

## Effects of Lignin Removal on Substrate Enzymatic Hydrolysis of SPORL-high-pH Pretreated Bagasse

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The performance of a process called sulfite pretreatment to overcome recalcitrance of lignocelluloses (SPORL), which was carried out at high pH, was preliminarily investigated as a means of improving bagasse substrate enzymatic digestibility (*SED*). The lignin removal significantly affected the *SED* resulting from the SPORL-high-pH treatment. Lignin removal was found to be well-correlated with *SED* when the Boltzmann function was used to fit the curve. In this fitting curve, *SED* increased slowly initially and then rose up quickly with lignin removal (from 0% to 10%, and then from 10% to 35%) and finally reached an asymptotic value (80%, based on o.d. glucan in the pretreated substrate). Removing lignin to about 35% to 40% would be sufficient to significantly improve the *SED* of bagasse to the asymptotic value (80%) during enzymatic hydrolysis, while low cellulase and  $\beta$ -glucosidase (15 FPU/g and 22.5 CUB/g o.d. cellulose in the substrate, respectively) were loaded. Furthermore, combining this Boltzmann function and the SPORL-high-pH delignification model would be useful for predicting neutral-sulfite pretreated *SED*.

*Keywords:* SPORL-high-pH pretreatment; Substrate enzymatic digestibility (*SED*); Lignin removal; Accessibility; H-factor

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### INTRODUCTION

Because of the dwindling and unstable supply of petroleum and the rapidly growing demand for energy, the pursuit of alternative and renewable energy sources has widely attracted researchers' attention worldwide (Mustafa 2011). With the shortage of forest resources, non-woody biomass (such as bamboo) has long been recognized as a potential sustainable source of sugars for the production of bio-ethanol in China (Min 2006; Peng *et al.* 2012), while woody biomass, such as hardwoods and softwoods, still has great interest in the U.S. and in European countries (Zhu and Zhuang 2012). However, the complex and rigid structure of lignocellulose (in both woody and non-woody biomass) makes it strongly resistant to biological conversion. Effectively overcoming that recalcitrance is therefore an important and urgent research problem for any implementation of a lignocellulosic biofuels platform (Himmel *et al.* 2007).

Numerous researchers (Ishizawa *et al.* 2009; Lee *et al.* 2009; Yang and Wyman 2004) have confirmed that removing the non-cellulosic components (mainly lignin and hemicellulose) through various pretreatments could effectively increase cellulose accessibility in pretreated lignocellulose and further improve its digestibility for

fermentable sugars production. At present, a large number of pretreatment methods, including physical (such as milling and grinding), chemical (such as acid, alkali, and organic solvents), and physico-chemical (such as steam explosion and ammonia fiber explosion) methods, have been developed for a variety of feedstocks (Alvira *et al.* 2010; Gable and Zacchi 2007; Yang and Wyman 2008). Direct physical pretreatment is very energy-intensive and has low efficiency, even if it is environmentally friendly (Yang and Wyman 2008). Although acidic pretreatment can easily remove hemicellulose and then significantly expose cellulose to enzymes, it involves equipment-corrosion problems and influences downstream processes, resulting in such issues as low enzyme recycle efficiency due to the irreversible adsorption of lignin in residues and the higher concentration of inhibitors such as furfural (F) and 5-hydroxymethyl furfural (HMF) in hydrolysates (Galbe and Zacchi 2007; Larsson *et al.* 1999). Alkaline pretreatment, on the contrary, can remove lignin to improve the digestibility of enzyme to cellulose, but residual alkali in solid substrates exhibits high inhibition or toxicity to enzymes if not enough water is used to wash it away (Alvira *et al.* 2010; McIntosh and Vancov 2010).

Recently, a sulfite pretreatment, known as sulfite pretreatment to overcome recalcitrance of lignocellulose (SPORL), was applied to pretreat different kinds of biomass (including woody and non-woody biomass) and obtained good results (Zhu *et al.* 2009). For example, a more than 90% cellulose conversion was achieved when 14.6 FPU cellulase and 22.5 CBU  $\beta$ -glucosidase per gram of substrate were loaded during the enzymatic hydrolysis process. Moreover, the amount of inhibitors (F and HMF) formed with SPORL was obviously less than that formed with the dilute acid pretreatment (Shuai *et al.* 2010). Using an adapted strain (*Saccharomyces cerevisiae* Y5), an ethanol yield of about 270 L/ton wood was obtained from lodgepole pine by co-fermentation of pretreated cellulosic solids substrate and pretreatment hydrolysate without detoxification (Tian *et al.* 2010). However, considering the equipment-corrosion problems and the safety problems induced by the volatile sulfide on the conductor during the acidic SPORL pretreatment, SPORL-high-pH pretreatment is therefore especially indicated. Hopefully, another new route toward not only high cellulose digestibility but also environmentally friendly operability and industrial scalability will be opened via the exploratory study of SPORL-high-pH.

Thus, this study was conducted to evaluate the efficiency of SPORL-high-pH pretreatment for converting bagasse cellulose to glucose by enzymatic hydrolysis. Differentiated from the low solubility of lignin but high removal of hemicellulose in the SPORL-low-pH pretreatment, SPORL-high-pH pretreatment may provide high lignin removal, thereby also resulting in higher cellulose digestibility during the enzymatic hydrolysis process. Moreover, the effect of lignin removal on substrate enzymatic hydrolysis of SPORL-high-pH pretreated bagasse in particular was studied.

