Modeling the sugar and byproducts from acid bisulfite pretreatment and enzymatic hydrolysis of Douglas-fir

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Abstract:

The aim of this work was to investigate the kinetics of multiple chemicals in acid bisulfite pretreatment and the relationship between total sugar yields and pretreatment factors (temperature and time). The results showed Saeman model accurately fitted the pretreatment process. According to this kinetic model, a maximum hemicellulose hydrolysis yield was achieved at a treatment time of 75 min with a temperature of 145 °C. Meantime, the concentrations of acetic acid, hydroxymethylfurfural (HMF), and furfural were 1.54, 0.60, and 1.15 g L⁻¹, respectively. Also, a Lorentzian function described the relationship between total sugar yield and pretreatment factors: temperature and time. The regression parameters from this mathematical fitting have accurately reflected the maximum total sugar yield and the optimal treatment conditions were determined to be 145 °C and 110 min.

Keywords:

Sulfite pretreatment, Micronized wood, Kinetic model, Lorentzian function, Lignosulfonates

1. Introduction

Cellulose, hemicellulose, and lignin are the major components of softwood lignocellulosic material. Besides carbohydrates, the characteristics and applications of lignin have been investigated in many studies (Howe et al., 2016; Li & McDonald, 2014; Zhou et al., 2013).

When utilizing cellulose, pretreatment was required to overcome the recalcitrance from lignin. Historically, acid bisulfite method was employed to prepare high-quality pulp through extensive hemicellulose and lignin removal (Rowell et al., 2005; Sjostrom, 1993). The acids in the chemical liquor release protons (H^+), which break the ether bonds between the monomer sugar units in the hemicellulose and cellulose (Aguilar et al., 2002; Jeffries, 1994). The active agents in the cooking liquor (H^+ and HSO_3^-) also attacked the α -ether bond in lignin resulting in the cleavage of the α -ether group (Sjostrom, 1993). As a result, monomeric and oligomeric sugars, as well as lignosulfonates are released into the spent liquor. Also, lignosulfonates can be a coproduct in biofuel production substituting for the complicated process of lignin base polymer production (Li et al., 2015a; Li et al., 2014; Li et al., 2015b; Sivasankarapillai et al., 2015; Sivasankarapillai et al., 2012). Given the effectiveness of both lignin and hemicellulose removal, Zhu et al.(2010) adapted this process as a pretreatment method for softwoods.

However, the monosaccharides in the spent liquor can be further decomposed into furfural and hydroxymethylfurfural (HMF) due to the dehydration of pentose and hexoses, while acetic acid is formed by hydrolysis of acetyl groups (Gómez et al., 2006). These degradation products can be promising resources for producing bio-chemicals, however their existence in the spent liquor may inhibit the fermentation of monosaccharide in the spent liquor. Depending on the intended purpose of the spent liquor, the pretreatment conditions can be adjusted. Therefore, investigating the evolution of monosaccharides and degradation products can assist in optimizing the

pretreatment conditions.

The Saeman model was first proposed for investigating Douglas-fir wood saccharification kinetics with respect to hydrolysis of cellulose and degradation of monomer sugars in dilute acid treatment (Saeman, 1945). In this study, two consecutive reactions are reflected: hydrolysis of polysaccharides into monosaccharides and degradation of monosaccharides into furfural and HMF. Many studies (Aguilar et al., 2002; Lenihan et al., 2011; Liu et al., 2012; Rahman et al., 2006; Zhuang et al., 2009) have been conducted to investigate the effectiveness of the model in describing the dilute acid or hot water pretreatment of various biomass types. Also, the two-fraction or biphasic model was also proposed based on Saeman model but differentiating the fast hydrolysis and slow hydrolysis fractions of the polysaccharides (Brennan & Wyman, 2004). For this purpose, a regression parameter α (mass fraction of the susceptible polymers in the raw material) was included in the model (Gómez et al., 2006; Guo et al., 2008; Lu & Mosier, 2008; Orozco et al., 2013; Vázquez et al., 2007).

