraded into organic acids. These compounds from sugar degradation not only reduce sugar yield but also affect downstream processing, such as fermentation, as they can inhibit most microorganisms. Hemicellulose dissolution and sugar degradation take place simultaneously in pretreatment of lignocelluloses. Sequential reactions, however, were assumed here to simplify process modeling. Furthermore, furans (furfural and hydroxymethylfurfural (HMF)) were assumed as indicator product by neglecting further degradation of furans to acids, and all sugar degradation reactions were lumped into one pool. A degradation reaction rate constant k_d was assumed to follow Arrhenius temperature dependence (Saeman 1945; Xiang and others 2004; Qi and Lu 2007):

$$k_d = e^{(\alpha_d - E_d/RT)} \tag{8}$$

where α_d is an adjustable parameter, E_d is apparent activation energy, R is the universal gas constant of 8.314 J/mole/K, and T is temperature in kelvins. The rate of sugar degradation or furan formation (D) in the pretreatment liquor can be expressed as the rate of reduction of dissolved carbohydrate:

$$\frac{dD}{dt} = k_d(1 - X_R) \tag{9}$$

where $(1 - X_R)$ represents the sum of the amount of carbohydrates dissolved by hydrolysis reactions in a pretreatment process. Neglecting cellulose dissolution, which is minimal for most pretreatments, Equation (7) can be substituted into Equation (9) to result in the following expression for furan formation through integration (Zhang and other 2014):

$$D = k_d \cdot t\left[1 - \frac{1-\theta}{\mathrm{CHF}}\left(1 - e^{-\mathrm{CHF}}\right) - \frac{\theta}{f\,\mathrm{CHF}}\left(1 - e^{-f\,\mathrm{CHF}}\right)\right] \tag{10}$$

where t is time in minutes at pretreatment temperature T.

4. Process Optimization and Scale-up Design Using CHF

4.1 Dissolution of Hemicelluloses and Its Relation to Sugar Yield

The significance of CHF is that it can not only accurately predict hemicellulose dissolution but also correlate enzymatic saccharification and sugar yield. Furthermore, it can predict furan formation. This provides the opportunity to use CHF for process optimization and scale-up design. As shown in Figure 4, the experimentally measured hemicellulose dissolution of aspen (Zhu and othes 2012), poplar NE222 (Zhang and others 2015), lodgepole pine (Zhou and others 2013b), and Douglas-fir forest residue (Leu and others 2013) under various pretreatment severities using DA and SPORL can be accurately predicted by Equation (7). Separate fitting of the xylan and mannan dissolution data produced better results (Fig. 4d). The fitting parameters of these studies are listed in Table 2. In these studies, sulfuric acid (H_2SO_4, catalyst A) and sodium bisulfite ($NaHSO_3$, catalyst B) were used. The results in Table 2 suggest that E = 100,000 J/mole is a very good number to use as a known quantity for softwoods in data fitting, and $\gamma = -10$ can be used for all wood species.

The importance of hemicellulose dissolution in removal of recalcitrance of lignocelluloses for enzymatic saccharification cannot be overstated (Leu and Zhu 2013). With the excellent prediction of hemicellulose dissolution by CHF, good correlations between enzymatic saccharification efficiency or sugar yield and CHF are ensured. This can be clearly seen from the SED of many DA- and SPORL-pretreated aspen substrates (Zhu and others 2012) (Fig. 5a), enzymatic hydrolysis glucose yield (EHGY) from SPORL-pretreated lodgepole pine WIS (Zhou and others 2013b) (Fig. 5b), and EHGY and xylose yield from SPORL-pretreated poplar NE222 (Zhang and others 2015) (Fig. 5c, d). The results in Figure 5 clearly indicate that maximal sugar yield can be achieved at a minimal CHF of approximately 20 (CHF_{opt} = 20 for lodgepole pine; CHF_{opt} = 4 for poplar NE222). As long as the desired CHF_{opt} is achieved, whether through a higher temperature with a short reaction time or a lower temperature with a longer reaction time, optimal sugar yield is ensured. In other words, instead of using conventional statistical experimental designs to optimize each pretreatment condition, such as temperature and time, we can simply use the optimal CHF to design desired pretreatment conditions that can meet other constraints, especially in commercial-scale productions due to facility limitations. Furthermore, in pretreatments such as SPORL with multiple process variables, there are often several optimal sets of pretreatment conditions or multiple optimal points. Traditional statistical process design may produce only a local optimal, depending on the ranges of process variables studied.

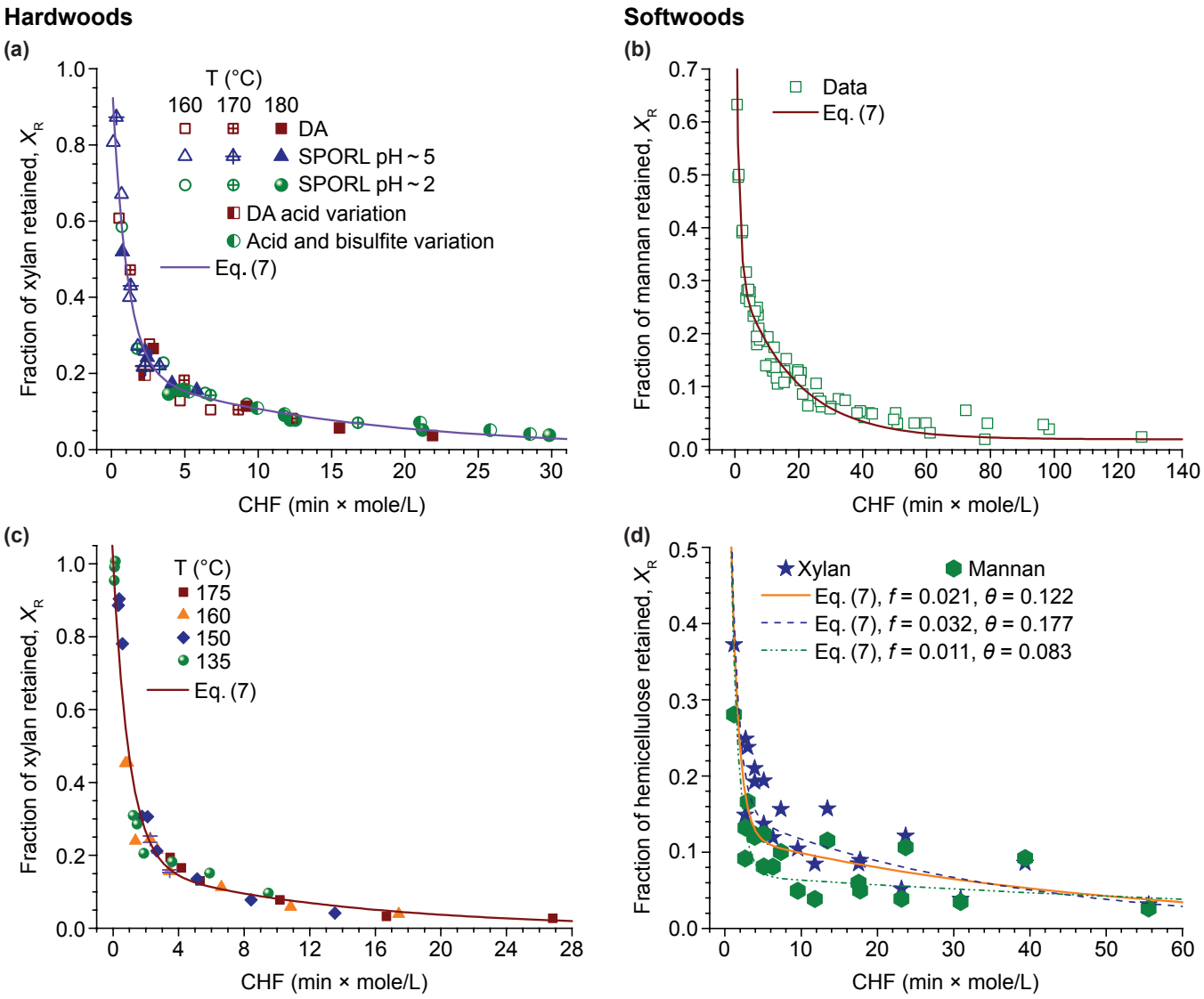

Figure 4—Using pretreatment severity CHF for predictions of dissolution of hemicelluloses by DA and SPORL: (a) aspen (Zhu and others 2012); (b) lodgepole pine (Zhu and others 2012); (c) poplar NE222 (Zhang and others 2015); (d) Douglas-fir forest residue (FS-03) (Leu and others 2013).

Table 2—Fitting parameters in Eqs. (5) and (7) for predicting hemicellulose dissolution of different feedstock pretreated by dilute acid and SPORL using catalysts: A = H_2SO_4, B = $NaHSO_3$ (C_B = 0 for dilute acid)[a]

Parameters	Aspen	NE222	Lodgepole pine	Douglas-fir	Douglas-fir residue
α	25.6	34.5	28.5	28.5	28.5
β (L/mole)	34.5	18.6	17.0	17.0	17.0
γ (L/mole)	−9.9	−9.7	−9.8	−10.0	−10.0
E (J/mole)	90,888	126,200	100,000	100,000	100,000
θ	0.205	0.178	0.320	0.180	0.122
f	0.065	0.078	0.056	0.100	0.021

[a]For softwoods, α, β, γ, and E were assumed not to be species dependent. Their values were based on fitting the hemicellulose (mannan and xylan) dissolution of lodgepole pine. Data from Zhu and others (2012) and Zhang and others (2014, 2015).

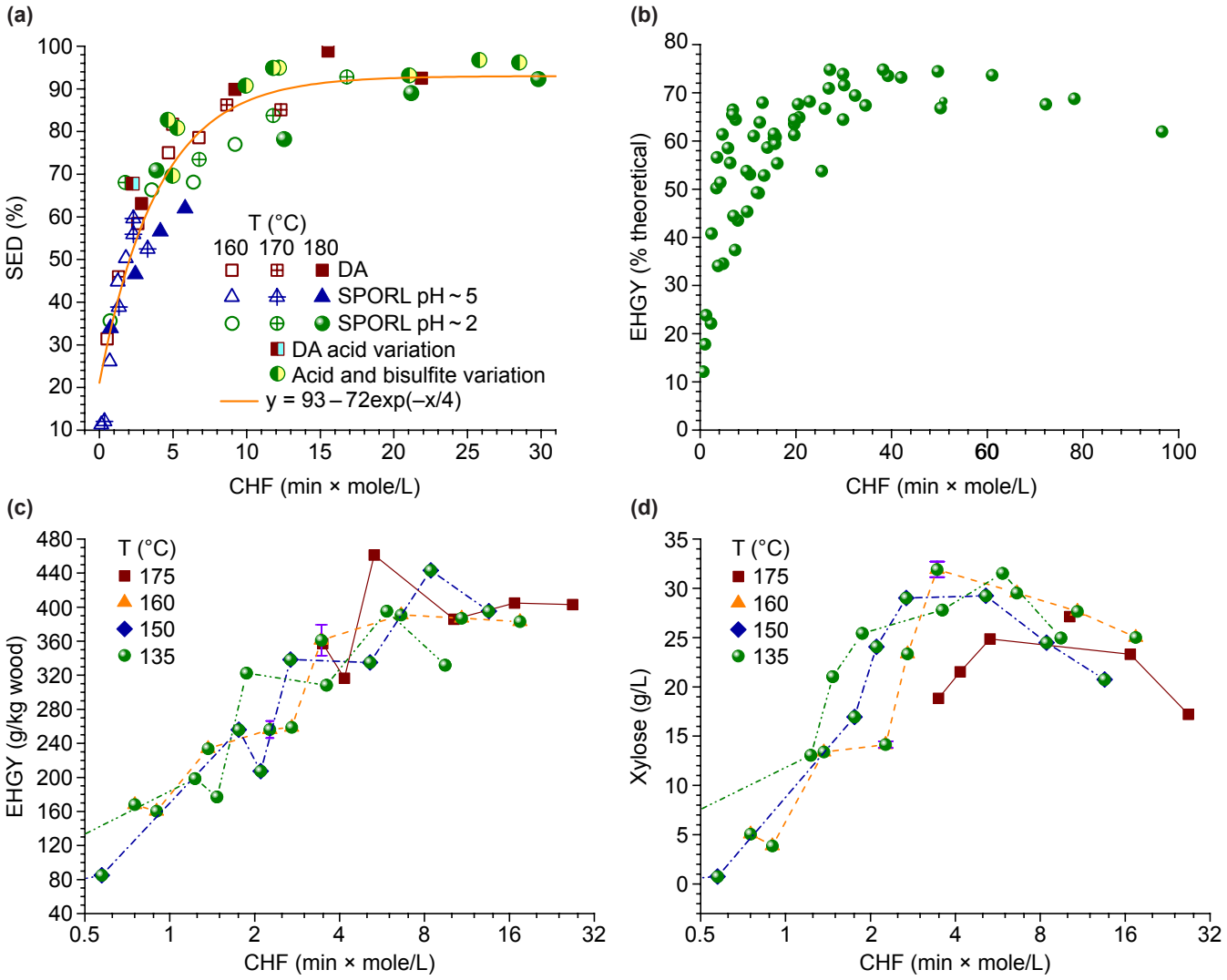

Figure 5—Correlating CHF with various sugar recovery measures: (a) aspen substrate enzymatic digestibility (SED) (Zhu and others 2012); (b) lodgepole pine enzymatic hydrolysis glucose yield (EHGY) (Zhou and others 2013b); (c) poplar NE222 EHGY (Zhang and others 2015); and (d) poplar NE222 xylose yield (Zhang and others 2015).

4.2 Balance Sugar Yield with Degradation Using Low-Temperature Pretreatment

Using CHF to optimize pretreatment also allows balancing sugar yield with inhibitor formation without sacrificing enzymatic saccharification efficiency of pretreated substrate. Sugar degradation is well known to often have a much higher activation energy than that for hemicellulose dissolution for most feedstock (Kamireddy and others 2014). Therefore, a lower pretreatment temperature but with a longer pretreatment duration can be used while maintaining the same optimal pretreatment severity CHF_{opt} to reduce sugar degradation. Specifically, paired pretreatments at two different temperatures, $T1 > T2$, with identical pretreatment severity CHF and chemical loadings can be evaluated. The ratio of sugar degradation for any pair pretreatments can be obtained based on Equation (10) as

$$\frac{D_{T1}}{D_{T2}} = \frac{k_d^{T1}}{k_d^{T2}} \cdot \frac{t^{T1}}{t^{T2}} = \exp\left[\frac{E - E_d}{R}\left(\frac{1}{T1} - \frac{1}{T2}\right)\right] \quad (11)$$

When $E_d > E$ and $T1 > T2$, $D_{T1} > D_{T2}$. The validity of Equation (10) for predicting sugar degradation was verified using 59 dilute acid and SPORL pretreatments of lodgepole pine in a separate study as shown in Figure 6a (Zhou and others 2013b).