## EXPERIMENTAL

### Materials

Fresh bagasse was generously provided by the Guigang Sugar Mill (Guangxi, China). The obtained bagasse was screened to remove all fibrils greater than 20 mm and less than 5 mm in length. The pentosan, total lignin, and  $\alpha$ -cellulose contents in screened bagasse were determined to be 18.88%, 21.40%, and 41.0% (based on o.d. bagasse), respectively. Before conducting the pretreatment experiment, the screened bagasse was

stored in a polyethylene bag with a zip lock to maintain an unchanged moisture content. Commercial enzymes, Celluclast 1.5 L (cellulase) and Novozyme 188 ( $\beta$ -glucosidase), were used as received from Sigma–Aldrich (Shanghai, China). Sodium sulfite was purchased from Tianjin Kernel Chemical Reagent (Tianjin, China) and used without purification.

### SPORL-high-pH Pretreatment

SPORL-high-pH pretreatments were conducted in a water bath digester (designed and manufactured by Shaanxi University of Science & Technology, Xi'an, Shaanxi, China). First, 200 g bagasse (o.d.) was directly introduced to a 2.5-L stainless steel pressure vessel. Sodium sulfite solution was prepared in a beaker, then poured into the vessel. The remainder of the clean water used to wash the beaker was then added to the same vessel, with a ratio of pretreatment liquor to bagasse (o.d.) of 8 (v/w). Four 2.5-L stainless steel pressure vessels were finally mounted in a large pulping digester (15 L) and heated externally using a water bath. The reactors were fixed on a shelf and rotated at the speed of 3 rpm to mix the bagasse and the chemicals during pretreatment. The sodium sulfite charge on the o.d. bagasse varied from 12% to 20% (w/w). The pretreatment temperature (T) and duration (t) were set at 100 °C, 130 °C, and 160 °C for 30 min, 60 min, and 90 min, respectively. The heating time to reach reaction temperatures ranged from 15 min to 30 min. At the end of the pretreatment, the solid was separated by filtering the liquor on a Büchner funnel with two layers of filter paper (diameter of 12.5 cm, slow type, Xinhua, Hangzhou, China). The solid was washed with triple-volume de-ionized water three times and then collected in a polyethylene bag with a zip-lock for subsequent enzymatic hydrolysis of the cellulose. The spent pretreatment liquor was also collected for residual sulfur concentration analysis.

### Enzymatic Hydrolysis

Enzymatic hydrolysis of the substrates was preliminarily carried out at a substrate consistency of 5% (w/v) in 50 mL sodium acetate buffer (pH 4.8, 50 mmol/L) at 50 °C in a shaking incubator (Thermo Fisher Scientific, MaxQ 420 HP, Waltham, MA) at 150 rpm. A mixture of Celluclast 1.5 L (cellulase) and Novozyme 188 ( $\beta$ -glucosidase) was used for enzymatic hydrolysis. The activity loadings of cellulase and  $\beta$ -glucosidase were set at 15 FPU and 22.5 CBU/g od cellulose, respectively. Hydrolysates for all samples were sampled only at the 72-h period for glucose analysis.

### Analytical Methods

The concentration of glucose from enzymatic hydrolysis was measured using high-performance anion exchange chromatography (ICS-3000, Dionex, Sunnyvale, CA) equipped with pulsed amperometric detection (HPAEC-PAD) and an anion exchange column (Dionex, CarboPac PA1) (Davis 1998). Detailed gradient and other chromatography conditions are referenced elsewhere (Davis 1998). The pretreatment solid yield was determined by the gravimetric method after balancing the moisture content of the collected solid substrates and original bagasse initially used in pretreatment experiments. The concentration of residual sulfur (calculated as total SO<sub>2</sub>) in the pretreatment liquor was titrated according to an iodometric method provided by TAPPI (T 604 om-04). The absorption spectra of the lignin in the neutral-sulfite pretreatment solutions were recorded by a UV–Vis spectrophotometer (Agilent 8453, Palo Alto, CA).

The absorbance at 280 nm was used for quantification (Ji 2007). Based on these measurements, the following equations were used to calculate the lignin concentration in the pretreatment liquor, the delignification, and the cellulose conversion based on od glucan in pretreated solid substrate during enzymatic hydrolysis. According to Lambert-Beer's law and the absorption coefficient given by Ji (2007), the concentration of lignin in the pretreatment liquor can be quantitatively calculated as,

$$C_{lignin} = 0.043 \chi A \quad (1)$$

where  $C_{lignin}$  is the concentration of lignin in the pretreatment liquor (g/L);  $\chi$  and  $A$  are the dilution time and the value of absorption, respectively. Then, the delignification of the bagasse can be thus expressed as,

$$D = \frac{C_{lignin} m \rho}{1000 m \alpha} \times 100 \% = \frac{C_{lignin} \rho}{10 \alpha} \quad (2)$$

where  $D$  is the delignification of bagasse during SPORL-high-pH pretreatments (%);  $m$  is the od mass of bagasse used for SPORL-high-pH pretreatments (g); and  $\alpha$  and  $\rho$  are the total lignin content (%) in bagasse and the ratio of liquor to solid during SPORL-high-pH pretreatments, respectively. According to the definition from the National Renewable Energy Laboratory (Golden, CO), substrate enzymatic digestibility ( $SED$ ) at 72 h based on the glucan content of the pretreated substrate is preliminarily given as,

$$SED = \frac{0.9[(C_{glucose} m_1 / \psi) / 1000]}{m_1 \beta} \times 100 = \frac{0.09 C_{glucose}}{\beta \psi} \quad (3)$$