Extensive kinetic studies have been conducted on the hemicellulose hydrolysis of agricultural or grass biomass with dilute acid pretreatment methods (Hong et al., 2013; Jin et al., 2011; Lavarack et al., 2002; Liu et al., 2012; Sun et al., 2011; Yan et al., 2014). The hydrolysis kinetics of xylan into xylose was specifically explored as it is the main components in hemicellulose of employed biomass (Shen & Wyman, 2011; Zhao et al., 2012). As the kinetic parameters strongly depend on pretreatment methods and substrate materials (Lenihan et al., 2011; Liu et al., 2012), kinetic studies on the acid bisulfite method with softwood as the feedstock are scarce. Acid sulfite cooking at 130-140 °C with sodium base chemicals was used to study the dissolution of lignin and hemicellulose in spruce wood powder (Hagberg & Schöön, 1974; Hagberg & Schöön, 1973). But, the liquid to wood solid ratio is 20:1 in those investigations, which is much higher

than current methods (6:1 to 3:1) (Gao et al., 2013; Zhu et al., 2015).

Current studies have typically evaluated wood chips as feedstock for pretreatment, however, a 180-min pretreatment time was required to obtain the maximum total sugar yield (i.e. a combination of sugars in spent liquor and enzymatic hydrolysates) at a generally low treatment temperature (130 -140 °C). In order to display a complete hydrolysis curve of hemicellulose, a micronized wood powder was used in this study to decrease the required hydrolysis time during pretreatment process.

While supporting a larger study to use forest residuals from the Northwest United States to produce aviation biofuels using a sugar process (NARA Renewables), the current study address the mathematical modeling of sugar and byproducts in acid bisulfite pretreatment and enzymatic hydrolysis. Specifically, our objectives are to (1) discern the polymeric sugars reaction curve, which is beneficial for industrial production and (2) obtain a mathematical model for optimizing pretreatment time and temperature for maximizing total sugar yields. Therefore, it would be of significant importance to establish a kinetic study of acid bisulfite pretreatment process with Douglas-fir as substrate materials.

2. Materials and methods

2.1 Materials

Micronized Douglas-fir (*Pseudotsuga menziesii*) powder was prepared by an impact mill (Zhengzhou Tianyuan Environmental Protection Machinery Co. Ltd, China) with a 30 min milling of 2 mm wood chips. The particle size of the resulting wood powders was characterized using a Malvern particle size analyzer (Mastersizer 3000 laser diffraction). The median particle size (D50) of this sample is 32 µm with a standard deviation 1.2 µm. The glucan, xylan/mannan, galactan, arabinan, and lignin content of Douglas-fir are 43.3%, 15.2%, 2.8%, 1.2%, and 31.7%,

respectively. Note that xylan and mannan are indicated as a single group. For Douglas-fir, mannan is the predominate sugar type (Socha et al., 2013). The cooking liquor for acid bisulfite treatment has a 3.34% of calcium bisulfite and a 1.06% of free sulfur dioxide.

2.2 Methods

2.2.1 Acid bisulfite pretreatment

The over dried wood to chemical liquor ratio in this pretreatment is 1:4. The equivalent sulfur dioxide loading on wood was 6%, which was selected according to previous studies (Gao et al., 2013; Zhu et al., 2015). A Parr reactor (100 ml, No. 4845) was employed for pretreatments. Three treatment temperatures were selected 135, 145, and 155 °C. The heat-up stage required approximately 25, 30, and 35 minutes to reach 135, 145, and 155 °C, respectively. It took approximately 15 minutes to cool the reactor down to room temperature. Under each temperature, cook time intervals (i.e. the duration time at target temperature) of 20, 40, 60, 120 and 180 minutes were evaluated with two replicates. After pretreatment, the slurry was separated by vacuum filtration. Spent liquor was collected for sugar, HMF, furfural, acetic acid, and lignosulfonates analysis. The pretreated solids were washed and stored in 4 °C refrigerator until use.