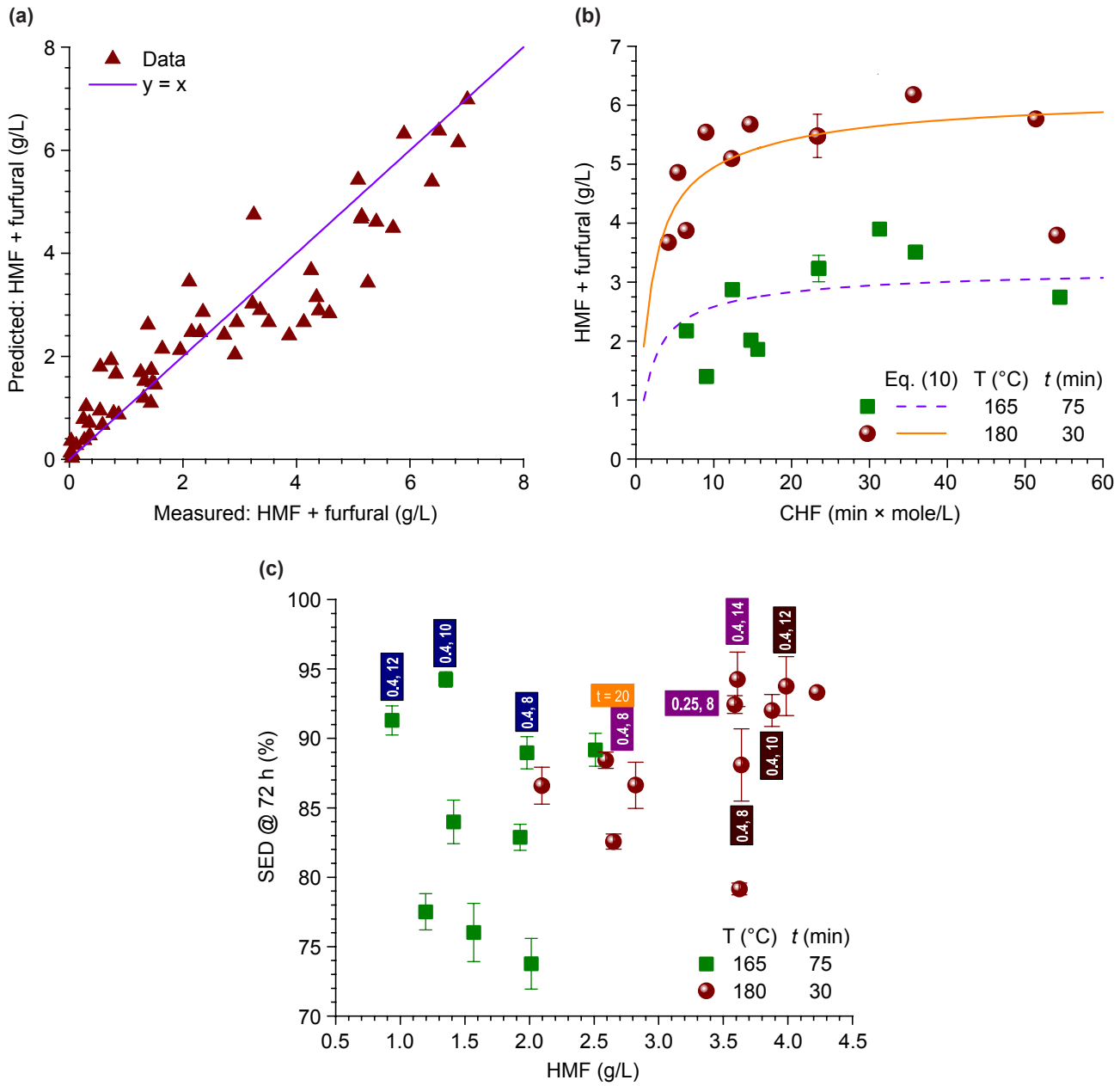

Figure 6—Balancing sugar yield with sugar degradation in SPORL: (a) validating sugar degradation model using experimentally measured furan data—lodgepole pine (Zhou and others 2013b); (b) predicting sugar degradation using pretreatment severity CHF at two temperatures—Douglas-fir (Zhang and others 2014); (c) comparisons of substrate enzymatic digestibility (SED) and HMF formation at two SPORL temperatures—Douglas-fir (Zhang and others 2014). Numbers in each vertical box are acid concentration (v/v%) in the pretreatment liquor and sodium bisulfite charge on wood, respectively.

Paired SPORL pretreatments on Douglas-fir wood chips at $T1$ = 180 °C and $T2$ = 165 °C were conducted to verify the validity of Equations (10) and (11) (Zhang and others 2014). Fitting the furans (HMF + furfural) data produced E_d = 160, 930 J/mole with E = 100,000 J/mole. As shown in Figure 6b, the amount of measured furans from a SPORL pretreatment at 180 °C is consistently greater than the value from the corresponding pretreatment at 165 °C with the same severity CHF. Furthermore, the predicted sugar degradations from Equation (10) are in good agreement with the measured furan data. The low measured furan value at the high severity CHF = 54 is due to the high acid loading that induces substantial degradation of furan to organic acids. Moreover, the ratio of the measured furans ($D_{T1=180}$/$D_{T2=165}$) for each paired SPORL pretreatment was slightly below 1.91, as predicted by Equation (11) for most of the pairs (not shown), suggesting slightly more furan degradation to organic acids at a higher temperature.

Enzymatic saccharification of these paired SPORL-pretreated WIS suggests that using the same CHF (at the same chemical loadings) but at different temperatures (180 °C and 165 °C) can indeed produce similar SED. Several runs at 165 °C produced SED near or over 90%, although more runs at 180 °C achieved the similar SED level, whereas the amount of furan formation can be reduced by two to four times, as shown in Figure 6c. Furthermore, pretreatment at a lower temperature tends to produce slightly higher WIS yields to result in a higher EHGY. The approach taken here of using low-temperature pretreatment is very different from low-severity pretreatment(s) reported in the literature (Chen and others 2012), which reduced inhibitor formation at the expense of enzymatic saccharification efficiency and EHGY. In those cases, additional process steps, such as further size reduction or xylanase supplementation, were employed to compensate for the reduced SED, which increases capital and operating costs.

4.3 Using pH-profiling in SPORL to Further Reduce Carbohydrate Degradation

The concept of pH-profiling was developed to further reduce sugar degradation in pretreating feedstock with high lignin content, such as forest residues and softwoods, when using SPORL (Cheng and others 2015). In this concept, the application of acid, such as H_2SO_4 or SO_2, was delayed but without changing total acid application (Fig. 7). Theoretically, both temperature and pH profiles can be controlled and acid injection can be continuous. The delayed application of acid facilitated delignification in the early stage with relatively high pH, whereas reduced acidic reaction time resulted in reduced sugar degradation and hemicellulose dissolution. The concept is based on two understandings: (1) the roles of acid and sulfite on hemicellulose dissolution (Zhu and others 2012), delignification through sulfonation (Ingruber 1985), sugar degradation (Zhang and others 2014), and deacetylation (Tune and Van Heiningen 2008); and (2) the quantitative effects of delignification and dissolution of hemicelluloses on improving cellulose accessibility and SED (Leu and Zhu 2013; Zhang and others 2014; Wang and others 2012a; Yang and Wyman 2004)—that is, the negative effect of reduced hemicellulose dissolution due to reduced acidic reaction time on substrate cellulose accessibility and digestibility can be compensated for by improved delignification. Therefore, equivalent enzymatic saccharification can be achieved but with lower sugar degradation. SPORL is uniquely suited for adopting the pH-profiling concept because delignification can be achieved under acidic conditions with sulfite, which can limit the pH range that needs to be controlled. The concept is similar to two-stage sulfite pulping (Croon 1965; Lagergren 1964; Sanyer and others 1962), but with the goal of reducing sugar degradation.

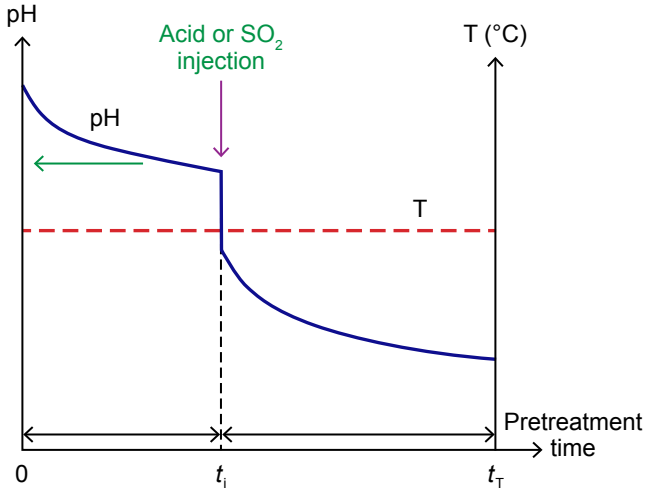

Figure 7—A pH–time diagram that schematically shows the concept of pH-profiling for pretreating lignocelluloses (Cheng and others 2015).

The pH-profiling concept was carried out with Douglas-fir forest residue using $NaHSO_3$ and H_2SO_4 in a laboratory at 165 °C with a total reaction time of 75 min. Different delay times for applying H_2SO_4 were used. The results were compared with the control run with no delay in H_2SO_4 application. As shown in Table 3, the three pH-profiling SPORL runs produced more delignification but dissolved less hemicellulose than the control run. Furthermore, the amounts of furfural, HMF, and acetic acid formation were substantially lower than the corresponding value for the control run. SED was not significantly affected. EHGY was slightly higher for the pH-profiling runs with acid injection delay times of 25 and 35 min. Enzymatic saccharification and fermentation of the pretreated whole slurry of the Douglas-fir forest residue at 21% total solids loading produced substantially higher ethanol yields from the two SPORL profiling runs than from the control run. The ethanol yield for the SPORL pH-profiling runs with 25 min acid delay time was 297 ± 9 L/t at a titer of 48.9 g/L compared with 215 ± 42 L/t at 38.6 g/L for the control run.

4.4 SPORL Optimization for Hardwoods and Herbaceous Biomass Using CHF

Unlike softwood species, hardwoods and herbaceous lignocelluloses contain a substantial amount of acetyl groups that can be converted to acetic acid in acidic pretreatments. Acetic acid inhibits most organisms such as yeasts and is difficult to remove. The discussion in Section 4.2 deals only with sugar degradation inhibitors and is not sufficient to address acetic acid inhibition. We developed an optimization strategy using CHF to address yeast fermentation inhibition by acetic acid at high solids loadings without detoxification (Zhang and others 2015). In this strategy, the optimal CHF

Table 3—Comparisons of pretreatment performance between normal SPORL (control) and SPORL with pH-profiling at different acid injection times[a]

	Pretreatment conditions					Pretreated washed solids (g/1000g FS-10)					Spent liquor (g/1000g FS-10) (only monomeric sugars were reported)[b]						Enzymatic saccharification		Fermentation at 21% solids
Run label	Initial pH	H_2SO_4 at $t = 0$ (wt%)	Delay time t_i (min)	H_2SO_4 at t_i (wt%)	Final pH	Glucan	Xylan	Man-nan	K lignin	Washed solid yield	Glu-cose	Xy-lose	Man-nose	Fur-fural	HMF	Acetic acid (g/L)	SED (%)	EHGY (% theoreti-cal)	Ethanol (% theoretical; g/L)
Untreated FS-10						410	57	97	293	1,000									
t0A1B12 (control)	1.79	2.2	0	0	1.45	388.4	13.7	15.8	170.6	612.7	21.8	17.7	54.6	5.0	9.3	5.3	97	83.9	52.8; 38.6
t25A4B12	4.06	0	25	2.2	1.72	405.6	22.7	23.7	130.1	603.9	10.0	13.5	32.8	2.2	4.0	3.8	95	85.1	73.1; 48.9
t35A4B12	4.06	0	35	2.2	1.40	410.7	20.4	21.8	137.7	616.5	15.7	15.1	38.2	2.0	3.8	4.1	95	86.8	
t45A4B12	4.06	0	45	2.2	1.66	406.5	21.0	25.7	131.9	622.3	9.1	14.2	30.9	2.0	2.9	3.4	93	80.2	63.7; 45.9

[a]All pretreatments were conducted using a Douglas-fir forest residue (FS-10) at liquor-to-wood ratio of 3 (L/kg) at 165 ºC for total of 75 min and sodium bisulfite ($NaHSO_3$) charge on wood of 12 wt%. Data from Cheng and others 2015.
[b]Glucose, xylose, mannose, furfural and HMF were reported as glucan, xylan, mannan, pentosan, and hexosan, respectively.

was not determined purely based on sugar yield, but rather based on near complete removal of fast xylan (remaining xylan $X_R = \theta$). Sugar yield at this pretreatment severity $CHF_{X_{R=\theta}}$ is near optimal (Fig. 5c, d). $CHF_{X_{R=\theta}}$ is used as the starting point for optimizing pretreatment of feedstock with high acetyl content. Mild pretreatments with severity $CHF < CHF_{X_{R=\theta}}$ may be necessary to further reduce acetic acid formation (Fig. 8a) to achieve maximal biofuel yield (Fig. 8b).

4.5 SPORL Process Scale-up Design

Process optimization using conventional statistical experimental design at large scales is always economically prohibitive. The low-temperature pretreatment approach discussed above can also be applied for process scale-up design. Specifically, optimal pretreatment severity measured by CHF_{opt} can be obtained from laboratory bench-scale studies as demonstrated in Figure 5. Based on Eq. (5), there are many possibilities to design a scale-up pretreatment at a severity of CHF_{opt} by using different combinations of pretreatment temperature and time at fixed chemical loadings. If the scale-up facility has constraints in operating temperature due to equipment pressure limitation or material corrosion, a low-temperature pretreatment can be designed with an extended pretreatment duration. On the other hand, if production capacity is the bottleneck, a short pretreatment at an elevated temperature can be used. For a given set of chemical loadings, we can calculate this scale-up scheme using Eq. (5) (Zhou and others 2015b) as

$$t^{T_{up}} = \exp\left[\frac{E}{R}\left(\frac{1}{T_{up}} - \frac{1}{T_{op}}\right)\right] t^{T_{op}} \qquad (12)$$

where subscript "up" denotes scale-up.

Table 4—Process scale-up design model Eq. (12) predicted pretreatment temperature and time for softwood species using SPORL at minimal chemical loadings[a] along with calculated normalized furan formation using simple sugar degradation model Eq. (11)

T (°C)	t (min)	Normalized furans
180	25–30	1
175	34–40	0.834
170	46–55	0.694
165	62–74	0.575
160	85–102	0.474
155	118–141	0.389
150	164–197	0.317
145	231–277	0.258
140	327–393	0.209

[a]SO_2 = 6.5 wt% and CaO = 1.8 wt%; or bisulfite loading 8–12 wt% and H_2SO_4 loading approximately 2.2 wt%.