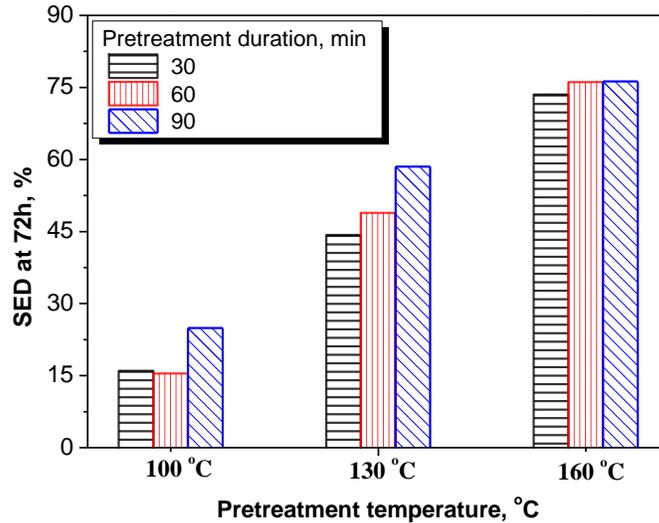
where  $SED$  is the enzymatic cellulose conversion at 72 h based on the glucan content of the pretreated substrate (%);  $C_{glucose}$  is the concentration of glucose in the enzymatic hydrolysis liquor (g/L);  $m_1$  is the mass of od substrate used in the enzymatic hydrolysis (g);  $\beta$  is the glucan content in the pretreated substrate (%); and  $\psi$  is the initial solid substrate consistency used for enzymatic hydrolysis (%).

## RESULTS AND DISCUSSION

### Effects of Pretreatment Temperature and Time on Substrate Enzymatic Digestibility

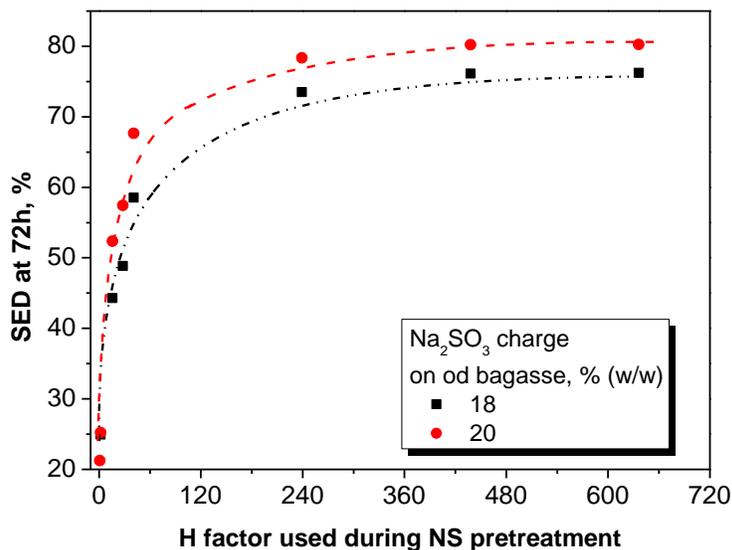
Previous sodium carbonate–sodium sulfite pretreatment reported by Yang *et al.* (2013) illustrated that the enhancement of the cellulose enzymatic hydrolysis conversion in rice straw was mainly contributed by removing partial lignin from lignocellulosic materials and sulfonating the residual lignin. As shown in Fig. 1, the varied pretreatment temperature and time were also evaluated for partial removal of lignin, reserving of most of the polysaccharides, thus enhancing the enzymatic hydrolysis conversion of cellulose (equal to substrate enzymatic digestibility,  $SED$ ) in pretreated substrates of bagasse. It was found that, when the pretreatment was conducted at 100 °C to 160 °C for 60 min with an  $\text{Na}_2\text{SO}_3$  charge of 18% (on od untreated bagasse weight), the  $SED$  at 72 h was

improved from 15% to 76%. Moreover, an extended pretreatment time from 30 min to 90 min at 130 °C increased the *SED* from 44% to 59%. This was mainly caused by the significant removal of lignin and the partial hydrolysis of hemicellulose. For example, about 15% to 50% of the lignin (data not shown) was removed from the bagasse when the pretreatment temperature was enhanced from 100 °C to 160 °C for 60 min.



**Fig. 1.** Effects of pretreatment temperature and time on substrate enzymatic digestibility (*SED*) during SPORL-high-pH pretreatments

The above results clearly indicate that both pretreatment temperature and time significantly affected *SED*. To simplify the process parameters during SPORL-high-pH pretreatments, the H-factor was used as a means of reducing the reaction temperature and time to a single variable (Vroom 1957; Yoon *et al.* 2008).