2.2.2 Enzymatic hydrolysis

The resulting solids from the acid bisulfite pretreatments were enzymatically hydrolyzed to assess the efficacy of the pretreatment process. In this evaluation, the solid loading was 2% of a total 50 ml volume. Cellic[®] CTec2 and HTec2 were complementarily provided by Novozymes North America (Franklinton, NC). The pretreated solids were hydrolyzed for 72 hours with a 4% (w/w) CTec2 and 0.4% (w/w) HTec2 (based on oven-dry wood basis) enzyme dosage. One percent (w/w) sodium azide were used for antibacterial function in hydrolysis slurry. The

reaction mixture was mechanically shaken in an orbital shaking incubator (Amerex Instruments, Inc. Lafayette, CA) at 50 °C for 72 h. The monomeric sugar concentrations in the liquid were measured at the time intervals of 0, 3, 6, 12, 24, 48, and 72 h. One ml slurry was collected at each time point and centrifuged to obtain supernatant for sugar quantification. In addition, the enzyme dosage of 8.8% was also used to investigate the total sugar yield.

2.2.3 Sugar analysis

The monomeric sugar concentrations were measured by an ionic chromatography system (Dionex ICS-3000), equipped with CarboPac PA20 Guard column: 4 × 50 mm and IonPac AS11-HC analytical column: 4 × 250 mm, ED40 electrochemical detector, AS40 autosampler, degassed E-pure water, 50mM and 200mM NaOH solution as eluent with a flow rate 0.5 ml/min. Oligomeric sugars in the spent liquors were hydrolyzed into the monomeric sugars according to NREL standard (Sluiter et al., 2006) and were then measured. Due to the co-elution of xylose and mannose, the two sugars are reported together in this study.

2.2.4 HMF, Furfural, and acetic acid analysis

The concentration of furfural, HMF, and acetic acid were measured using high performance liquid chromatography (HPLC) (Waters, Milford, MA) equipped with differential refractive index detector (ERC-5710, ERMA), a Rezex ROA organic acid column (7.8 mm × 30 cm, Phenomenex, Torrance, CA), on elution with 0.005 N aqueous H₂SO₄ with a flow rate of 0.5 mL / min at 65 °C. An aliquot portion of hydrolysate (1 mL) was taken and filtered (0.45 mm) into an HPLC vial. Data was acquired and analyzed using the N2000 chromatography software.

2.2.5 Lignosulfonate analysis

Lignosulfonate concentration was measured with a UV-Visible spectrophotometer (Perkin Elmer Lambda 35, PerkinElmer, Inc. Waltham, MA). The scan range is from 200 to 400 nm with a step

interval of 0.5 nm. It was calculated according to the absorbance at the wavelength of 232.5 nm with an extinction coefficient of 24.5 (Llano et al., 2012; Marqués et al., 2009).

2.2.6 Total sugar yields analysis

The total sugar yield included two fractions of monomeric sugars produced in the (1) spent liquor and (2) enzymatic hydrolysis. The monomeric sugar was normalized by a hydro molecular weight correction factor that varies from 0.88 or 0.9 depending on the sugar type. The total sugar conversion yield can then be obtained by dividing the total carbohydrates in the original raw material.

2.3 Modeling theory

Acid bisulfite pretreatment is a complex acid hydrolysis process involving a solid state substrate and a liquid state catalyst. The reaction rate of this process depends on a number of variables, such as temperature, acid concentration, time and substrate concentration (Lenihan et al., 2011). The main objectives of pretreatment process kinetic modeling are 1) understanding the reactions kinetics of multiple products in pretreatment process and 2) establishing an index (reaction rate) for multiple products evaluation.

The Saeman model (Saeman, 1945) was established under the assumption that the reactions occurred during acid hydrolysis are irreversible pseudo-homogeneous first order reactions. The process can be expressed as follows:

$$\frac{dP}{dt} = -k_1 P \quad (1)$$

$$\frac{dC}{dt} = k_1 P - k_2 C \quad (2)$$

Where: k_1 = The rate constant of polymer to monomer, min^{-1}

k_2 = The rate constant of monomer to degradation products, min^{-1}

P, C = Concentrations of polymeric and monomeric sugar, g L^{-1}

These polymers being reduced to simple sugars include glucan, xylan, mannan, galactan, and arabinan. From this reaction mode and solving differential equations (1) and (2), monomer concentration (C) as a function of time (t) can be represented by (Malester et al., 1992; Rahman et al., 2006; Saeman, 1945; Zhuang et al., 2009):

$$C_m = \frac{P_0 k_1}{(k_2 - k_1)} \times (e^{-k_1 t} - e^{-k_2 t}) \quad (3)$$

$$Y = \frac{C_m}{P_0} = \frac{k_1}{k_2 - k_1} \times (e^{-k_1 t} - e^{-k_2 t}) \quad (4)$$