Table 4 lists the calculated pretreatment temperature and time scheme for softwoods at the minimal sulfite loading and hydroxide loading on wood (SO_2 = 6.5 wt%, CaO = 1.8 wt%) or bisulfite loading between 8 and12 wt% and H_2SO_4 loading approximately 2.2 wt%. We also calculated the normalized furan formation at different temperatures using Eq. (11). The reduction of furan formation (Table 4) is a significant advantage using low-temperature pretreatment. Pretreatment duration greater than 120 min may be too long and can negatively affect productivity. Increasing SO_2 loading over its minimal loading of 6.5 wt% (on wood)

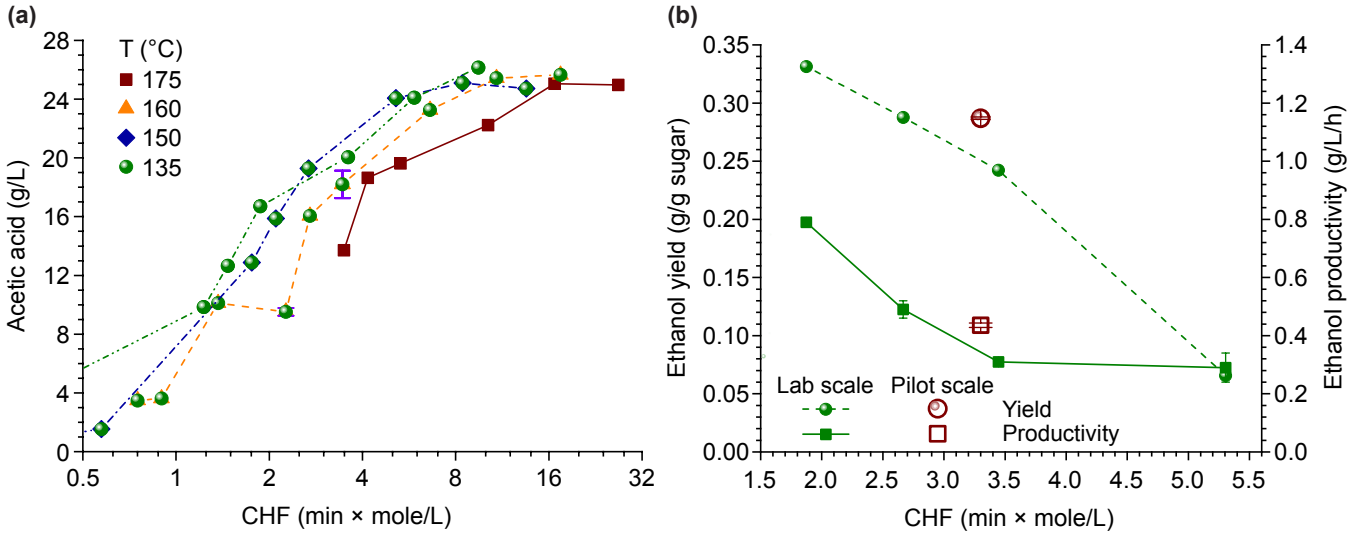

Figure 8—Using combined hydrolysis factor (CHF) for bioconversion of poplar wood NE222 using SPORL: (a) CHF on acetic acid formation (Zhang and others 2015); (b) CHF on ethanol yield and productivity (Zhou and others 2015a).

can substantially reduce pretreatment time and thereby further reduce furan formation, as we recently found in our laboratory.

Table 4 indicates that a pretreatment time of 6 h (240 min) or more is required at temperature below 145 °C to obtain good pretreatment. Although furan formation can be reduced by a factor of 4 at $T < 145$ °C (Table 4), such a prolonged reaction time substantially reduces production capacity and increases capital cost for more reactors. One way to maintain reaction severity at low temperatures is to increase chemical loading without increasing reaction time. Such an attempt has been carried in our laboratory with limited experiments (Gu and others 2016). Specifically, we found that increasing SO_2 loading was very effective in reducing reaction time. Furan formation was very low because of the low pretreatment temperature of 140 °C and the leveling of pH of the sulfite solution to approximately 1.5 at increased SO_2 loadings. One should be able to develop an equation similar to Equation (12) to scale reaction time with SO_2 loading (note that Eq. (12) was developed under fixed chemical loading). However, more experiments than those we carried out are needed.

5. Lignin Sulfonation on Nonproductive Cellulase Binding

Nonproductive binding of cellulase to lignin has been recognized as a leading mechanism of lignin inhibition of enzymatic saccharification of cellulose (Mansfield and others 1999; Sewalt and others 1997; Berlin and others 2006; Palonen and others 2004). To reduce nonproductive cellulase binding, washing pretreated solids to remove dissolved lignin is often practiced in most reported studies (Nagle and others 2002; Tengborg and others 2001). However, the remaining unsolubilized lignin on pretreated solids can still bind cellulase to reduce activity. Because of the dissolution of hemicelluloses in acidic pretreatments, lignin content in pretreated solids is often enriched. Therefore, nonproductive cellulase binding to lignin is unavoidable. Instead of using an expensive delignification approach to remove lignin and reduce nonproductive binding, lignin modification resulting in a lignin with low affinity to cellulase is a better alternative. One of the unique characteristics of SPORL is the lignin sulfonation that solubilizes approximately 20% to 40% of lignin as lignosulfonate into the pretreatment spent liquor. The remaining undissolved lignin on solids is also sulfonated. Lignin sulfonation produces a hydrophilic lignin that has a low affinity to cellulase because cellulase binding is mainly through hydrophobic interactions (Hansen and others 1988; Nakagame and others 2011b; Haynes and Norde 1994). Therefore, lignin sulfonation plays a very important role in improving sugar yield through reducing nonproductive cellulase binding and process simplification by eliminating washing of pretreated solids as discussed in Section 5.1.

5.1 Lignosulfonate to Enhance Enzymatic Saccharification

Non-ionic surfactants have been recognized as being capable of keeping the remaining lignin on a lignocellulosic solid substrate from binding cellulase to result in enhanced cellulose saccharification (Eriksson and others 2002; Liu and others 2010; Borjesson and others 2007; Zheng and others 2008). Lignosulfonate is a surfactant and can be used to block lignin on solid substrate from interacting with cellulase; however, it is also a lignin that has a hydrophobic end, and therefore can bind to cellulase to reduce enzyme activity on cellulose (Wang and others 2013a; Liu and others 2010). Different fractions of commercial lignosulfonate with different molecular weight and sulfonation were applied to enzymatic saccharification of a hardwood (aspen) and a softwood (lodgepole pine) pretreated by SPORL, DA, and alkaline processes. The results indicated that application of lignosulfonate enhanced saccharification (Zhou and others 2013a). However, the degree of enhancement depended on (1) the molecular weight and degree of sulfonation of the lignosulfonate applied—the smaller molecular weight fraction that also tends to be more sulfonated performed better than the larger molecular weight fraction; (2) the substrate lignin—the enhancement for the alkaline-pretreated substrate is greater than for the SPORL-pretreated substrate because the SPORL substrate lignin is sulfonated and already has relatively low affinity to cellulase; and (3) the loading of lignosulfonate (Fig. 9)—increasing lignosulfonate loading resulted in increased saccharification.

Lignosulfonate produced from SPORL tends to have lower molecular weight than commercial lignosulfonate but is equally sulfonated. When SPORL-pretreated WIS from lodgepole pine were mixed with the spent liquor from the same SPORL pretreatment and containing lignosulfonate, SED of the washed WIS was enhanced by approximately 60% or more. Furthermore, the enhancement increased with an increase in spent liquor application dosage (the effect of dissolved sugar in the liquor was accounted for) (Fig. 10). The lignosulfonate concentration at 1 g/L in Fig. 10a represents the lignosulfonate level in the pretreated whole slurry. This indicated that liquor and solid separation and solid washing are not only unnecessary, but also not preferred; saccharification and fermentation should use the pretreated whole slurry, which can significantly simplify process integration (Fig. 2). This is one of the main advantages of SPORL over competing pretreatment technologies.

5.2 pH-Induced Electrostatic Repulsion between Sulfonated Lignin and Cellulase

Electrostatic interaction was recently found to play an important role in cellulase binding to lignin through pH-induced lignin surface charge (Lou and others 2013) despite it being recognized as unimportant for protein binding to solid surfaces (Haynes and Norde 1994). Remaining lignin on SPORL-pretreated WIS is sulfonated. The surface charge

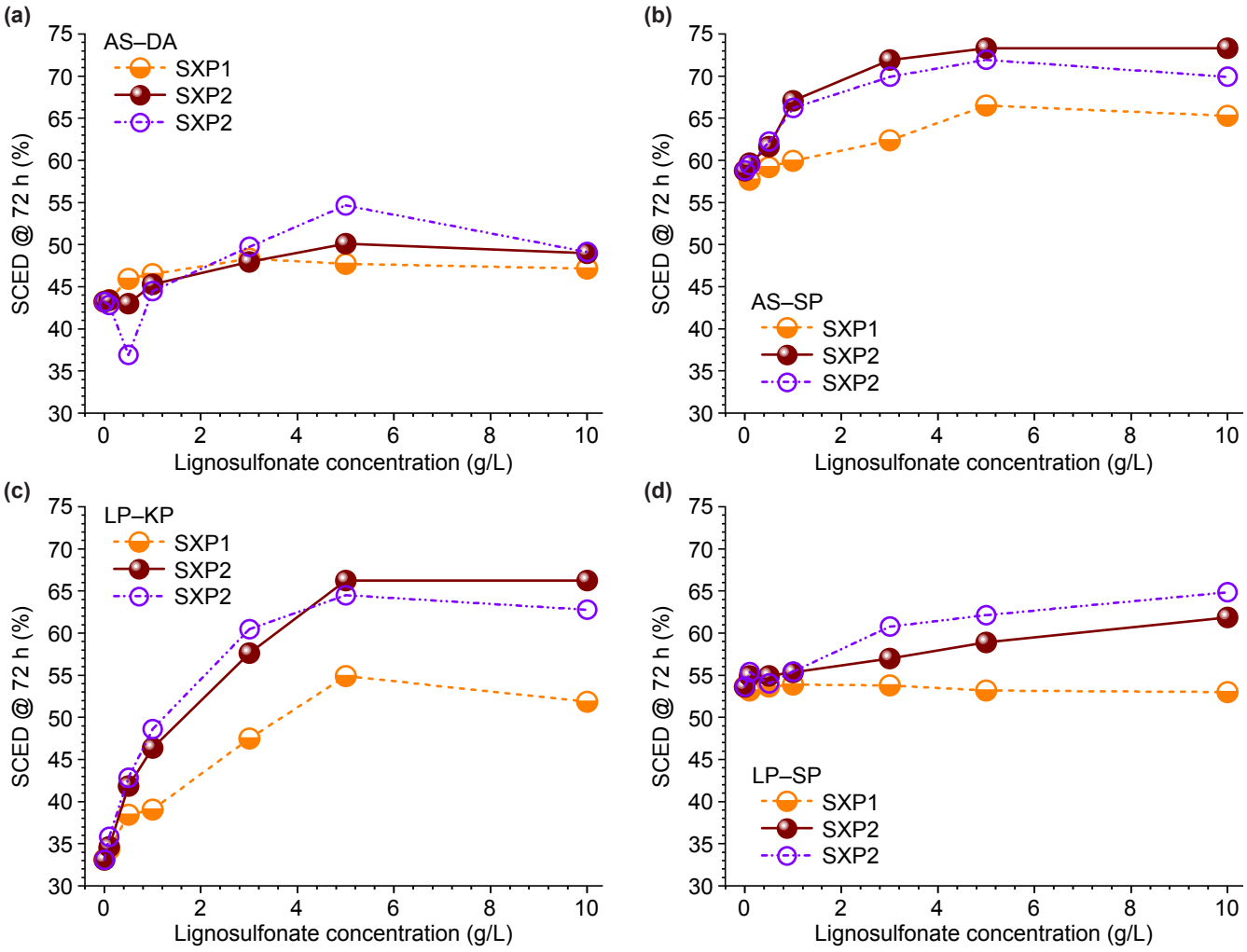

Figure 9—Applications of lignosulfonates of different molecular weight (and sulfonation) on substrate cellulose enzymatic digestibility (SCED) of different substrates (Zhou and others 2013a): (a) dilute-acid-pretreated aspen; (b) SPORL-pretreated aspen; (c) alkaline-pretreated lodgepole pine; (d) SPORL-pretreated lodgepole pine.

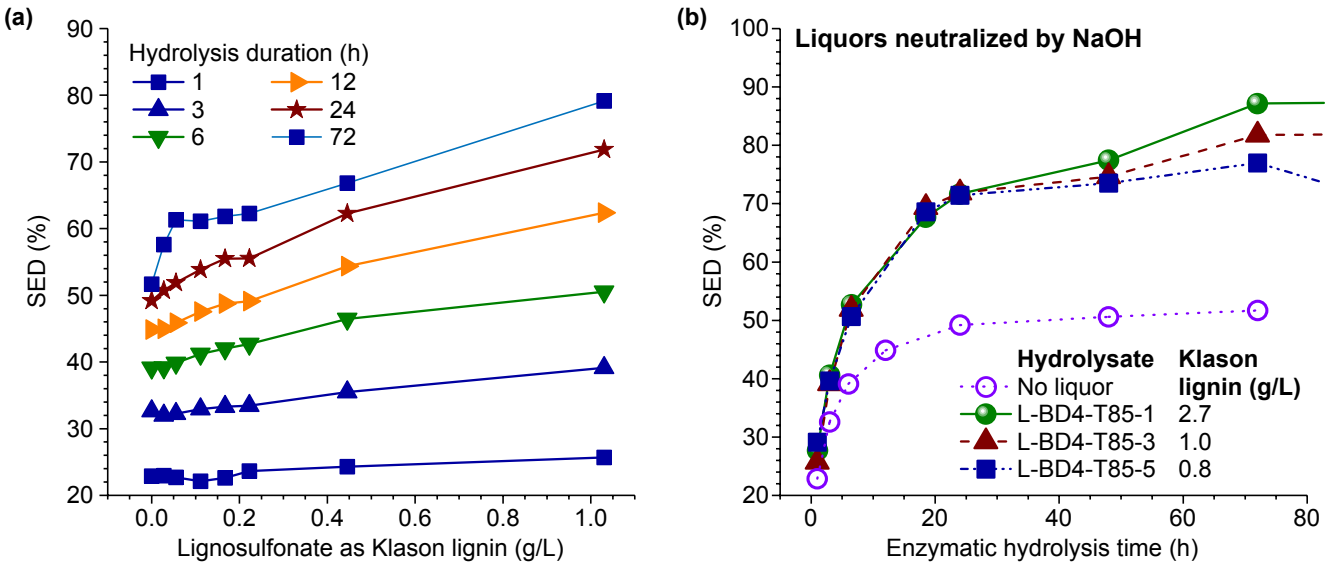

Figure 10—Application of SPORL spent liquor on SPORL water insoluble solids (WIS) on substrate enzymatic digestibility (SED) (Wang and others 2013a): (a) effect of spent liquor dosage; (b) effect of different SPORL spent liquor.

of this sulfonated lignin can be further increased at elevated pH. This was verified by using four enzymatic hydrolysis residue lignin substrates produced through extensive enzymatic hydrolysis of one dilute acid and three SPORL-pretreated lodgepole pine WIS samples (Lou and others 2013). The remaining protein from the enzymes was thoroughly removed using proteinase. The measured zeta-potential (absolute value) was found to increase linearly with pH. At an elevated pH > pI (isoelectric point) of the enzyme, the cellulase will also be negatively charged and result in a repulsive interaction with the negatively charged lignin. The measured cellulase binding to lignin decreased rapidly with increase in pH (Fig. 11a). Notice that the amounts of enzyme (CTec2) binding to two very different lignin substrates (L-DA with no sulfonic acid group and L-SP-B6 highly sulfonated) were not much different at pH 4.5 (close to pI of CTec2), suggesting that the lignin surface property does not affect cellulase binding at a low pH close to the enzyme pI due to lack of electrostatic interactions. However, the difference increased when both lignin and enzyme became negatively charged at elevated pH (> pI). The amount of CTec2 binding to L-SP-B6 was near zero at pH 6.0.

To further illustrate the electrostatic interaction between lignin and enzyme, the measured amounts of CTec2 binding to the four lignin substrates were plotted against the measured zeta potentials of these lignin substrates at different pH (Fig. 11b). CTec2 bindings to lignin were not affected by lignin surface charge (also other surface properties, such as sulfonic acid group content) at low pH of 4.5 and 4.8. This is in agreement with discussion above. CTec2 has little charge at this pH range because its pI is approximately between 4.5 and 4.8 (based on the pI of its major components) (Nakagame and others 2011a; Vinzant and others 2001; Chirico and Brown 1987; Hui and others 2001) and therefore lacks electrostatic repulsion between cellulase and lignin. Nonproductive cellulase binding to lignin takes place through hydrophobic interactions. At elevated pH > pI, the CTec2 binding to lignin decreased linearly with increase in lignin surface charge measured by zeta potential (Fig. 11b). Furthermore, the lignin substrates (L-SP-B6) with the highest sulfonic acid group content resulted in the highest zeta-potential and the lowest cellulase binding to lignin (Fig. 11b,c).