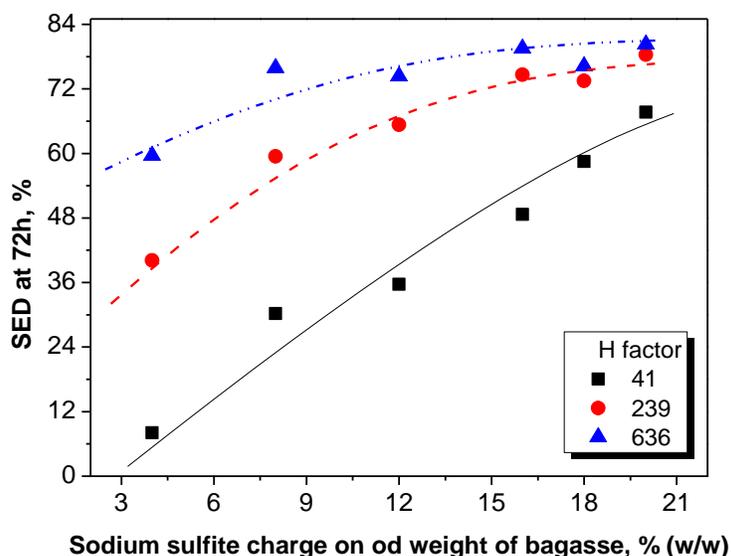


**Fig. 2.** The correlation between the H-factor and substrate enzymatic digestibility (*SED*) during SPORL-high-pH pretreatments

The correlation between the H-factor and substrate enzymatic digestibility (*SED*) during SPORL-high-pH pretreatments is shown in Fig. 2. Regardless of different SPORL-high-pH pretreatments temperatures and times, a single relationship between the H-factor and *SED* was obtained at a given sodium sulfite charge of 18% or 20% on o.d. bagasse. The results shown in Fig. 2 also demonstrate that (1) when the H-factor was lower than 240, *SED* increased with increasing H-factor at the same  $\text{Na}_2\text{SO}_3$  charge; and (2) *SED* increased rapidly to a certain value (*SED*s of 72% and 78% for  $\text{Na}_2\text{SO}_3$  charges of 18% and 20%, respectively) at an H-factor of about 240, and the increase then leveled off at higher H-factors. This analysis implies that the H-factor can be regarded as an ideal process parameter to describe the change in *SED* during SPORL-high-pH pretreatments when the chemical charge is fixed.

### Effect of the Sodium Sulfite Charge on Substrate Enzymatic Digestibility

The effect of the sodium sulfite charge on substrate enzymatic digestibility (*SED*) originating from SPORL-high-pH pretreatments is shown in Fig. 3. It was found that both the H-factor and the sodium sulfite charge affected the *SED*. The sodium sulfite charge at a low H-factor had a much greater effect on *SED* than that at a high H-factor level (curves of H-factors at 41 and 636 in Fig. 3). For all three levels of H-factors, at 41, 239, and 636, *SED* increased with increasing sodium sulfite charge. For the H-factor at 41, the *SED* was improved from approximately 7% to 61%, while the sodium sulfite charge increased from 4% to 20%. At the same range of the sodium sulfite charge, the *SED* climbed up from about 60% to 80% for an H-factor of 636, and to about 40% to 76% for an H-factor of 239, respectively. Moreover, the maximal *SED* of about 80% could be found in the curve of the H-factor at 636 (Fig. 3); the corresponding sodium sulfite charge was 16% (on od weight of bagasse). This difference is probably due to the disparate range of lignin removal resulting from the three H-factors used in this study. Combining the results shown in Figs. 3 and 4, there is a detailed explanation for this difference that will be fully elucidated below.



**Fig. 3.** Effect of sodium sulfite charge on substrate enzymatic digestibility (*SED*) during SPORL-high-pH pretreatments

## The Change in Substrate Enzymatic Digestibility (*SED*) on Lignin Removal

Although xylan removal has been well-correlated to *SED* during the acidic sulfite pretreatment (Zhu *et al.* 2012) of aspen, lignin removal also has been proven to be critical for increasing cellulose accessibility to enzymes. This is because limitations on accessibility due to hemicellulose can be removed by the SPORL-high-pH pretreatments (Yang *et al.* 2013) of rice straw. The relationship between *SED* and lignin removal during the SPORL-high-pH pretreatments of bagasse is shown in Fig. 4. To identify the exact relationship between them, *SED* datasets of different sodium sulfite charges were fitted with delignification using the Boltzmann function:

$$y = A_2 + \frac{A_1 - A_2}{1 + \exp[(x - x_0) \cdot dx]} \quad (4)$$

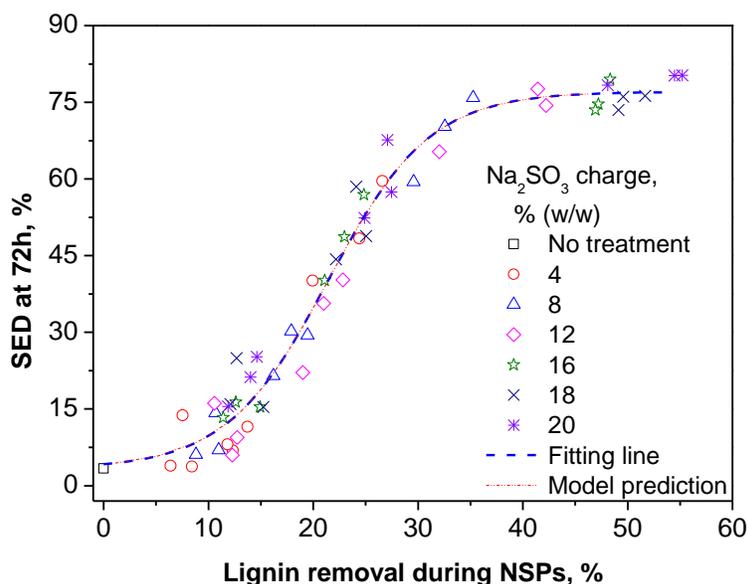
The data and fitting line in Fig. 4 clearly indicate that the *SED* initially increased slowly as the lignin removal was still lower than 10% (calculated as lignin content in od bagasse). However, probably because the cellulose was still compactly filled with residual lignin and hemicellulose and therefore not significantly accessible to the enzymes, the *SED* of the untreated bagasse had no evident improvement. Thus, this value, represented by  $A_1$  in Eq. (4), can be designated  $SED_0$ . Once more lignin was removed, the cellulose was gradually exposed to the enzymes, resulting in a fast increase in *SED*. The delignification that corresponds to the rapid increase in *SED* is  $x_0$  in Eq. (4), and it can be represented as  $D_x$ . Finally, the *SED* increased slowly and reached an asymptotic value ( $A_2$  in Eq. (4)). This asymptotic value can be regarded as potential *SED* and labeled  $SED_p$ . The corresponding lowest delignification of this  $SED_p$  is labeled  $D_T$  (Fig. 4). The parameter  $dx$  in Eq. (4) should be the relative change rate of the variable in fitting the curve, given as  $\Delta D$ .

After fitting the *SED* against delignification with the Boltzmann function, the *SEDs* of the pretreated bagasse substrates with different sodium sulfite charges and H-factors were found to fall on a single curve (Fig. 4). Therefore, it is conceivable to reflect the increase in *SED* using delignification ( $R^2 = 0.97$ ). The values of the parameters in the Boltzmann function (Eq. 4) were thus obtained (Table 1) via curve fitting, experimental measurement, and subjective estimation. The result clearly demonstrates that it is unnecessary to completely remove lignin from bagasse through SPORL-high-pH pretreatments to increase the cellulose accessibility to enzymes.

**Table 1.** Obtained Values of Parameters of the Boltzmann Function (Eq. (4)) via Curve Fitting, Experimental Measurement, and Subjective Estimation

	Measured Value	Fitted Value
$SED_0$	$3.40 \pm 1.28$	$3.33 \pm 2.79$
$SED_p$	$80.26 \pm 1.28$	$77.08 \pm 1.40$
$D_x$	— <sup>a</sup>	$21.44 \pm 0.56$
$D_T$	$40.00 \pm 0.00$	— <sup>b</sup>
$\Delta D$	— <sup>a</sup>	$4.86 \pm 0.51$
$R^2$	— <sup>a</sup>	0.97

<sup>a</sup> These values cannot be obtained from the experimental measurement.  
<sup>b</sup> This parameter is not included in the Boltzmann function but is estimated subjectively from the tendency of the fitting curve in Fig. 4.



**Fig. 4.** Nonlinear fittings and model prediction of SED to a Boltzmann function and Eq. (9), based on lignin removal during SPORL-high-pH pretreatments