Where: C_m = Monomeric sugar concentration, g L^{-1}

P_0 = Initial Polymer concentration, g L^{-1}

Y = Monomeric sugar yield in percent, %

As known, HMF and furfural are main products from dehydration of pentose and hexose. Acetic acid was produced from cleavage of acetyl groups in hemicellulose. These three chemicals are the main by-products in the spent liquor. Their production can be modeled as (Aguilar et al., 2002; Gámez et al., 2006; Rueda et al., 2015; Yan et al., 2014):

$$C_D = C_0(1 - e^{-k_3 t}) \quad (5)$$

Where: C_D = Concentrations of HMF, furfural, and acetic acid, g L^{-1}

C_0 = Regression parameters, potential concentrations, g L^{-1}

k_3 = Rate constants of reactions, min^{-1}

Once the rate constant of reaction (k_i) is determined, the other important kinetic parameters can be correlated with temperature using the Arrhenius Equation (Gámez et al., 2006):

$$k_i = A_i e^{\frac{-E_a}{RT}} \quad (6)$$

Where: k_i = Rate constant of reaction ($i = 1, 2$ or 3), min^{-1}

A_i = Pre-exponential factor ($i=1, 2$ or 3), min^{-1}

E_a = Activation Energy, J mol^{-1}

R = Gas Content, $8.314 \text{ J mol}^{-1} \text{ K}^{-1}$

T = Temperature, K

In all, the production of monomeric sugars and degradation products in spent liquor during acid bisulfite pretreatment can be estimated by the above models.

3. Results and discussion

3.1 Modeling of monomeric sugars

The monomeric sugar production in the hydrothermal chemical pretreatment was exemplified by an initial increase and a subsequent decrease curve (Figure 1). This behavior is consistent with the findings by others (Lenihan et al., 2011; Saeman, 1945) and results from the acid hydrolysis of the polymeric sugars to monomer species. After conversion, the monomeric sugars are then susceptible to thermal degradation to byproducts such as furfural or HMF. Because the activation energy for these two reactions differ, the concentration of monomeric sugar decreases following a maximum yield was achieved. In order to simulate the development of this curve, we measured the actual concentrations of monomeric sugars at specific pretreatment time and then employed Saeman model to simulate the curves of the monomeric sugars in the system. The activation energy and pre-exponential factors were obtained by applying Arrhenius equation.

3.1.1 Reaction rate constants calculation

The measured concentrations of monomeric sugars in the spent liquor are plotted in Figure 1 along with solid lines that represent the fitted curves from the Saeman model. The best fit for the Saeman model was obtained by minimizing the error sum of squared between measured concentration and predicted concentration ($\sum(C_{mea} - C_{pre})^2$). The resulting reaction rate

constants k_1 and k_2 were summarized in Table 1.

All monomeric sugars except glucose displayed a peak in the concentration curve under all three pretreatment temperatures. A high pretreatment temperature resulted in earlier peak concentration. For example, the maximum sugar yield for xylose/mannose was reached in approximately 41 min at 155 °C, 75 min at 145 °C and 132 min at 135 °C. As for glucose, sugar concentration continuously increased throughout the 180 min cooking process. Approximately 39% of Douglas-fir is composed of glucan, which is primarily present in the crystalline structure of the cellulose. Consequently, it is logical that the hydrolysis of glucan would require additional time to complete compared to amorphous carbohydrate polymers.