This finding of pH-induced electrostatic interaction that results in reduction in nonproductive cellulase binding to lignin was also verified by a recent study (Rahikainen and others 2013). This finding suggested that enzymatic saccharification of lignocelluloses should be conducted at elevated pH > pI of enzyme, rather than at the level suggested by enzyme manufacturers (pH 4.8) based on enzymatic hydrolysis of pure cellulosic substrates. The benefit can be clearly seen from two sets of controlled enzymatic saccharification studies using differently pretreated lodgepole pine (SPORL and alkaline) and aspen (SPORL and DA) at 2% WIS loading (Lan and others 2013). The pH of the

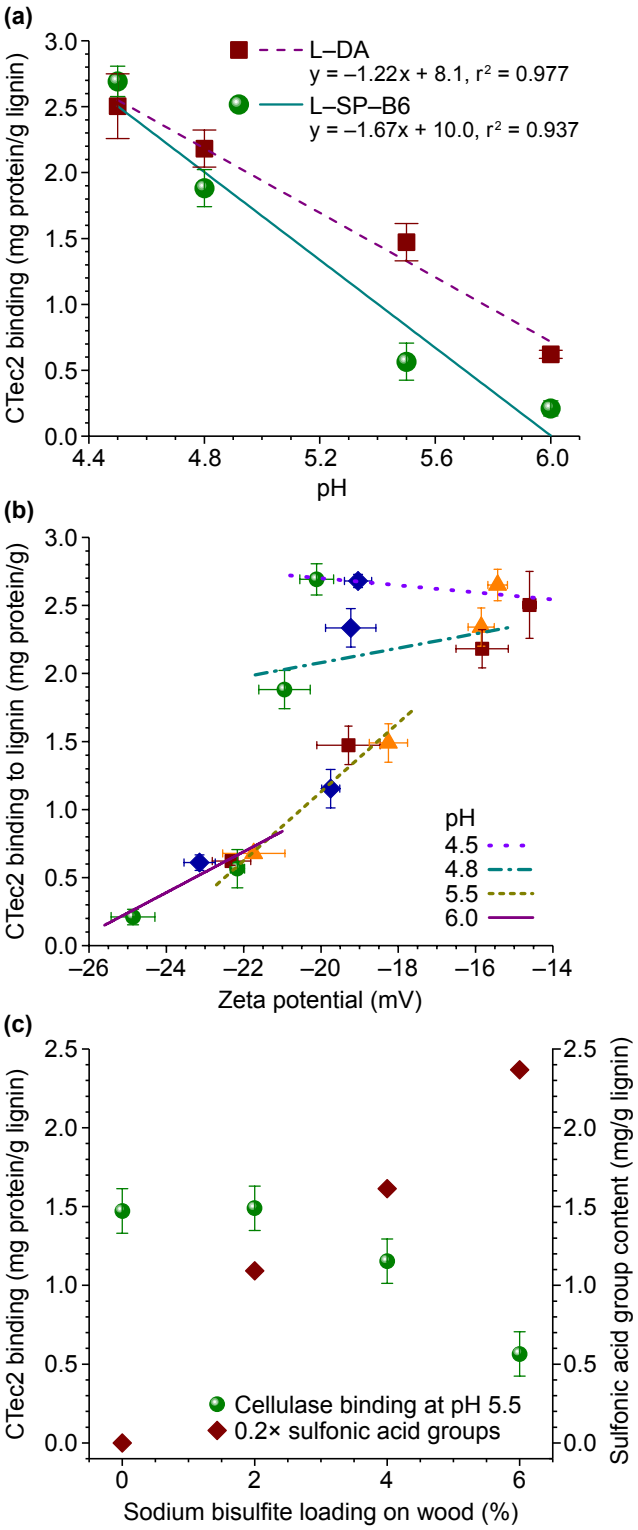

Figure 11—Illustrations of pH-induced electrostatic interactions between a commercial cellulase (CTec2) and lignin on nonproductive binding of cellulase to lignin (Lou and others 2013): (a) effect of pH on nonproductive CTec2 binding; (b) effects of lignin surface charge (zeta-potential) on nonproductive CTec2 binding at different pH; (c) effects of sulfite charge in SPORL on sulfonic acid group content in undissolved solid lignin and nonproductive CTec2 binding at pH 5.5.

lignocellulosic substrate suspension was buffered in a range of 4–7. Furthermore, measured pH (rather than buffer pH) of the suspension was used in data plotting. Results indicate that the optimal pH for enzymatic saccharification of all lignocellulosic substrates all shifted to a higher pH of approximately of 5.5, whereas optimal pH for Whatman paper (pure cellulose) was at approximately 4.8. The gain in enzymatic saccharification efficiency (compared with the efficiency at the enzyme manufacturer recommended pH 4.8) varied with pretreatment methods used. The maximum gain was from lodgepole pine pretreated by SPORL under acidic conditions, whose lignin is highly sulfonated, in agreement with the results shown in Fig. 11c. A very high pH 6.0 did not produce the highest gain in enzymatic saccharification despite nonproductive cellulase binding being the lowest (Fig. 11a). This is because enzyme activity on pure cellulose is optimal at pH 4.8 and is reduced by approximately 10% at pH 6.0, as can be seen from the saccharification of both Whatman paper (Fig. 12) and bleached kraft pulp fibers that do not contain lignin (Lou and others 2013). Direct correlation between gains in saccharification efficiency and reductions in nonproductive binding was observed (Lou and others 2013).

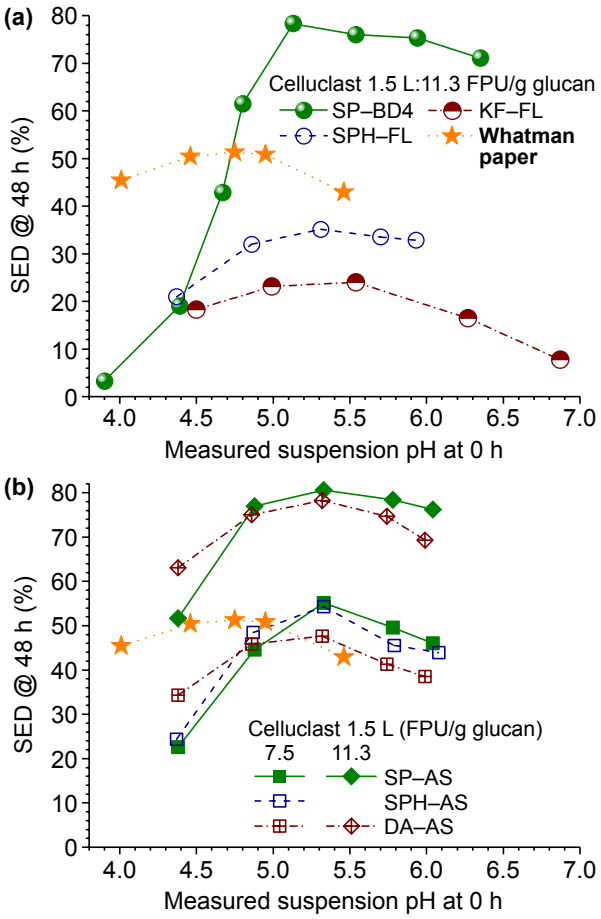

Figure 12—Effect of pH on enzymatic saccharification of different lignocelluloses measured by substrate enzymatic digestibility (SED) (Lan and others 2013): (a) differently pretreated lodgepole pine; (b) differently pretreated aspen.

6. SPORL Process Performance and Integration for Biofuel and Lignin Co-Product Production

All the features of SPORL for robust bioconversion of lignocellulosic biomass have been discussed so far. This section outlines SPORL process performance by first comparing it with other pretreatment methods and then presenting bioethanol and lignin co-product production from woody biomass, including underutilized lignocelluloses such as forest harvest residue.

6.1 Comparison of SPORL with Dilute Acid Pretreatment—Enzymatic Saccharification and Fermentation of Washed Water-Insoluble Solids

Two studies were specifically designed to compare SPORL with DA pretreatment. These two studies simply examined the effect of sulfite on the effectiveness of pretreatment of a spruce (Shuai and others 2010) and an aspen (Zhu and other 2011). All pretreatment conditions such as temperature, time, liquor-to-wood ratio, and H_2SO_4 loadings were identical for DA and SPORL except that $NaHSO_3$ at 3 wt% and Na_2SO_3 at 9 wt% on wood were added in SPORL for aspen and spruce, respectively. For the study using spruce (Shuai and others 2010), it was obvious that DA could not effectively remove the recalcitrance of softwood spruce. The advantages of SPORL were very obvious in terms of enzymatic saccharification efficiency of the washed pretreated WIS (Zhu and others 2011a). SPORL-pretreated aspen WIS was also more digestible than DA with lower inhibitor formation. Similar comparative results were also obtained when pretreating eucalyptus (Wang and others 2009) and poplar woods (Wang and others 2012b).

The second study was carried out according to Scheme II shown in Figure 2. Enzymatic saccharification and fermentation runs were conducted at high solid loadings between 12% and 18% using washed DA- and SPORL-pretreated aspen WIS. The DA and SPORL aspen substrates were produced under identical conditions except 3 wt% on wood $NaHSO_3$ was applied in SPORL (Zhu and others 2011a). Under the same cellulase loading of 10 FPU/g glucan, less torque and mechanical energy for shear mixing were required to liquefy the washed WIS from SPORL than the WIS from DA pretreatment. The advantage of SPORL was more obvious at a low cellulase loading of 6 FPU/g glucan, as shown by the ethanol concentration in the fermentation broth in Figure 13a. Because the SPORL WIS had a higher glucan content of 66.2% than 61.6% for the DA substrate, a comparison of simultaneous saccharification and fermentation efficiency at different cellulase loadings was made. The efficiency achieved at 10 FPU/g glucan for the SPORL substrate was equivalent to that achieved at 15 FPU/g glucan for the substrate pretreated by DA (Fig. 13b).

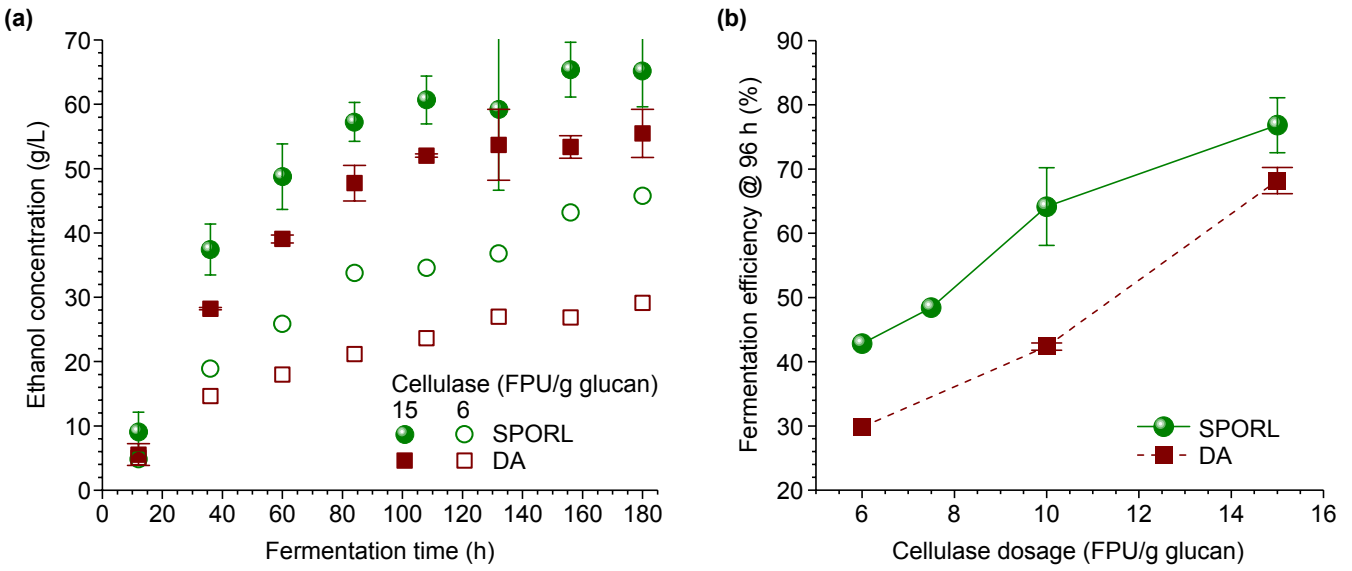

Figure 13—Comparison of high titer ethanol productions from washed water insoluble solids (WIS) fraction of aspen pretreated by dilute acid (DA) and SPORL (Zhu and others 2011a): (a) time-dependent ethanol concentrations in the fermentation broth at two cellulase loadings; (b) terminal ethanol fermentation efficiencies at different cellulase loadings.

6.2 Bioethanol Production from Lodgepole Pine—Process Scale-up Demonstration and Comparison with SO_2 Steam Explosion

The concepts of process scale-up design and low-temperature pretreatment to balance sugar yield and degradation presented in Section 4 were demonstrated here using a softwood—lodgepole pine. The first study focused on scale-up to 2 kg in a 23-L wood pulping digester based on SPORL optimization using 150 g wood chips in a 1-L reactor (Zhou and others 2013b). The lodgepole pine wood chips BD4 were produced from a mountain pine beetle killed tree harvested from the Canyon Lakes Ranger District of the Arapaho–Roosevelt National Forest, Colorado (Luo and others 2010; Zhu and others 2011b). The 2-kg scale-up study was carried out at two temperatures so that the advantage of the low-temperature pretreatment could be shown (Zhou and others 2014). The second study scaled the SPORL process up to 50 kg wood chips in a pilot-scale 390-L wood pulping digester based on the 2-kg study at the same pretreatment temperature of 165 °C. The lodgepole pine wood chips BKLP were produced from mountain pine beetle killed lodgepole pine trees. These trees were approximately 28 cm in diameter at breast height (DBH) and harvested from Canyon Lakes Ranger District of the Arapaho–Roosevelt National Forest, Colorado (GPS location: 13 T 458469 4492172 (NAD27), elevation 2,620 m).

Enzymatic saccharification results from 59 pretreatment runs at a scale of 150 g wood chips were used to design the two scale-up studies. As shown in Fig. 5b, the 150-g scale study indicated that EHGY was approximately maximized (plateaued) at the minimal pretreatment severity CHF of approximately 20. Based on this CHF, pretreatment at 180 °C can be designed for 25 min at $NaHSO_3$ and H_2SO_4 loadings of 8 and 2.2 wt%, respectively, on wood with liquor-to-wood ratio 3 (L/kg), which gives an actual CHF = 22.5 (calculated using Eq. (5) with parameters listed in Table 2) for the scale-up pretreatment at 2 kg. Using Eq. (12), a low-temperature pretreatment at 165 °C can be designed for 75 min under the same chemical loadings for scale-up pretreatments using 2 kg and 50 kg wood chips.