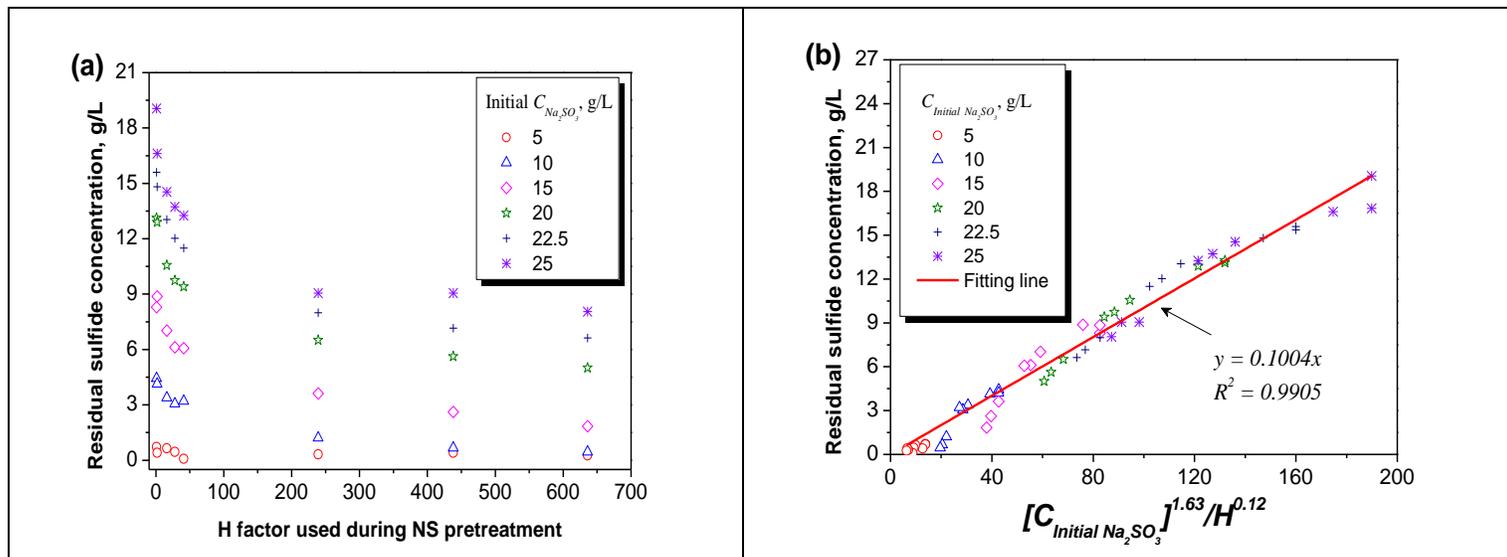
Based on the parameters in Table 1, the observation reflected in Fig. 3 can be fully explained. For the low H-factor (41) with the highest sodium sulfite charge (20%), the highest lignin removal was about 27% (based on lignin content in o.d. bagasse, data not shown). This is much lower than the target lignin removal ( $D_T$ ), which is the prerequisite for sufficiently increasing the cellulose accessibility to enzymes. Thus,  $SED$ s falling inside the curve of H-factor 41 exhibited steady growth in the form of the Boltzmann curve. For the high H-factor (636) with the sodium sulfite charge ranging from 4% to 20%, the lignin removal varies from 27% to 55% (data not shown), which includes target lignin removal ( $D_T$ ). A high H-factor induced  $SED$  to rise with the increase of lignin removal before reaching  $D_T$  and then to gradually tend to an asymptotic value ( $SED_p$ ). Therefore, it is certain that, for SPORL-high-pH pretreatments with different conditions, the  $SED$  of pretreated substrates can be correctly controlled using the corresponding delignification. Moreover, the present results confirm that both lignin and hemicelluloses are major obstacles to enzymatic hydrolysis of cellulose in lignocellulosic materials because they prevent enzyme accessibility. Partially removing or disrupting one of them will make cellulose in lignocellulosic materials more accessible to enzyme molecules. Similar to hemicelluloses, a strong correlation between lignin extracted and cellulose digestibility had been obtained during SPORL-high-pH pretreatments.

### The Delignification Model Can Be Used to Predict and Control SED

Because some of the lignin was dissolved during the non-isothermal process of SPORL-high-pH pretreatments, a kinetic model of delignification should be developed with the H-factor, which is often utilized to unify reaction temperature and time as a single variable during chemical pulping. Based on the reaction scheme and activation energy of neutral sulfite pulping (Basu *et al.* 1974), the kinetic equation can be defined as,

$$\frac{dL_d}{dH} = K (L_0 - L_d)^m S_d^n \quad (5)$$

where  $L_0$  is the maximum lignin concentration in the pretreatment liquor (assuming that all the lignin in bagasse is completely dissolved when  $H$  is infinite) (g/L);  $L_d$  is the lignin concentration in the pretreatment liquor at a given  $H$ -factor (g/L);  $S_d$  is the residual sulfide concentration in the pretreatment liquor (g/L);  $K$  is a reaction constant; and  $m$  and  $n$  are the reaction orders of lignin and sulfide, respectively.



**Fig. 5.** (a) Dependence of the residual sulfide concentration with different initial sodium sulfite charges in the pretreatment liquor on the  $H$ -factor; (b) The relationship between the residual sulfide concentration in the pretreatment liquor and the combination of the initial sodium sulfite charge and the  $H$ -factor ( $[C_{\text{Initial Na}_2\text{SO}_3}]^{1.63}/H^{0.12}$ )

Based on the mathematical fit among  $S_d$ , the  $H$ -factor, and the initial sodium sulfite charge (calculated as the concentration in the pretreatment liquor, g/L), the relationship between them is given in Fig. 5b and expressed as,

$$S_d = \alpha S_0^a / H^b \quad (6)$$

where  $S_0^a$  is the initial dissolved sulfide concentration in the pretreatment liquor without zero heating and reaction time, g/L;  $\alpha$ ,  $a$ , and  $b$  are also constants.

As  $S_d$  depends on the  $H$ -factor and the initial sodium sulfite charge (shown in Fig. 5a), combining Eq. (5) and Eq. (6) and integrating Eq. (5) leads to the following expressions:

$$-\frac{(L_0 - L_d)^{1-m}}{1-m} = K (AS_0^a)^n \frac{H^{1-bn}}{1-bn} + C \quad (7)$$

According to the initial reaction conditions, without SPORL-high-pH pretreatments ( $H = 0$ ),  $H^{1-bn}$  and  $L_d$  are equal to 0. Thus, the constant  $C$  can be calculated as:

$$C = -L_0^{1-m} / (1 - m) \quad (8)$$

After determining the constant  $C$ , Eq. (7), induced delignification, can be rearranged as

$$D = \left[ 1 - \frac{L_0^{(1-m)} + [(m - 1)K (\alpha S_0^a)^n H^{(1-bn)} / (1 - bn)]^{-(1-m)}}{L_0} \right] \times 100 \% \quad (9)$$