3.1.2 Activation energy calculation

After obtaining k_1 and k_2 , Arrhenius equation (Eq. 6) was employed to calculate the activation energy E_a and pre-exponential factor A_i . The obtained values were summarized in Table 2 with respect to k_1 and k_2 . The lowest activation energy for polymeric sugar hydrolysis into monomeric sugar was xylan/mannan, whereas the lowest activation energy of monomeric sugar degradation was galactose. Glucan exhibits the highest activation energy for both monomer sugar production and degradation. Since glucan has a known, stable crystalline structure compared to the other carbohydrate polymers, a higher activation energy results when hydrolyzing to the monomeric sugar. The two-fraction model was also used to characterize the glucan hydrolysis. The obtained activation energy for monomeric sugar production and degradation were 98.6 and 129.0 kJ mol⁻¹, which were similar to the results from the Saeman model.

3.2 Modeling of HMF, furfural, and acetic acid

The concentrations of HMF, furfural, and acetic acid all increased with increasing pretreatment temperature and time (Figure 2). However, a slight decrease in HMF and furfural was often

observed for the 180 min cook times. This can be explained by subsequent degradation of the HMF and furfural to organic acids (Yan et al., 2014). It is known that HMF can be degraded into levulinic acid and formic acid, while furfural can also be degraded into formic acid (Jönsson et al., 2013).

To estimate the yield of HMF, furfural, and acetic acid, the Saeman model was used with similar steps as monomeric sugar modeling process. The obtained kinetic parameters were summarized in Table 3. As shown, the HMF and furfural required a much higher activation energy than the acetic acid, indicating that cleavage of acetyl groups was much easier than dehydration of monomeric sugars. Also, the regression parameter C_o for HMF was substantially higher than furfural, which is consistent with the high percentage of pentose in the raw materials. As known, the maximum xylose/mannose yield was achieved at 145 °C and 75 min, the corresponding concentrations of acetic acid, HMF, and furfural were 1.54, 0.60, and 1.15 g L⁻¹ obtained from above models.

3.3 Lignosulfonates analysis

The concentration of lignosulfonates also increased with an increase of cooking temperature and time as shown in Figure 2(d). There is no obvious difference of lignosulfonates concentration between 120 and 180 min cooking at 145 and 155 °C. As proposed by many researchers, lignin condensation occurred during the hydrothermal chemical treatment especially at the acidic conditions (Sannigrahi et al., 2011; Sannigrahi et al., 2008; Shimada et al., 1997; Sun et al., 2013; Yin et al., 2017). In this process, dissolved lignin segments may be attached to the lignin matrix, resulting in decreased lignosulfonates concentration in spent liquor.

3.4 Sugar yield in enzymatic hydrolysis

The glucose conversion in enzymatic hydrolysis was calculated based on original carbohydrates content in the raw material. The evolution of glucan hydrolysis in 72 h was recorded as shown in Figure 3. For materials pretreated at 135 °C, the 180 min treated solids reached their highest glucan conversion of 39% followed by the 120 and 60 min pretreated solids. While at 145 and 155 °C, 120 min cooking achieved highest glucan conversion 53% and 49%, respectively. It is obvious that the 180 min cooking at 145 and 155 °C resulted in a decrease of the glucan hydrolysis yield. The potential reason for this is lignin condensation, which can inhibit enzyme accessibility to cellulose (Hu et al., 2012). This is indirectly supported by the decrease in lignosulfonates concentration at 180 min cooking.

3.5 Total sugar yields analysis

As shown in Figure 4 (a), a minimum total sugar yield was obtained at approximately 40 min cooking under 145 and 155 °C. This minimum yields can be attributed to the increased monomeric sugar degradation and inefficient enzymatic hydrolysis yield. In order to investigate the relationship between total sugar yield and pretreatment time, as well as temperature, a Lorentzian function (Eq. 7) was used to fit the data. The practical significance of regression parameters a , x_0 , and y_0 are maximum total sugar yield, optimal pretreatment temperature and time. According to the obtained regression parameters, the maximum total sugar yield was 62% under the pretreatment condition of 143 °C and 111 min, which approaches the actual situation 65% at 145 °C and 120 min (as shown in Table 4).

$$z = \frac{a}{\left[1 + \left(\frac{x - x_0}{b}\right)^2\right] \left[1 + \left(\frac{y - y_0}{c}\right)^2\right]} \quad (7)$$

Where: x = Treatment temperature, °C

y = Treatment time, min

z = Total sugar yield, %

x_0 , y_0 , a , b , and c = Regression parameters

When enzyme dosage was increased to 8.8% (Figure 4 (b)), the obtained regression parameter “ a ” increased to 75%, which approaches 77% in the actual situation. Other regression parameters changed little. Therefore, we can conclude that this equation can simulate the relationship between total sugar yield and pretreatment condition: temperature and time. With this finding, it can be used to estimate the maximum total sugar yield and simplify the optimization process of pretreatment.