The 2-kg scale study was carried out according to the experimental flow diagram shown in Figure 14 at both 165 and 180 °C. The 23-L rotary wood pulping digester was heated through a steam jacket. At the end of pretreatment, the digester was cooled down by flushing tap water into the jacket. The drainable spent liquor was sampled and then collected and neutralized. The neutralized liquor was mixed and disk-refined with the collected pretreated wet solids that contain approximately two-thirds of the spent liquor. The refined whole slurry was directly used for quasi-enzymatic saccharification and fermentation (q-SSF) using *Saccharomyces cerevisiae* YRH400 (Hector and others 2011) after neutralization to approximately pH 6 without any detoxification, solids and liquor separation, solids washing, or supplementing nutrients. Pretreatment at 165 °C resulted in a higher solids and carbohydrate recovery than at 180 °C (Table 5). For example, glucan loss was only approximately 2% at 165 °C compared with 15% at 185 °C, whereas hemicellulose recovery was approximately 65% at 165 °C compared with 50% at 180 °C. Furthermore, total furan formation was reduced by approximately 50% from 180 to

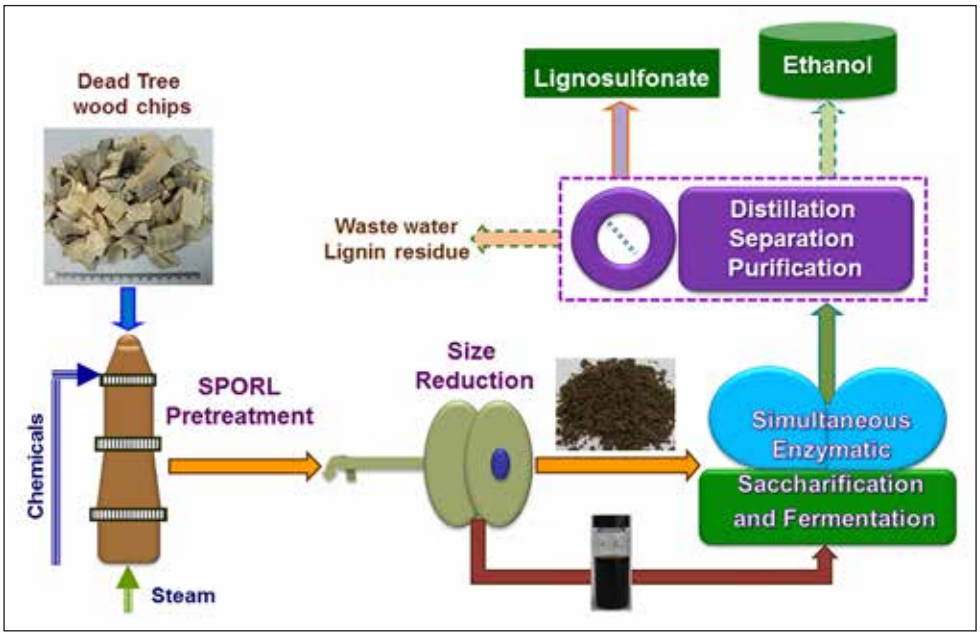

Figure 14—Schematic process flow diagram for high titer ethanol and lignosulfonate production using SPORL at laboratory bench scale (Zhou and others 2013b).

165 °C, in agreement with the prediction by Eq. (11). These advantages produced tangible benefits in q-SSF at high solids of 18% despite the washed WIS from 165 °C being less digestible than that from 180 °C (Fig. 15a). Sugar consumption and ethanol production were much faster for the whole slurry produced at 165 °C than that at 180 °C (Fig.15b) with an equivalent terminal ethanol titer of approximately 47 g/L. Because of the higher carbohydrate recovery, pretreatment at 165 °C produced a higher ethanol yield of 72% theoretical based on wood glucan and mannan (306 L/t) than 61% (260 L/t) at 180 °C (Zhou and others 2014). Ethanol yield of 306 L/t is excellent, considering xylose utilization by YRH400 was negligible due to low concentration. This suggests that using CHF to scale the SPORL process from 150 g up to 2 kg and designing low-temperature pretreatment at 165°C worked well.

Using the CHF design scheme (Eq. (12)) to conduct a SPORL pilot-scale study using 50 kg wood chips in a 390-L wood pulping digester (Fig. 16) worked equally well. The experiment was carried out following the flow diagram similar to Fig. 14 except that the digester content was discharged under pressure to a blow tank at the end of pretreatment (Zhou and others 2015b). Noncondensable gases, including SO_2, were vented to a sodium hydroxide scrubber (Fig. 16). The SED of the washed WIS from the pilot scale agreed with the results from the 2-kg study (Fig. 15a). Component recoveries are listed in Table 6. Very similar major carbohydrate recoveries were achieved when comparing the pretreatment at 2-kg (Table 5) with that at 50-kg scale (Table 6) at 165 °C, except that a higher mannan recovery was obtained at the pilot scale perhaps due to small differences in the wood used. The furans formed were also very comparable with higher HMF formed at 2-kg scale, consistent with the higher mannan dissolution observed. Furthermore, q-SSF of the pretreated whole slurry at 20% total solids loading without nutrient supplementation produced an ethanol yield of 289 L/t, or 72% theoretical based on wood glucan, xylan, and mannan content, similar to that achieved at 2-kg scale using a different lodgepole pine (BD4). Detailed comparisons between the two scale-up studies at 2 kg and 50 kg are shown in Table 7, where ethanol yields were based on wood glucan and mannan only.

Few processes demonstrated the capability for high-titer ethanol production from softwoods without detoxification. The only data available were for spruce using SO_2-catalyzed steam explosion (Hoyer and other 2013) and were compared with the two lodgepole pine scale-up studies discussed above. As shown in Table 7, the SO_2 steam explosion study consumed more energy input in pretreatment at 205 °C, had better mixing in enzymatic saccharification with mechanical actions, and used 50 times more yeast loading, along with supplementation of several nutrients, but produced a lower ethanol yield of 72% theoretical (based on wood glucan and mannan only) as compared to approximately 80% theoretical obtained from the two SPORL studies.

6.3 Bioethanol Production from a Softwood Forest Residue—Scale-up Evaluation

Building upon the several process technologies discussed in Sections 4 and 5, process integration was carried out for the production of high-titer ethanol and lignosulfonate from forest harvest residue using the SPORL technology without detoxification and solids washing (Zhu and other 2015).

Table 5—Chemical composition of untreated lodgepole pine BD4 wood chips and wood component recoveries from SPORL at two temperatures[a]

	Untreated wood	Unwashed solids[b]		Collected spent liquor[b]		Total recovery (%)	
		165 °C	180 °C	165 °C	180 °C	165 °C	180 °C
Wet weight (kg)	2.281	6.227	5.620	1.726	2.250		
Solids content (%)	87.7	31.86	29.98	8.37	9.09		
Solids (kg)[c]	2.0	1.984; 90.0%	1.685; 76.4%	0.144; 6.6%	0.205; 9.3%	96.6	85.7
Klason lignin (%)	28.6	28.31; 99.0%	24.45; 85.5%	0.29; 1.0%	4.15; 14.5%	100.0	100.0
Arabinan (%)	1.7	0.58; 34.2%	0.39; 23.0%	0.06; 3.3%	0.07; 3.9%	37.6	26.9
Galactan (%)	2.9	1.43; 49.3%	1.04; 35.9%	0.40; 13.9%	0.55; 18.9%	63.1	54.8
Glucan (%)	41.9	40.64; 97.0%	34.68; 82.8%	0.60; 1.4%	1.03; 2.5%	98.4	85.2
Mannan (%)	11.7	5.98; 51.1%	4.21; 36.0%	1.70; 14.5%	2.39; 20.4%	65.6	56.4
Xylan (%)	5.5	2.86; 52.0%	1.87; 33.9%	0.53; 9.7%	0.70; 12.7%	61.6	46.6
HMF (%)[d]		0.43; 3.7%	0.72; 6.1%	0.18 (1.7); 1.5%	0.41 (3.0); 3.5%	5.2	9.6
Furfural (%)[d]		0.31; 5.7%	0.40; 7.3%	0.13 (1.1); 2.3%	0.23 (1.6); 4.2%	8.0	11.5
Acetic acid (%)		1.87	2.05	0.76	1.17		

[a]Pretreatments were conducted at sulfuric acid and sodium bisulfite loading on wood of 2.2 and 8%, respectively, for 25 and 75 min at 180 °C and 165 °C, respectively. Data from Zhou and others (2014).
[b]The numbers after ";" are wt% of theoretical based on untreated wood carbohydrate content.
[c]Oven dry (od) weight.
[d]Reported as xylan and mannan for furfural and HMF, representing percent of xylan and mannan converted to furfural and HMF, respectively. Numbers in parentheses are concentrations measured in the collected spent liquor in g/L.

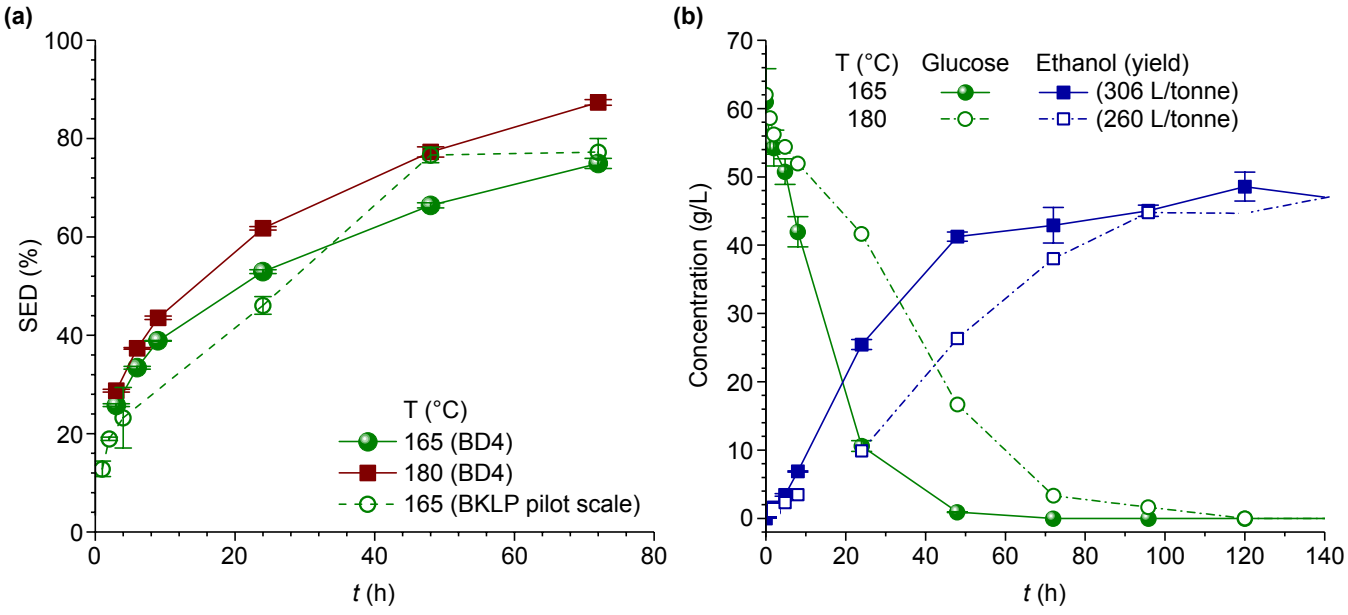

Figure 15—Comparison of high titer ethanol production from SPORL-pretreated lodgepole pine at two temperatures: (a) comparisons of water insoluble solids (WIS) substrate enzymatic digestibility (SED), including data from a substrate from pilot-scale of 50-kg pretreatment (modified from (Zhou and others (2014)); (b) time-dependent glucose utilization and ethanol concentration in the fermentation broth of the pretreated whole slurry (Zhou and others 2014).

Table 6—Chemical composition of untreated BKLP wood chips and wood component recoveries from a pilot-scale SPORL run in a 390-L wood pulping digester[a]

	Untreated wood	Unwashed solids[b]	Collected spent liquor[b]	Total recovery (%)	Washed solids[b]
Wet weight (kg)	49.95	109.45	39.95		
Solids content (%)	80.1	31.9	15.8		
Solids (kg)[c]	40.0	34.86; 87.2%	6.31; 15.8%	102.9	26.40; 66.0%
Klason lignin (%)	29.85 ± 0.01	22.48 ± 0.39; 75.3%	2.32; 7.8%	83.1	23.01 ± 0.33; 77.1%
Arabinan (%)	1.76 ± 0.02	0.55 ± 0.01; 31.2%	0.00	31.2	0.00
Galactan (%)	3.56 ± 0.04	1.54 ± 0.02; 44.3%	0.74; 20.8%	64.1	0.12 ± 0.11; 7.0%
Glucan (%)	39.00 ± 0.03	36.95 ± 1.53; 94.7%	1.13; 2.9%	97.6	37.46 ± 0.94; 88.4%
Mannan (%)	9.46 ± 0.11	5.95 ± 0.14; 61.4%	2.49; 26.4%	87.8	1.42 ± 0.16; 21.3%
Xylan (%)	7.23 ± 0.01	3.64 ± 0.11; 50.4%	1.16; 16.0%	66.4	1.57 ± 0.12; 37.6%
HMF (%)[d]		0.30; 3.1%	0.11 (0.8); 1.1%	4.3	
Furfural (%)[d]		0.36; 5.0%	0.13 (1.0); 1.8%	6.8	
Acetic acid (%)		1.17	0.43 (4.3)		

[a]Pretreatment was conducted at sulfuric acid and sodium bisulfite loading on wood of 2.2 and 8%, respectively, for 60 min at 165 °C with ramping time to 165 °C of 38 min. Data from Zhou and others (2015b).
[b]The first numbers of the chemical component data are based 100 kg of untreated wood; the % data are theoretical yields based on total solids or component of untreated wood.
[c]Based on oven dry (od) basis of untreated wood
[d]Conversion rates of the compounds indicated. The numbers in parentheses are concentrations measured in the collected spent liquor in g/L.

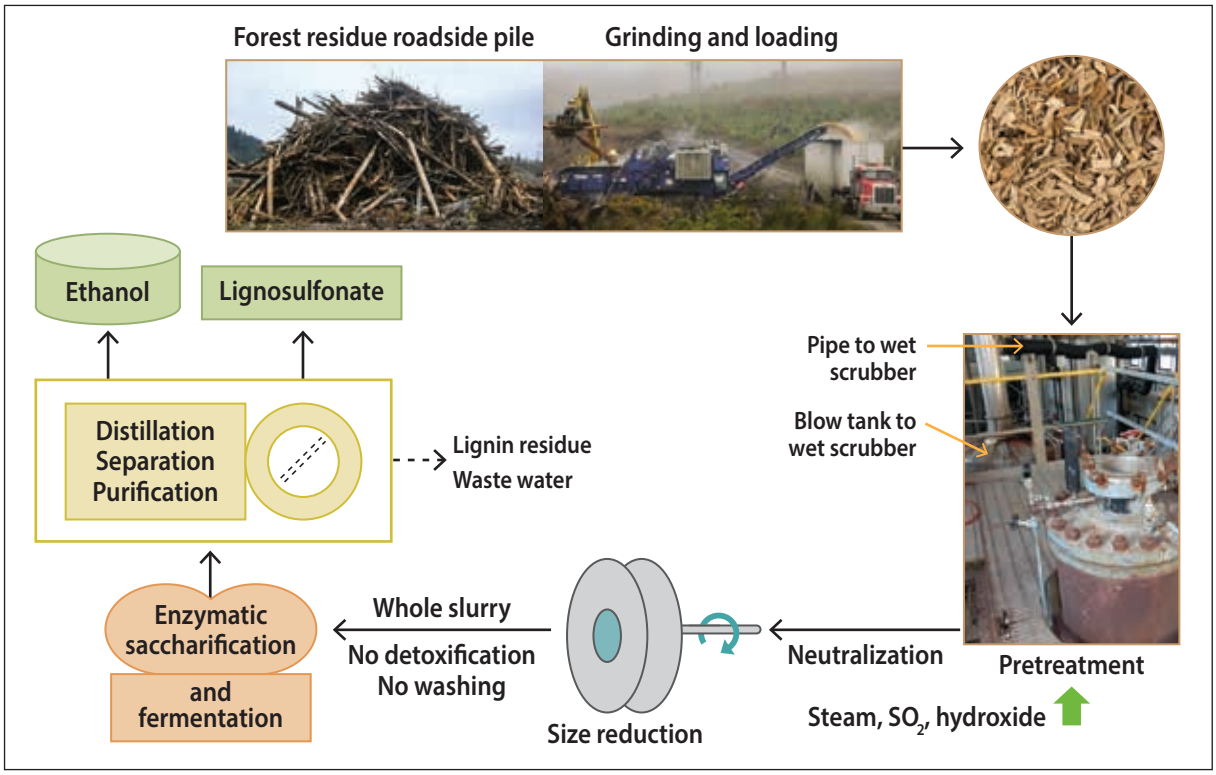

Figure 16—Schematic process flow diagram showing high titer ethanol and lignosulfonate production from forest residue using SPORL at a pilot scale (Zhou and others 2013b), including grinding and loading of roadside piles of harvest residue and pictures of pilot-scale wood pulping digester and blow tank and caustic wet scrubbing of noncondensable gases from SPORL.