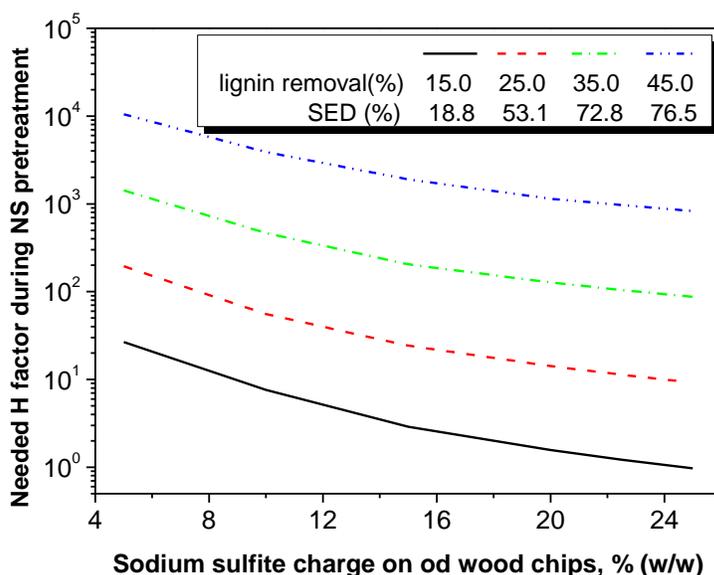
The estimates of the kinetic parameters in the delignification model (Eq. (9)) during SPORL-high-pH pretreatments were determined using the least squares method in Microsoft Excel 2003. The parameters of  $m$ ,  $n$ ,  $\alpha$ ,  $a$ ,  $b$ , and  $K$  were found to be 1.315, 0.700, 0.537, -0.156, 1.512, and 3.126, respectively.

According to Eq. (4) and Eq. (9),  $SED$  can be regarded as an indirect function of  $S_0$  and the H-factor. A simple expression of the relationship between  $f(H, S_0)$  and  $SED$  can be obtained as

$$D(f(H, S_0)) = \frac{\text{Log}_e[(SED - SED_0) / (SED_p - SED_0)]}{\Delta D} + D_x \quad (10)$$

Based on all previously determined parameters in Eq. (4) and Eq. (9), values for simultaneously predicting / approximately obtaining SPORL-high-pH pretreatments induced  $SED$  and  $D$  of bagasse substrates could be easily obtained by adjusting the H-factor ( $H$ ) and the initial sodium sulfite charge. The results are presented in Fig. 6. To obtain an  $SED$  of 72.8 at a corresponding  $D$  of 35.0, the H-factor should be adjusted from about 105 to 1427 while the initial sodium sulfite charge ranges from 5% to 25%. Moreover, co-controlling the H-factor and the initial sodium sulfite charge will not only achieve the targeted  $SED$  and  $D$  but will also provide multiple choices to evaluate the process energy output efficiency (PEOE). Sulfides, namely  $\text{SO}_2$ ,  $\text{SO}_3^{2-}$ , and  $\text{HSO}_3^-$ , have been identified as strong inhibitors of fermentation microorganisms during co-fermentation of pretreated solid substrate and hydrolysate from SPORL pretreatment (Luo 2011).

The results presented in Fig. 6 demonstrate that a certain  $SED$  can be obtained not only by SPORL-high-pH pretreatment with a low H-factor (low heat energy input) and high  $\text{Na}_2\text{SO}_3$  charge (high sulfide concentration in the pretreatment liquor), but also by SPORL-high-pH pretreatment with a high H-factor and low initial  $\text{Na}_2\text{SO}_3$  charge. Due to the strong inhibition caused by sulfides with high concentrations, the fermentation efficiency of the former might be lower than that of the latter. Thus, the final PEOE of the former also may be lower than that of latter, even though low heat energy was input during SPORL-high-pH pretreatments. Therefore, to reach a presupposed  $SED$ , co-adjusting the parameters of the H-factor and the initial sodium sulfite charge will be an effective way to address specific operation requirements.



**Fig. 6.** Desired *SED* and *D* curves at different combinations of H-factor and initial sodium sulfite charge

## CONCLUSIONS

1. Once lignin removal was more than 40%, cellulose conversion over 80% was steadily achieved after 72 h of enzymatic hydrolysis with cellulase loadings of 15 FPU/g and 22.5 CUB/g of cellulose in the substrate. Thus, it could be preliminarily concluded that partially removing lignin seems to be sufficient to significantly improve the conversion of glucan to glucose during enzymatic hydrolysis.
2. Moreover, experimental measured lignin removal was preliminarily found to correlate very well with substrate enzymatic digestibility (*SED*) using the Boltzmann function.
3. Based on this correlation and the developed delignification model, *SED* seems be flexibly controlled by co-adjusting the H-factor and the initial sodium sulfite charge, which can facilitate the scale-up of SPORL-high-pH pretreatment experiments.