4. Conclusion

The Saeman model accurately represented the process of polymeric sugar hydrolysis and subsequent monomeric sugar degradation in the acid bisulfite pretreatment. According to this model, the maximum yield of xylose/mannose was achieved at 75 min and 145 °C with the concentration of 1.54, 0.60, and 1.15 g L⁻¹ for acetic acid, HMF, and furfural. A Lorentzian function was used to describe the relationship between total sugar yield and pretreatment factors: temperature and time. The obtained maximum total sugar yield and optimal treatment condition from the fitted model were 75%, 145 °C and 112 min, which approaches the actual situation.

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Table Captions

Table 1. Reaction rate constants and maximum yield time predicted by the Saeman model

Table 2. Activation energy of each sugar type obtained through the Arrhenius law

Table 3. Activation energy of HMF, furfural, and acetic acid obtained through the Arrhenius law

Table 4. Total sugar yield released during the pretreatment and the enzymatic hydrolysis under 4.4% and 8.8% enzyme dosage

Figure Captions

Figure 1. Experimental data of sugar concentrations in the spent liquors at different pretreatment times and the predicted data by the Seaman model

Figure 2. Concentrations of (a) HMF, (b) furfural, (c) acetic acid, and (d) lignosulfonates in the spent liquor:

Figure 3. Glucan conversion with the time during enzymatic hydrolysis after pretreated under three treatment temperatures of (a) 135 °C, (b) 145 °C, and (c) 155 °C

Figure 4. Modeling of total sugar yield with a Lorentzian function under (a) 4.4% and (b) 8.8% enzyme dosage.

Table 1

Sugar type	135 °C			145 °C			155 °C		
	k ₁	k ₂	Time ^a (min)	k ₁	k ₂	Time ^a (min)	k ₁	k ₂	Time ^a (min)
Arabinose	0.0897	0.0068	31	0.1686	0.0096	18	0.3300	0.0225	9
Galactose	0.0142	0.0044	119	0.0272	0.0062	70	0.0474	0.0121	39
Glucose	0.0005	0.0021	931	0.0015	0.0087	247	0.0018	0.0100	210
Xyl/mannose	0.0110	0.0049	132	0.0208	0.0079	75	0.0346	0.0164	41

a: Time consumed for achieving maximum sugar yield, obtained by Saeman model.

Table 2

Sugar type	Reaction rate constants	E_a (kJ mol ⁻¹)	Ln A_i	R^2
Arabinan	k_1	95.1	25.5	0.99
	k_2	86.8	20.4	0.94
Galactan	k_1	93.5	21.6	0.99
	k_2	73.1	16.0	0.96
Glucan	k_1	99.0	21.5	0.87
	k_2	115.9	28.0	0.83
Xylan/mannan	k_1	85.4	20.2	0.99
	k_2	88.4	20.3	0.98

Table 3

Products	C_0 (g L ⁻¹)	E_a (kJ mol ⁻¹)	Ln A_i	R^2
HMF	44.2	108.9	22.7	0.88
Furfural	4.5	105.8	24.9	0.99
Acetic acid	9.7	71.5	14.5	0.87

Table 4

Pretreatment time (min)	135 °C				145 °C				155 °C			
	4.4%		8.8%		4.4%		8.8%		4.4%		8.8%	
	Ave.	SD	Ave.	SD	Ave.	SD	Ave.	SD	Ave.	SD	Ave.	SD
60	51.9	0.03	59.2	0.08	58.6	1.07	68.6	1.03	52.4	0.45	66.0	1.36
120	55.6	0.21	64.9	0.08	65.5	0.03	77.1	0.01	52.2	0.21	65.7	0.33
180	52.3	0.01	63.1	0.03	50.7	0.06	66.0	1.70	45.1	1.00	55.8	0.28