Table 7—Comparison of ethanol production of two SPORL studies using lodgepole pine and a literature work based on SO_2 steam explosion from spruce[a]

	SPORL, lodgepole pine (BKLP) (pilot-scale 50 kg)	SPORL, lodgepole pine (BD4) (bench-scale 2 kg)	SO_2 steam explosion, spruce
Pretreatment			
Temperature (°C)	165	165	205
Duration (min)	60 (38-min ramping)	75 (10-min ramping)	6–7
Liquor to wood ratio (L/kg)	3.0	3.0	~3.0
Q-SSF			
Water-insoluble solids (WIS) (%)	15.1	12.0	13.7
Total wet mass (g)	50	50	1,300
Mixing mode	Shaking bed: poor	Shaking bed: poor	Mechanical mixing: good
Cellulase (FPU/g WIS)	11.5 CTec3	11.1 CTec2	10.0 CTec2 + β-glucosidase
Yeast (g dry cell/L)	~0.1	~0.1	5
Nutrients for fermentation			
$(NH_4)_2HPO_4$ (g/L)	None	None	0.5
$MgSO_4 \cdot 7H_2O$ (g/L)	None	None	0.025
Yeast extract (g/L)	None	None	1.0
Liquefaction time (h)	24–26	22	22
Final ethanol production			
Ethanol concentration (g/L)	52.2	47.1	47.8
Ethanol yield (% of theoretical)[a]	82.5	79.2	72.0

[a]Data from Zhou and others (2013b) and Hoyer and others (2013).
[b]Theoretical yield based on glucan and mannan in the untreated wood.

Roadside piles of Douglas-fir plantation harvest residue were ground and then loaded into a truck (Fig. 16). The ground materials can be either screened on the conveyer belt while loading to the truck or separately screened to remove the fine fraction, which has high bark and ash contents (Cheng and others 2015; Zhang and others 2012b). The screened forest residue (FS-10) had a lower bark and ash content than the as-ground material. A pretreatment using 61.75 kg of FS-10 at a moisture content of 18.6% was conducted in the 390-L rotating wood pulping digester at 145 °C with a minimal total SO_2 loadings of 6.6 wt% on wood (oven-dry base), or approximately 23 g/L in the pretreatment liquor. Loading of $Ca(OH)_2$ on wood was 2.4 wt%. Final liquor-to-wood ratio was 3.55 (L/kg) including the amount of moisture used in pre-steaming. The heat-up period to 145 °C was 37 min. The calculated required pretreatment duration was 231–277 min based on the scale-up design of Eq. (12) (Table 4) using laboratory bench-scale optimal time of 25–30 min at 180 °C (Leu and others 2013). Considering the longer heat-up period of 37 min required for the 390-L digester relative to that for the 23-L bench-scale digester, a pretreatment duration of 240 min was used at 145 °C. At the end of pretreatment, the digester contents were discharged to a blow tank that was connected to a wet scrubber to eliminate any noncondensable gases, including SO_2 (Fig. 16). The free drainable liquor was collected, neutralized, and fed to a disk refiner together with the collected wet solids, which contains approximately two-thirds of the pretreatment spent liquor, resulting in the whole slurry.

The q-SSF was carried out by first liquefying the pretreated whole slurry using CTec3 after neutralizing pH to approximately 6.0 and then adding *Saccharomyces cerevisiae* YRH400 for fermentation. The yeast was grown on YPD agar plates (Zhou and others 2013b). This q-SSF was conducted without supplementation of nutrients, solids and liquor separation, solids washing, or detoxification. At the end of fermentation, the residual solids, which contained primarily water-insoluble sulfonated lignin, were separated using a centrifuge. The supernatant was fed into an in-house built membrane filtration system to separate lignosulfonate from smaller molecular weight ethanol and remaining soluble sugars. The entire process is relatively simple for simultaneous production of bio-ethanol at high titer and lignosulfonate.

The overall mass balance of the process is shown in Figure 17 for q-SSF at 18% total solids with a cellulase (CTec3) loading of 35 mL/kg FS-10. A terminal ethanol yield of 284 L/t FS-10, or 70% theoretical based on glucan, mannan, and xylan content in FS-10, at a titer of 41.9 g/L was achieved. SPORL dissolved approximately 40% of the lignin as lignosulfonate, which is readily marketable without

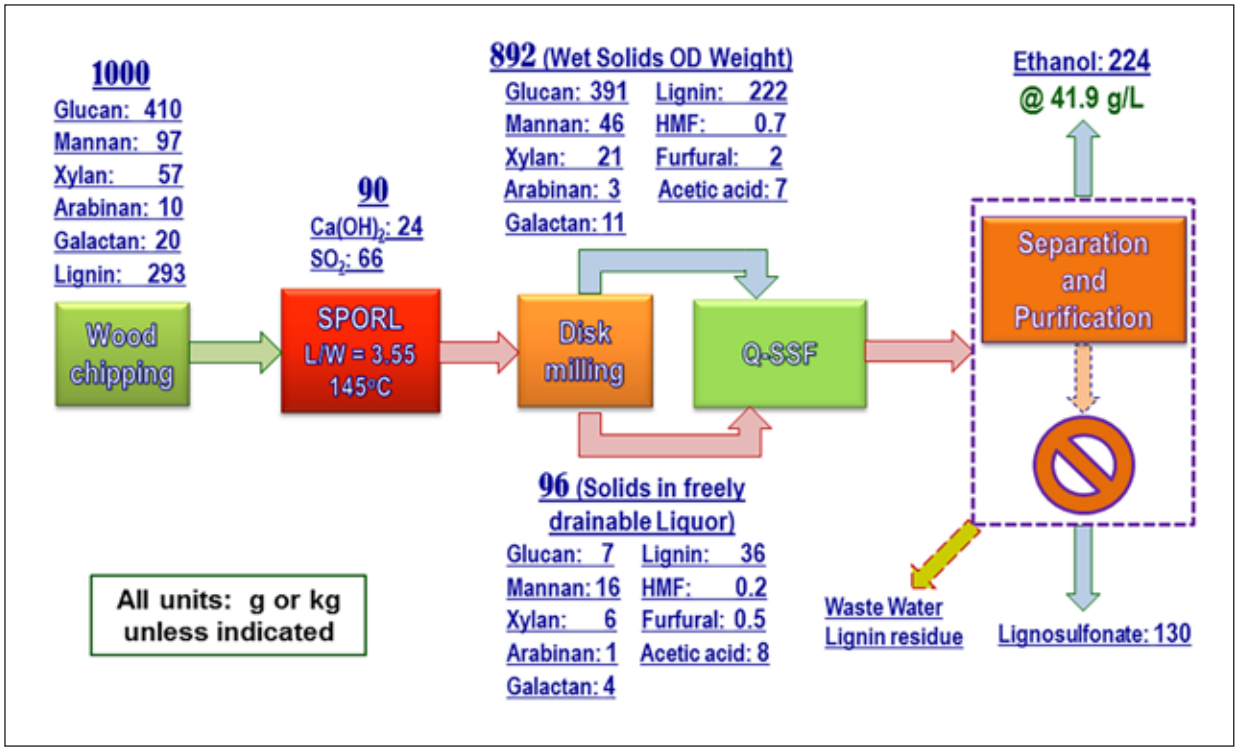

Figure 17—Overall mass balance for productions of bioethanol and lignosulfonate from a Douglas-fir forest residue (FS-10) using SPORL at a pilot-scale (Zhu and others 2015).

further processing—a significant economic advantage over competing technologies. Even without mechanical shear mixing, an equivalent ethanol yield of 68% theoretical was achieved at a lower CTec 3 loading of approximately 26 mL/kg FS-10 (Zhu and others 2015). It is expected that mechanical mixing can further enhance ethanol yield and that a low cellulase loading of approximately 25 mL/kg forest residue or 15 FPU/g glucan is sufficient to produce an excellent ethanol yield.

To reduce pretreatment duration at low pretreatment temperatures, we increased SO_2 loading (as discussed in Section 4.5). An increase in total SO_2 charge on wood by a factor of 4 to a targeted value of 32 wt% with a combined SO_2 (with Mg) of 4.4 wt% can reduce pretreatment duration to 60 min at 140 °C to produce excellent digestible substrate from a Douglas-fir forest residue FS-10 (Gu and others 2016). Furan formation was very low, less than 0.5 g/L, using liquid-to-wood ratio of 4. Compared with the low SO_2 loading study (6.6 wt% on wood) at 145 °C with long pretreatment time of 240 min (Zhu and others 2015), the high SO_2 loading study produced a maximal terminal ethanol yield of 321 L/t FS-10 at a titer of 56.3 g/L, compared with 284 L/t FS-10 at 41.9 g/L from the run with low SO_2 loading of 6.6 wt% on wood under the same CTec3 loading of 35 mL/kg FS-10.

6.4 Bioethanol Production from Poplar—Scale-up Evaluation

Poplar has been identified as a short-rotation energy crop that can grow on marginal land, recycle nutrients, and sequester carbon (Vance and others 2010). Bioconversion of poplar wood to biofuels through fermentation of hydrolyzed sugars is quite challenging. As a woody biomass, poplar species have a relatively higher lignin content than herbaceous biomass and can be highly recalcitrant to enzymatic saccharification (Wang and others 2012b; Studer and others 2011). As discussed in Section 4.4, poplar species also have a relatively high content of acetyl group (Gille and Pauly 2012) that can be easily converted to acetic acid (Tune and Van Heiningen 2008; Tian and others 2011), a fermentation inhibitor that is difficult to neutralize or distill (Xavier and others 2010) and cannot be metabolized by yeast (Wei and others 2013). Typical yeast can tolerate only approximately 15 g/L of acetic acid (Keating and others 2006). De-acetylation using hydroxide requires additional processing (Chen and others 2012; Kundu and others 2014). The compounding effect of acetic acid with furan and aromatics makes fermentative biofuel production from poplar very difficult (Palmqvist and others 1999), with few successes reported.

Here bioethanol production is demonstrated from poplar NE222 using the same 390-L pilot-scale digester for

pretreatment as described in the Section 6.3. The experimental design was based on the results from a 1-L scale study using CHF. As shown in Figure 8b, the laboratory 1-L scale study (Zhang and others 2015) indicated that pretreatment severity CHF needed to be reduced from the optimal CHF for maximal sugar yield (Fig. 5c,d). A target CHF of 2.7 was used based on the results in Fig. 8b (Zhou and others 2015a). Using Eq. (5), the pretreatment time of 45 min was determined for pretreatment at 160 °C with a liquor-to-wood ratio of 3 L/kg and sulfuric acid and sodium bisulfite charge on wood of 1.1 and 4.0 wt%, respectively. The actual CHF was 3.3 with a shorter pretreatment time of 40 min (due to the longer temperature ramping time of 30 min in the 390-L digester) and a sodium bisulfite loading of 3 wt%. The experimental details for pretreatment and q-SSF were similar to those described in Section 6.3. Again, the disk-refined whole slurry was not detoxified and nutrients were not supplemented for direct q-SSF after neutralization to pH 6.0 using a CTec3 loading of 26 mL/kg untreated wood.

Mass balance for the pretreatment is shown in Table 8 along with the furans and acetic acid concentrations of approximately 3 and 13 g/L, respectively, in the collected spent liquor. Glucan loss was minimal at approximately 5%. Xylan and mannan dissolutions were approximately 75% and 55%, respectively. Major sugar consumption and ethanol production through q-SSF using YRH400 at three different yeast loadings are shown in Figure 18. Glucose was rapidly consumed except at very low yeast loading of 0.1 mg/g. With the high xylan content of poplar and therefore high xylose concentration, YRH400 was able to consume approximately 25% of the xylose. The low xylose consumption was due to the presence of fermentation inhibitors and because *S. cerevisiae* relies on hexose transport, which has low affinity to xylose. Mannose was not completely consumed due to low concentration. Overall, the scale-up results agreed with laboratory bench-scale experiment (Fig. 8b), suggesting that using CHF can properly scale up SPORL pretreatment of poplar. A final ethanol yield of 247 L/t wood, or 54.7% theoretical based on NE222 glucan, xylan, and mannan content, at titer of 43.6 g/L was obtained using a shaker flask for fermentation without shear mixing.

6.5 Properties of Lignosulfonate from SPORL

Very high ethanol yield has been achieved using SPORL with limited room for further improvement (as discussed in Section 6.4). The fuel market is very large, with a very low risk of market saturation. However, fuel is a low-value commodity and the return from fuel production alone is not economically viable for commercialization. Therefore, producing lignin co-products are vital. SPORL has the advantages of being able to partially solubilize lignin through sulfonation. The dissolved lignin is a directly marketable product as lignosulfonate, which can produce another stream of revenue for improving economics.

Table 8—Wood component recoveries from a pilot-scale SPORL run using poplar NE222 along with the chemical composition of the untreated NE222[a]

	Untreated wood	Unwashed solids[b]	Collected spent liquor[b]	Total recovery (%)	Washed solids
Wet weight (kg)	81.90	110.70	32.15		
Solids content (%)	49.9	32.9	11.1		
Solids (kg)[c]	40.9	36.45; 89.2%	3.58; 8.8%	97.9	27.52; 67.3%
Klason lignin (%)	23.43 ± 2.03	18.56 ± 0.05; 79.1%	4.87; 20.8%	100.0	16.69 ± 0.03; 71.2%
Arabinan (%)	0.61 ± 0.43	0.21 ± 0.03; 35.1%	0.07; 12.0%	47.1	0.00 ± 0.00; 0.0
Galactan (%)	0.61 ± 0.06	0.32 ± 0.01; 52.6%	0.13; 21.9%	74.6	0.00 ± 0.00; 0.0
Glucan (%)	46.76 ± 3.32	44.36 ± 0.52; 94.9%	0.10; 0.2%	95.1	40.83 ± 0.69; 87.3%
Mannan (%)	2.84 ± 0.03	1.86 ± 0.20; 65.6%	0.18; 6.5%	72.1	1.31 ± 0.03; 46.3%
Xylan (%)	12.74 ± 0.05	8.65 ± 0.06; 67.9%	1.74; 13.6%	81.5	3.24 ± 0.26; 25.4%
HMF (%)[d]		0.11; 3.9%	0.05 (0.5); 1.7%	5.5	
Furfural (%)[d]		0.59; 4.6%	0.26 (2.4); 2.0%	6.7	
Acetic acid (%)		2.43	1.05 (13.4)		

[a]Pretreatment was conducted in a 390-L rotating wood pulping digester with sulfuric acid and sodium bisulfite loading on wood 1.1 and 3 wt%, respectively, for 40 min at 160 °C. Data from Zhou and others (2015a).
[b]The first numbers of chemical component data are based on 100 kg of untreated wood; the numbers after ";" are wt% of theoretical based on total solids or component mass in the amount of untreated wood.
[c]Oven dry (od) weight based on untreated wood.
[d]Reported as xylan and mannan for furfural and HMF, representing percentage of xylan and mannan converted to furfural and HMF, respectively. The numbers in parentheses are concentration measured in the collected spent liquor in g/L.