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## REFERENCES CITED

- Alvira, P., Tomás-Pejó, E., Ballesteros, M., and Negro, M. J. (2010). "Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review," *Bioresour. Technol.* 101(13), 4851-4861.
- Basu, S., Krause, T., and Schurz, J. (1974). "Delignification kinetic studies of NSSC pulping and its correlation with H-factor for pulp scheduling," *Holzforschung* 28(4), 121-130.
- Davis, M. W. (1998). "A rapid modified method for compositional carbohydrate analysis of lignocellulosics by high pH anion-exchange chromatography with pulsed amperometric detection (HPAEC/PAD)," *J. Wood Chem. Technol.* 18(2), 235-252.
- Galbe, M., and Zacchi, G. (2007). "Pretreatment of lignocellulosic materials for efficient bioethanol production," *Adv. Biochem. Engin/Biotechnol.* 108, 41-65.
- Himmel, M. E., Ding, S. Y., Johnson, D. K., Adney, W. S., Nimlos, M. R., Brady, J. W., and Foust, T. D. (2007). "Biomass recalcitrance: Engineering plants and enzymes for biofuels production," *Science* 315(5813), 804-807.
- Ishizawa, C. I., Jeoh, T., and Adney, W. S. (2009). "Can delignification decrease cellulose digestibility in acid pretreated corn stover?," *Cellulose* 16(4), 677-686.
- Ji, Y. (2007). "Kinetics and mechanism of oxygen delignification," Ph.D. thesis, The University of Maine, Dept. of Chemical Engineering, Orono, ME.
- Larsson, S., Palmqvist, E., Hahn-Hägerdal, B., Tengborg, C., Stenberg, K., Zacchi, G., and Nilvebrant, N. O. (1999). "The generation of fermentation inhibitors during dilute acid hydrolysis of softwood," *Enzyme Microb. Technol.* 24(3/4), 151-159.
- Lee, H. S., Doherty, T. V., Linhardt, R. J., and Dordick, J. S. (2009). "Ionic liquid-mediated selective extraction of lignin from wood leading to enhanced enzymatic cellulose hydrolysis," *Biotechnol. Bioeng.* 102(5), 1368-1376.
- Luo, X. L. (2011). "Study on enzymatic hydrolysis and co-fermentation of solid and liquor after SPORL pretreatment of lignocellulose and simultaneous production of nanocellulose," Ph.D. thesis, South China University of Technology, Guangzhou, China.
- McIntosh, S., and Vancov, T. (2010). "Enhanced enzyme saccharification of sorghum bicolor straw using dilute alkali pretreatment," *Bioresour. Technol.* 101(17), 6718-6727.
- Min, E. Z. (2006). "Developing biorefinery by utilizing agriculture and forestry biomass resources: Striding forward the 'Carbohydrate' era," *Prog. Chem.* 18(2/3), 131-141.
- Mustafa, B. (2011). "Production of bioethanol from lignocellulosic materials via the biochemical pathway: A review," *Energy Convers. Manage.* 52(2), 858-875.
- Peng, H., Wang, N., Hu, Z. R., Yu, Z. P., Liu, Y. H., Zhang, J. S., and Ruan, R. (2012). "Physicochemical characterization of hemicelluloses from bamboo (*Phyllostachys pubescens* Mazel) stem," *Ind. Crops Prod.* 37(1), 41-50.
- Shuai, L., Yang, Q., Zhu, J. Y., Lu, F. C., Weimer, P. J., Ralph, J., and Pan, X. J. (2010). "Comparative study of SPORL and dilute-acid pretreatments of spruce for cellulosic ethanol production," *Bioresour. Technol.* 101(9), 3106-3114.
- Tian, S., Luo, X. L., Yang, X. S., and Zhu, J. Y. (2010). "Robust cellulosic ethanol production from SPORL-pretreated lodgepole pine using an adapted strain *S. cerevisiae* without detoxification," *Bioresour. Technol.* 101(22), 8678-8685.
- Vroom, K. E. (1957). "A means of expressing cooking times and temperatures as a single variable," *Pulp Paper Mag. Can.* 58(3), 228-231.

- Yang, B., and Wyman, C. E. (2004). "Effect of xylan and lignin removal by batch and flowthrough pretreatment on the enzymatic digestibility of corn stover cellulose," *Biotechnol. Bioeng.* 86(1), 88-95.
- Yang, B., and Wyman, C. E. (2008). "Pretreatment: The key to unlocking low-cost cellulosic ethanol," *Biofuel. Bioprod. Bior.* 2(1), 26-40.
- Yang, L. F., Cao, J., Mao, J. Y., and Jin, Y. C. (2013). "Sodium carbonate–sodium sulfite pretreatment for improving the enzymatic hydrolysis of rice straw," *Ind. Crops Prod.* 43, 711-717.
- Yoon, S. H., Macewan, K., and Van Heiningen, A. (2008). "Hot-water pre-extraction from loblolly pine (*Pinus taeda*) in an integrated forest products biorefinery," *Tappi J.* 7(6), 27-31.
- Zhu, J. Y., and Zhuang, X. S. (2012). "Conceptual net energy output for biofuel production from lignocellulosic biomass through biorefining," *Prog. Energ. Combust.* 38(4), 583-598.
- Zhu, J. Y., Pan X. J., Wang, G. S., and Gleisner, R. (2009). "Sulfite pretreatment (SPORL) for robust enzymatic saccharification of spruce and red pine," *Bioresour. Technol.* 100(8), 2411-2418.
- Zhu, W., Houtman, C. J., Zhu, J. Y., Gleisner, R., and Chen, K. F. (2012). "Quantitative predictions of bioconversion of aspen by dilute acid and SPORL pretreatments using a unified combined hydrolysis factor (CHF)," *Process Biochem.* 47(5), 785-791.

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