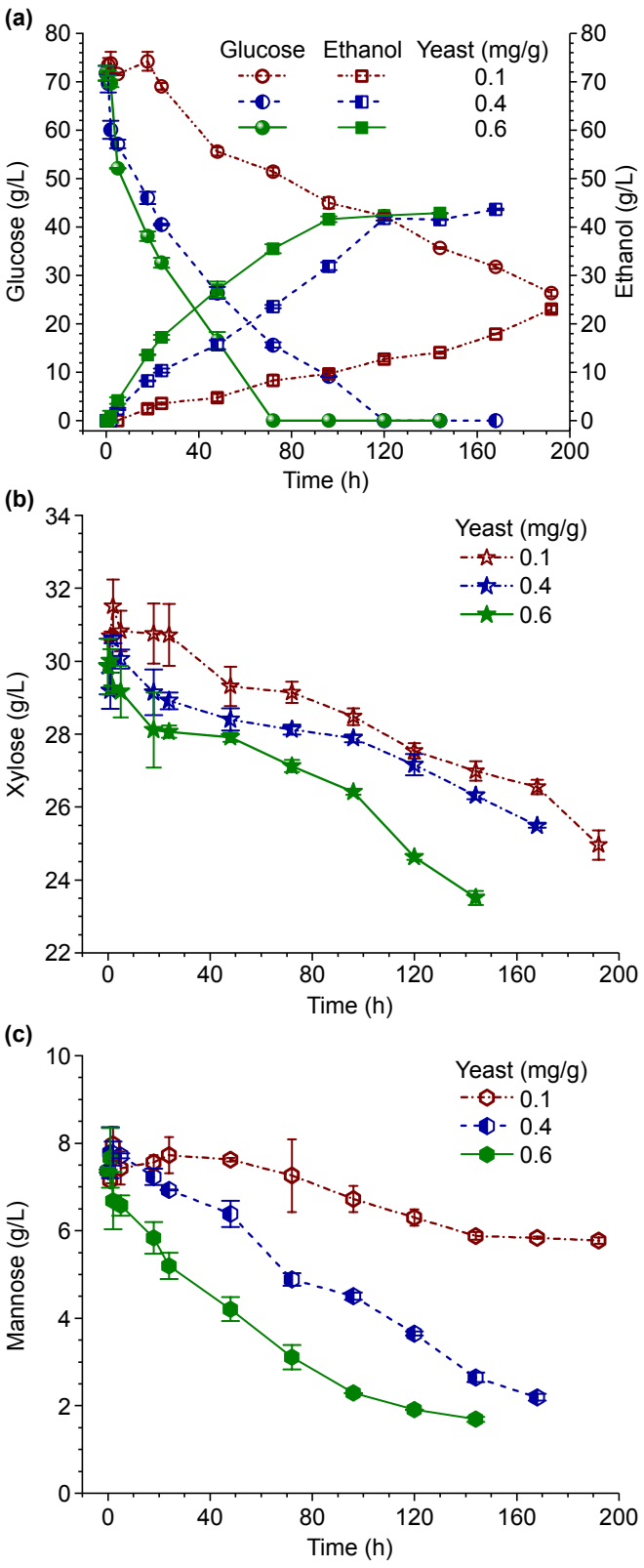

Figure 18—Time-dependent ethanol production and sugar consumptions during Q-SSF at 20% total solids loading without detoxification of the whole slurry of SPORL-pretreated NE222 at a pilot-scale (Zhou and others 2015a): (a) ethanol and glucose; (b) xylose; (c) mannose.

Molecular weight and degree of sulfonation are two important properties of lignosulfonate. The spent liquor from the pilot-scale pretreatment of BKLP at 165 °C was purified using dialysis through a membrane system. The purified lignosulfonate was analyzed and compared with a commercial purified softwood lignosulfonate D-748 (Zhou and others 2015b). Results indicate that SPORL lignosulfonate (LS-SP-BKLP) had similar sulfonation as D-748, with a sulfur content of approximately 6 wt%, but it had a lower molecular weight of approximately 10,000, compared with approximately 40,000 for D-748. Membrane separation of lignosulfonate after q-SSF was employed on the substrate produced in the pilot-scale studies described in Section 6.3. Similar results were also obtained for the lignosulfonate from a Douglas-fir forest residue pretreated at 145 °C (Zhu and others 2015) after correction for difference in molecular weight calibration. Preliminary testing showed that the performance of SPORL lignosulfonate was comparable or slightly better as a dispersant for coal water slurry.

6.6 Production of Lipid from a Softwood Forest Residue

SPORL-pretreated whole slurries of a Douglas-fir forest residue were used to produce intracellular microbial lipid using oleaginous microorganisms. In the first study (Harde and others 2016), two SPORL-pretreated Douglas-fir residue whole slurries at a high targeted SO_2 loading of 32 wt% on wood (Gu and others 2016) were fermented using two yeast strains, *Cryptococcus curvatus* NRRL Y-1511 and *Rhodosporidium toruloides* Y-1091, and two fungi, *Mortierella isabellina* NRRL 1757 and *Chaetomium globosum* NRRL 1870, all from the Agricultural Research Service (ARS) Culture Collection, Illinois. It was found that high sulfite in the whole slurry inhibited fermentation using these organisms. Fortunately overliming using calcium hydroxide at 50 °C for 30 min with the removal of overliming-produced solids through centrifuge was found sufficient to detoxify the whole slurry for effective fermentation. Maximal lipid yield of 0.15 g/g sugar at lipid concentration of 18.6 g/L was achieved from the detoxified whole slurry using *Mortierella isabellina* NRRL 1757.

In the second study (Dien and others 2016), two yeast strains, *L. tetrasporus* NRRL Y-11562 and *Y. lipolytica* NRRL YB-437, both from the Agricultural Research Service (ARS) Culture Collection, Illinois, were used to ferment two SPORL-pretreated Douglas-fir residue whole slurries produced using a low sulfite loading with and without pH-profiling (Cheng and others 2015). Using two multiple batch culture schemes, lipid yield of 0.174 g/g sugar at titer of 13.4 g/L and 0.104 g/g at titer 18.1 g/L were achieved without detoxifying the SPORL whole slurry sugars.

6.7 Production of Iso-Butanol and Bio-Jet from a Softwood Forest Residue

Attempts were made to produce biofuels other than ethanol from SPORL-pretreated softwood forest residue. With the financial support of a USDA-NIFA project (No. 2011-68005-30416) through the Northwest Advanced Renewables Alliance (NARA), SPORL-pretreated forest residue has been used to produce iso-butanol with subsequent conversion to isoparaffinic kerosene (IPK), or bio-jet. Approximately 60 t of Douglas-fir forest residue were pretreated using SPORL at an industrial facility of ZeaChem in Boardman, Oregon. The pretreated materials were shipped to an industrial saccharification and fermentation facility of ICM in St. Joseph, Missouri, to produce iso-butanol using a genetically modified yeast developed by GEVO. The iso-butanol will be then shipped to South Hampton Refining in Silsbee, Texas, to produce approximately 1,400 gallons of IPK. The IPK will be blended with petroleum jet using an ASTM-certified process for a commercial flight by Alaska Airlines in 2016. Laboratory studies at various scales produced excellent yield of iso-butanol by GEVO. Iso-butanol itself is a drop-in biofuel and a valuable building block for producing various chemicals.

7. Summary

SPORL as a pretreatment process demonstrated robust performance for bioconversion of very recalcitrant lignocelluloses such as woody biomass and especially softwood forest harvest residues. Lignin sulfonation reduces nonproductive binding of cellulase to lignin and facilitates the processing of the pretreated whole slurry without solid and liquor separation or solids washing, even at high solids loadings. Specifically, the sulfonated lignin on solid substrate is relatively hydrophilic and has low affinity to cellulase. Further reduction of nonproductive binding of cellulase can be achieved by conducting enzymatic saccharification at elevated pH around 5.5 through pH-induced electrostatic repulsion between cellulase and lignin on solid substrate. Moreover, the dissolved lignin as lignosulfonate is a surfactant and can enhance enzymatic saccharification. Low-temperature pretreatments can be designed using a combined hydrolysis factor (CHF) as a pretreatment severity indicator that can accurately predict hemicellulose dissolution and lead to reduced sugar degradation to furans for high-titer biofuel production without detoxification. Excellent ethanol yields of approximately 72% theoretical of available wood glucan, mannan, and xylan were achieved for softwoods through fermentation of the SPORL-pretreated whole slurry. The q-SSF fermentations generated high titers of over 40 g/L at relatively low enzyme dosages of 26 mL/kg wood without shear mixing during liquefaction of the pretreated solid substrates. SPORL is also effective for converting hardwood to biofuel at high titer without detoxification, despite the high acetyl group content in hardwood species. Dissolved lignin is a valuable co-product that can be directly marketed to improve biofuel production economics. Because SPORL was developed based on sulfite pulping, it is scalable for commercial production with low risks. Overall, SPORL has great commercial potential compared with competing technologies.

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Literature Cited

Abatzoglou, N.; Chornet, E.; Belkacemi, K.; Overend, R.P. 1992. Phenomenological kinetics of complex systems: the development of a generalized severity parameter and its application to lignocellulosics fractionation. Chemical Engineering Science. 47(5):1109-1122.

Berlin, A.; Balakshin, M.; Gilkes, N.; Kadla, J.; Maximenko, V.; Kubo, S.; Saddler, J. 2006. Inhibition of cellulase, xylanase and beta-glucosidase activities by softwood lignin preparations. Journal of Biotechnology. 125(2):198-209.

Borjesson, J.; Peterson, R.; Tjerneld, F. 2007. Enhanced enzymatic conversion of softwood lignocellulose by poly(ethylene glycol) addition. Enzyme and Microbial Technology. 40(4):754-762.

Brasch, D.J.; Free, K.W. 1965. Prehydrolysis-Kraft pulping of Pinus radiata grown in New Zealand. TAPPI. 48(4):245-248.

Bryce, J.R.G. 1980. Sulfite pulping. In: Casey, J., ed. Pulp and paper: chemistry and chemical technology, vol. I, third ed. New York: John Wiley & Sons: 291-376.

Chen, X.; Shekiro, J.; Franden, M.A.; Wang, W.; Zhang, M.; Kuhn, E.; Johnson, D.K.; Tucker, M.P. 2012. The impacts of deacetylation prior to dilute acid pretreatment on the bioethanol process. Biotechnology for Biofuels. 5:8.

Cheng, J.; Leu, S-Y.; Zhu, J.Y.; Gleisner, R. 2015. High titer and yield ethanol production from undetoxified whole slurry of Douglas-fir forest residue using pH-profiling in SPORL. Biotechnology and Biofuels. 8:22.

Chirico, W.J.; Brown, Jr., R.D. 1987. Purification and characterization of a β-glucosidase from *Trichoderma reesei*. European Journal of Biochemistry. 165(2):333-341.

Chum, H.L.; Johnson, D.K.; Black, S.K.; Overend, R.P. 1990. Pretreatment-catalyst effects of the combined severity parameter. Applied Biochemistry and Biotechnology. 24/25:1-14.

Croon, I. 1965. The flexibility of sodium-based two-stage neutral-acid sulfite pulping. . Pulp Paper Mag Canada. February:T71-T76.

Dien, B.S.; Zhu, J.Y.; Slininger, P.J.; Kurtzman, C.P.; Moser, B.; O'Bryan, P.J.; Gleisner, R.; Cotta, M.A. 2016. Conversion of SPORL pretreated Douglas-fir forest residue into microbial lipids with oleaginous yeasts. RSC Advances. 6(25):20695-20705.

Eriksson, T.; Borjesson, J.; Tjerneld, F. 2002. Mechanism of surfactant effect in enzymatic hydrolysis of lignocellulose. Enzyme and Microbial Technology. 31(3):353-364.

Gille, S.; Pauly, M. 2012. O-acetylation of plant cell wall polysaccharides. Frontiers in Plant Science. 3:12.

Gu, F.; Gilles, W.; Gleisner, R.; Zhu, J.Y. 2016. Fermentative high titer ethanol production from a Douglas-fir forest residue without detoxification using SPORL: high SO2 loading at a low temperature. Ind. Biotechnol. 12:168-175.

Hansen, J.; Ely, K.; Horsley, D.; Herron, J.; Hlady, V.; Andrade, J.D. 1988. The adsorption of lysozymes: A model system. Makromol Chem Macromol Symp. 17:135-154.

Harde, S.M.; Wang, Z.; Zhu, J.Y.; Pan, X. 2016. Microbial lipid production from SPORL-pretreated Douglas fir by *Mortierella isabellina*. Fuel. 175:64-74.

Haynes, C.A.; Norde, W. 1994. Globular proteins at solid/liquid interfaces. Colloids and Surfaces B: Biointerfaces. 2(6):517-566.

Hector, R.E.; Dien, B.S.; Cotta, M.A.; Qureshi, N. 2011. Engineering industrial *Saccharomyces cerevisiae* strains for xylose fermentation and comparison for switchgrass conversion. Journal of Industrial Microbiology and Biotechnology. 38(9):1193-1202.

Hoyer, K.; Galbe, M.; Zacchi, G. 2013. The effect of prehydrolysis and improved mixing on high-solids batch simultaneous saccharification and fermentation of spruce to ethanol. Process Biochemistry. 48(2):289-293.

Hui, J.P.M.; Lanthier, P.; White, T.C.; McHugh, S.G.; Yaguchi, M.; Roy, R.; Thibault, P. 2001. Characterization of cellobiohydrolase I (Cel7A) glycoforms from extracts of *Trichoderma reesei* using capillary isoelectric focusing and electrospray mass spectrometry. Journal of Chromatography B: Biomedical Sciences and Applications. 752(2):349-368.

Ingruber, O. 1985. Sulfite science, part I: Sulfite pulping cooking liquor and the four bases. In: Ingruber, O; Kocurek, M.; Wong, A., eds. Sulfite science and technology, vol. 4, third ed. Atlanta, GA: The joint textbook Committee of the Paper Industry, TAPPI/CPPA: 3-23.

Jacobsen, S.E.; Wyman, C.E. 2000. Cellulose and hemicellulose hydrolysis models for application to current and novel pretreatment processes. Applied Biochemistry and Biotechnology—Part A Enzyme Engineering and Biotechnology. 84-86:81-96.

Kamireddy, S.R.; Kozliak, E.I.; Tucker, M.; Ji, Y. 2014. Kinetic features of xylan de-polymerization in production of xylose monomer and furfural during acid pretreatment for kenaf, forage sorghums and sunn hemp feedstocks. International Journal of Agricultural and Biological Engineering. 7(4):86-98.

Keating, J.D.; Panganiban, C.; Mansfield, S.D. 2006. Tolerance and adaptation of ethanologenic yeasts to lignocellulosic inhibitory compounds. Biotechnology and Bioengineering. 93(6):1196-1206.

Kobayashi, T.; Sakai, Y. 1956. Hydrolysis rate of pentosan of hardwood in dilute sulfuric acid. Bulletin of the Agricultural Chemistry Society Japan. 20:1-7.

Kundu, C.; Lee, H.J.; Lee, J.W. 2014. Enhanced bioethanol production from yellow poplar by deacetylation and oxalic acid pretreatment without detoxification. Bioresource Technology. 178:28-35.

Lagergren, S. 1964. The Stora pulping process and its implications for European paper makers. Svensk Papperstidning. 67(238-243).

Lan, T.Q.; Lou, H.; Zhu, J.Y. 2013. Enzymatic saccharification of lignocelluloses should be conducted at elevated pH 5.2–6.2. Bioenergy Research. 6(2):476-485.

Larsson, S.; Palmqvist, E.; Hahn-Hagerdal, B.; Tengborg, C.; Stenberg, K.; Zacchi, G.; Nilvebrant, N-O. 1999. The generation of fermentation inhibitors during dilute acid hydrolysis of softwood. Enzyme and Microbial Technology. 24:151-159.

Leu, S.Y.; Zhu, J.Y. 2013. Substrate-related factors affecting enzymatic saccharification of lignocelluloses: Our recent understanding. Bioenergy Research. 6(2):405-415.

Leu, S-Y.; Gleisner, R.; Zhu, J.Y.; Sessions, J.; Marrs, G. 2013. Robust enzymatic saccharification of a Douglas-fir forest harvest residue by SPORL. Biomass and Bioenergy. 59:393-401.

Liu, H.; Zhu, J.Y.; Fu, S. 2010. Effects of lignin-metal complexation on enzymatic hydrolysis of cellulose. Journal of Agricultural Food Chemistry. 58:7233-7238.

Lou, H.; Zhu, J.Y.; Lan, T.Q.; Lai, H.; Qiu, X. 2013. pH-induced lignin surface modification to reduce nonspecific cellulase binding and enhance enzymatic saccharification of lignocelluloses. ChemSusChem. 6(5):919-927.

Lourenço, A.; Gominho, J.; Pereira, H. 2011. Modeling of sapwood and heartwood delignification kinetics of *Eucalyptus globulus* using consecutive and simultaneous approaches. Journal of Wood Science. 57(1):20-26.

Lu, Y.; Mosier, N.S. 2008. Kinetic modeling analysis of maleic acid-catalyzed hemicellulose hydrolysis in corn stover. Biotechnology and Bioengineering. 101(6):1170-1181.

Luo, X.; Gleisner, R.; Tian, S.; Negron, J.; Horn, E.; Pan, X.J.; Zhu, J.Y. 2010. Evaluation of mountain beetle infested lodgepole pine for cellulosic ethanol production by SPORL pretreatment. Industrial Engineering Chemistry Research. 49(17):8258-8266.

Maloney, M.T.; Chapman, T.W.; Baker, A.J. 1985. Dilute acid hydrolysis of paper birch: Kinetics studies of xylan and acetyl-group hydrolysis. Biotechnology and Bioengineering. 27(3):355-361.

Mansfield, S.D.; Mooney, C.; Saddler, J.N. 1999. Substrate and enzyme characteristics that limit cellulose hydrolysis. Biotechnology Progress. 15:804-816.

Miranda, I.; Pereira, H. 2002. Kinetics of ASAM and kraft pulping of eucalypt wood (*Eucalyptus globulus*). Holzforschung. 56(1):85-90.

Montané, D.; Salvadó, J.; Torras, C.; Farriol, X. 2002. High-temperature dilute-acid hydrolysis of olive stones for furfural production. Biomass and Bioenergy. 22(4):295-304.

Morinelly, J.E.; Jensen, J.R.; Browne, M.; Co, T.B.; Shonnard, D.R. 2009. Kinetic characterization of xylose monomer and oligomer concentrations during dilute acid pretreatment of lignocellulosic biomass from forests and switchgrass. Industrial and Engineering Chemistry Research. 48(22):9877-9884.

Nagle, N.J.; Elander, R.T.; Newman, M.M.; Rohrback, B.T.; Ruiz, R.O.; Torget, R.W. 2002. Efficacy of a hot washing process for pretreated yellow poplar to enhance bioethanol production. Biotechnology Progress. 18:734-738.

Nakagame, S.; Chandra, R.P.; Kadla, J.F.; Saddler, J.N. 2011a. The isolation, characterization and effect of lignin isolated from steam pretreated Douglas-fir on the enzymatic hydrolysis of cellulose. Bioresource Technology. 102(6):4507-4517.

Nakagame, S.; Chandra, R.P.; Saddler, J.N. 2011b. The influence of lignin on the enzymatic hydrolysis of pretreated biomass substrates. In: Zhu, J.Y.; Zhang, X.; Pan, X.J., eds. Sustainable production of fuels, chemicals, and fibers from forest biomass. Washington, DC: American Chemical Society: 145-167.

Palmqvist, E.; Grage, H.; Meinander, N.Q.; Hahn-Hägerdal, B. 1999. Main and interaction effects of acetic acid, furfural, and p-hydroxybenzoic acid on growth and ethanol productivity of yeasts. Biotechnology and Bioengineering. 63(1):46-55.

Palonen, H.; Tjerneld, F.; Zacchi, G.; Tenkanen, M. 2004. Adsorption of *Trichoderma reesei* CBH I and EG II and their catalytic domains on steam pretreated softwood and isolated lignin. Journal of Biotechnology. 107(1):65-72.

Perlack, R.D.; Stokes, B.J. 2011. U.S. billion-ton update: biomass supply for a bioenergy and bioproducts industry, vol. DE-AC05-00R22725. Oak Ridge: Oakridge National Laboratory.

Qi, J.; Lu, X. 2007. Kinetics of non-catalyzed decomposition of D-xylose in high temperature liquid water. Chinese Journal of Chemical Engineering. 15(5):666-669.

Rahikainen, J.L.; Evans, J.D.; Mikander, S.; Kalliola, A.; Puranen, T.; Tamminen, T.; Marjamaa, K.; Kruus, K. 2013. Cellulase-lignin interactions—the role of

carbohydrate-binding module and pH in non-productive binding. Enzyme and Microbial Technology. 53(5):315-321.

Saeman, J.F. 1945. Kinetics of wood saccharification: Hydrolysis of cellulose and decomposition of sugars in dilute acid at high temperature. Industrial Engineering Chemistry. 37(1):43-52.

Santos, A.; Rodríguez, F.; Gilarranz, M.A.; Moreno, D.; García-Ochoa, F. 1997. Kinetic modeling of kraft delignification of *Eucalyptus globulus*. Industrial and Engineering Chemistry Research. 36(10):4114-4125.

Sanyer, N.; Keller, E.L.; Chidester, G.H. 1962. Multistage sulfite pulping of jack pine, balsam fir, spruce, oak, and sweetgum. TAPPI. 45(2):90-104.

Sewalt, V.J.H.; Glasser, W.G.; Beauchemin, K.A. 1997. Lignin impact on fiber degradation. 3. Reversal of inhibition of enzymatic hydrolysis by chemical modification of lignin and by additives. Journal of Agricultural and Food Chemistry. 45(5):1823-1828.

Shuai, L.; Yang, Q.; Zhu, J.Y.; Lu, F.; Weimer, P.; Ralph, J.; Pan, X.J. 2010. Comparative study of SPORL and dilute acid pretreatments of softwood spruce for cellulose ethanol production. Bioresource Technology. 101:3106-3114.

Springer E.L. 1966. Hydrolysis of aspenwood xylan with aqueous solutions of hydrochloric acid. TAPPI. 49(3):102-106.

Springer, E.L.; Harris, J.F.; Neill, W.K. 1963. Rate studies of the hydrotropic delignification of aspenwood. TAPPI. 46(9):551-555.

Studer, M.H.; DeMartini, J.D.; Davis, M.F.; Sykes, R.W.; Davison, B.; Keller, M.T., G.A.; Wyman, C.E. 2011. Lignin content in natural *Populus* variants affects sugar release. Proceedings of the National Academy of Science (PNAS). 108(15):6300-6305.

Tengborg, C.; Galbe, M.; Zacchi, G. 2001. Reduced inhibition of enzymatic hydrolysis of steam-pretreated softwood. Enzyme and Microbial Technology. 28(9-10):835-844.

Tian, S.; Zhu, W.; Gleisner, R.; Pan, X.J.; Zhu, J.Y. 2011. Comparisons of SPORL and dilute acid pretreatments for sugar and ethanol productions from aspen. Biotechnology Progress. 27(2):419-427.

Tunc, M.S.; Van Heiningen, A.R.P. 2008. Hemicellulose extraction of mixed southern hardwood with water at 150 °C: effect of time. Industrial and Engineering Chemistry Research. 47(18):7031-7037.

Vance, E.D.; Maguire, D.A.; Zalesny, R.S.J. 2010. Research strategies for increasing productivity of intensively managed forest plantations. Journal of Forestry. 108:183-192.

Vinzant, T.B.; Adney, W.S.; Decker, S.R.; Baker, J.O.; Kinter, M.T.; Sherman, N.E.; Fox, J.W.; Himmel, M.E. 2001. Fingerprinting *Trichoderma reesei* hydrolases in a commercial cellulase preparation. Applied Biochemistry and Biotechnology—Part A Enzyme Engineering and Biotechnology. 91-93:99-107.

Vroom, K.E. 1957. A means of expressing cooking times and temperatures as a single variable. Pulp and Paper Magazine Canada. 58:228-231.

Wang, G.S.; Pan, X.J.; Zhu, J.Y.; Gleisner, R. 2009. Sulfite pretreatment to overcome recalcitrance of lignocellulose (SPORL) for robust enzymatic saccharification of hardwoods. Biotechnology Progress. 25(4):1086-1093.

Wang, Q.Q.; He, Z.; Zhu, Z.; Zhang, Y-H.P.; Ni, Y.; Luo, X.L.; Zhu, J.Y. 2012a. Evaluations of cellulose accessibilities of lignocelluloses by solute exclusion and protein adsorption techniques. Biotechnology and Bioengineering. 109(2):381-389.

Wang, Z.J.; Zhu, J.Y.; Zalesny, R.S.J.; Chen, K.F. 2012b. Ethanol production form poplar wood the through enzymatic saccharification and fermentation by dilute acid and SPORL pretreatments. Fuel. 95:606-614.

Wang, Z.J.; Lan, T.Q.; Zhu, J.Y. 2013a. Lignosulfonate and elevated pH can enhance enzymatic saccharification of lignocelluloses. Biotechnology for Biofuels. 6:9.

Wang, Z.; Zhu, J.Y.; Fu, Y.; Qin, M.; Shao, Z.; Jiang, J.; Yang, F. 2013b. Lignosulfonate-mediated cellulase adsorption: enhanced enzymatic saccharification of lignocellulose through weakening nonproductive binding to lignin. Biotechnology for Biofuels. 6:156.

Wang, G.S.; Pan, X.J.; Zhu, J.Y.; Gleisner, R.L. 2015. Sulfite pretreatment for biorefining biomass. U.S. Patent 9,090,915 B2.

Wei, N.; Quarterman, J.; Kim, S.R.; Cate, J.H.D.; Jin, Y.S. 2013. Enhanced biofuel production through coupled acetic acid and xylose consumption by engineered yeast. Nature Communications. 4:2580.

Xavier, AM.R.B.; Correia, M.F.; Pereira, S.R.; Evtuguin, D.V. 2010. Second-generation bioethanol from eucalyptus sulphite spent liquor. Bioresource Technology. 101(8):2755-2762.

Xiang, Q.; Lee, Y.Y.; Torget, R.W. 2004. Kinetics of glucose decomposition during dilute-acid hydrolysis of lignocellulosic biomass. Applied Biochemistry and Biotechnology. 113-16:1127-1138.

Yang, B.; Wyman, C.E. 2004. Effect of xylan and lignin removal by batch and flowthrough pretreatment on the enzymatic digestibility of corn stover cellulose. Biotechnology and Bioengineering. 86(1):88-95.

Zhang, D.; Ong, Y.L.; Li, Z.; Wu, J.C. 2012a. Optimization of dilute acid-catalyzed hydrolysis of oil palm empty fruit

bunch for high yield production of xylose. Chemical Engineering Journal. 181-182:636-642.

Zhang, C.; Zhu, J.Y.; Gleisner, R.; Sessions, J. 2012b. Fractionation of forest residues of Douglas-fir for fermentable sugar production by SPORL pretreatment. Bioenergy Research. 5(4):978-988.

Zhang, C.; Houtman, C.J.; Zhu, J.Y. 2014. Using low temperature to balance enzymatic saccharification and furan formation in SPORL pretreatment of Douglas-fir. Process Biochemistry. 49:466-473.

Zhang, J.; Gu, F.; Zhu, J.Y.; Zalesny, R.S. 2015. Using a combined hydrolysis factor to optimize high titer ethanol production from sulfite-pretreated poplar without detoxification. Bioresource Technology. 186:223-231.

Zhao, X.; Zhou, Y.; Liu, D. 2012. Kinetic model for glycan hydrolysis and formation of monosaccharides during dilute acid hydrolysis of sugarcane bagasse. Bioresource Technology. 105:160-168.

Zheng, Y.; Pan, Z.; Zhang, R.; Wang, D.; Jenkins, B. 2008. Non-ionic surfactants and non-catalytic protein treatment on enzymatic hydrolysis of pretreated creeping wild ryegrass. Applied Biochemistry and Biotechnology. 146:231-248.

Zhou, H.; Lou, H.; Yang, D.; Zhu, J.Y.; Qiu, X. 2013a. Lignosulfonate to enhance enzymatic saccharification of enzymatic saccharification of lignocelluloses: role of molecular weight and substrate lignin. Industrial and Engineering Chemistry Research. 52(25):8464-8470.

Zhou, H.; Zhu, J.Y.; Luo, X.; Leu, S-Y.; Wu, X.; Gleisner, R.; Dien, B.S.; Hector, R.E.; Yang, D.; Qiu, X. [and others]. 2013b. Bioconversion of beetle-killed lodgepole pine using SPORL: Process scale-up design, lignin coproduct, and high solids fermentation without detoxification. Industrial Engineering Chemistry Research. 52(45):16057-16065.

Zhou, H.; Leu, S-Y.; Wu, X.; Zhu, J.Y.; Gleisner, R.; Yang, D.; Qiu, X.; Horn, E. 2014. Comparisons of high titer ethanol production and lignosulfonate properties by SPORL pretreatment of lodgepole pine at two temperatures. RSC Advances. 4:27033-27038.

Zhou, H.; Zhu, J.Y.; Gleisner, R.; Qiu, X.; Horn, E. 2015a. High titer ethanol and lignosulfonate productions from SPORL pretreated poplar at pilot-scale. Frontiers of Energy Research. 3:16.

Zhou, H.; Zhu, J.Y.; Gleisner, R.; Qiu, X.; Horn, E.; Negron, J. 2015b. Pilot-scale demonstration of SPORL for bioconversion of lodgepole pine to bio-ethanol and lignosulfonate. Holzforschung DOI:101515/hf-2014-0332.

Zhu, J.Y.; Pan, X.J. 2010. Woody biomass pretreatment for cellulosic ethanol production: technology and energy consumption evaluation. Bioresource Technology. 101:4992-5002.

Zhu, J.Y.; Pan, X.J.; Wang, G.S.; Gleisner, R. 2009. Sulfite pretreatment (SPORL) for robust enzymatic saccharification of spruce and red pine. Bioresource Technology. 100(8):2411-2418.

Zhu, J.Y.; Gleisner, R.; Scott, C.T.; Luo, X.L.; Tian, S. 2011a. High titer ethanol production from simultaneous enzymatic saccharification and fermentation of aspen at high solids: a comparison between SPORL and dilute acid pretreatments. Bioresource Technology. 102(19):8921-8929.

Zhu, J.Y.; Luo, X.; Tian, S.; Gleisner, R.; Negrone, J.; Horn, E. 2011b. Efficient ethanol production from beetle-killed lodgepole pine using SPORL technology and *Saccharomyces cerevisiae* without detoxification. TAPPI Journal. 10(5):9-18.

Zhu, W.; Houtman, C.J.; Zhu, J.Y.; Gleisner, R.; Chen, K.F. 2012. Quantitative predictions of bioconversion of aspen by dilute acid and SPORL pretreatments using a unified combined hydrolysis factor (CHF). Process Biochemistry. 47:785-791.

Zhu, J.Y.; Chandra, M.S.; Gu, F.; Gleisner, R.; Reiner, R.; Sessions, J.; Marrs, G.; Gao, J.; Anderson, D. 2015. Using sulfite chemistry for robust bioconversion of Douglas-fir forest residue to bioethanol at high titer and lignosulfonate: a pilot-scale evaluation. Bioresource Technology. 179:390-397.