Bioresource Technology 226 (2017) 24–30

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Evaluating the effect of wood ultrastructural changes from mechanical treatment on kinetics of monomeric sugars and chemicals production in acid bisulfite treatment

Yalan Liu [a,*], Jinwu Wang [b], Michael P. Wolcott [a]

[a] Composite Materials & Engineering Center, Washington State University, Pullman, WA 99164-1806, United States
[b] United States Department of Agriculture, Forest Service, Forest Products Laboratory, 35 Flagstaff Road, Orono, ME 04469-5793, United States

HIGHLIGHTS

Correlating ultrastructural changes with monomeric sugar production and degradation.
Decreasing the severity of chemical treatment with a mechanical-chemical treatment.
Optimizing acid bisulfite pretreatment conditions with kinetic modeling.

ARTICLE INFO

Article history:
Received 13 October 2016
Received in revised form 1 December 2016
Accepted 2 December 2016
Available online 5 December 2016

Keywords:
Crystallinity
Kinetics
Monomeric sugar
Lignosulfonates
Sugar degradation
Enzymatic hydrolysis

ABSTRACT

Currently, various chemical-mechanical treatments were widely used in biofuel production to achieve high total sugar yields. However, the interaction between two treatments was scarcely investigated. In this study, we employed a ball milling process to create ultrastructural changes for Douglas-fir (*Pseudotsuga menziesii*) micronized wood powders. The 0, 30, and 60 min ball milled wood powders resulted in a crystallinity index of 0.41, 0.21, and 0.10 respectively. It was found that the ultrastructural changes accelerate monomeric sugars production without influencing the yield of sugar degradation products. The optimal acid bisulfite treatment time was substantially decreased from 120 min to 40 min as the cellulose crystallinity decreased. Meanwhile, total sugar yield increased from 65% to 92% and had a linear relation with a decrease of the cellulose crystallinity.

1. Introduction

Lignocellulosic materials are abundant and renewable resources on the earth. Their utilization for biofuel production has drawn substantial attentions due to fossil fuel crisis and environmental pollution (Himmel et al., 2007). However, the intrinsic structure of this material: crystalline structure of cellulose, surrounding by hemicellulose and lignin, inhibits total sugar yield from bioconversion process (Jeoh et al., 2007; Kumar and Wyman, 2014; Rowell et al., 2005). Therefore, many pretreatment methods were investigated to overcome the recalcitrance of lignocellulosic materials (Hendriks and Zeeman, 2009).

For instance, the dilute acid method is effective for herbaceous biomass and some hardwoods, sulfite methods are needed for adequate yield for softwood biomass, and simple size reduction increases pretreatment efficiency in both types of processes (Ballesteros et al., 2000; Liu et al., 2013; Shuai et al., 2010; Silva et al., 2012; Zhou et al., 2013). However, single chemical or mechanical treatment method may result in extreme pretreatment conditions or unsatisfactory total sugar yield (Ballesteros et al., 2000; Liu et al., 2013; Mais et al., 2002). As a result, some researchers have deployed combined treatments to achieve good treatment performance with low severity conditions (Jin and Chen, 2006; Li et al., 2016).

The combinations of mechanical and chemical treatment were investigated to improve the total sugar yield (Inoue et al., 2008; Miura et al., 2012; Peng et al., 2013; Yuan et al., 2015; Zakaria et al., 2014). Fine milling such as ball milling was widely adopted in combined treatments. Ball milling altered ultrastructural properties of materials, i.e. crystallinity and particle size (Schwanninger et al., 2004; Yang et al., 2014). However, the influence of ultrastructural changes in the kinetics of monomeric sugars

* Corresponding author.
E-mail addresses: yalan.liu@wsu.edu (Y. Liu), jinwuwang@fs.fed.us (J. Wang), wolcott@wsu.edu (M.P. Wolcott).

http://dx.doi.org/10.1016/j.biortech.2016.12.009
0960-8524/

production and degradation in chemical treatment have not been revealed. Also, the relationship between ultrastructure characteristics of raw materials and total sugar yield have not been correlated quantitively. Also, researchers may have a concern about the energy cost in the fine milling process. However, some of our ongoing works show that proper utilizing multi-step milling process and appropriate mill types can save a large amount of energy (Liu et al., 2016a).

In addition, monomeric sugars were core products in the bioconversion studies. Other products yielding in the chemical treatment process, such as acetic acid, furfural, hydroxymethylfurfural (HMF) and lignosulfonates (LS), may either bring profits in a biorefinery or inhibits enzymatic hydrolysis yields (Jönsson et al., 2013). Lignosulfonates can be a co-products in biorefinery instead of lignin based polymer, which has been widely investigated in current researches (Li and McDonald, 2014; Li et al., 2014, 2015a, b). Therefore, investigating the influence of ultrastructural changes on the yield of these products is meaningful.

Considering these gaps in current research we established this study to investigate the influence of ultrastructural changes in raw materials on the kinetics of monomeric sugars production and degradation in the chemical treatment process. Meantime, the response of co-products yield to ultrastructural changes was investigated. Also, the relationship between ultrastructural changes and total sugar yields in chemical treatment and enzymatic hydrolysis were correlated.

2. Materials and methods

2.1. Materials preparation and characterization

Douglas-fir (*Pseudotsuga menziesii*) micronized wood powder was prepared by an impact mill (Zhenzhou Tianyuan Environmental Protection Machinery Co. Ltd, China) with 30 min milling of 2 mm wood chips. The particle size of this wood powders was characterized by 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. A planetary ball mill (Across International, PQ-N04, two 100 ml steel jars, 100 steel balls with a diameter of 6 mm and 16 steel balls with a diameter of 10 mm in each jar) was employed to mill the wood powders at two conditions 30 and 60 min. Therefore, three types of raw materials were employed in this study, unball-milled materials (BM 0), ball milled 30 min (BM 30), and ball milled 60 min (BM 60).

Images of wood particles were obtained through Scanning electron microscopy (SEM) (FEI Quanta 200F, field emission gun, high vacuum, ETD detectors, FEI Company, Hillsboro, Oregon, USA) after sputter coating with gold.

2.2. Acid bisulfite pretreatment

Acid bisulfite method was used to prepare the materials for enzymatic hydrolysis. 145 °C was selected as the treatment temperature and treatment duration time was 20–80 min for ball milled materials, while 20–180 min for materials without ball milling (Liu et al., 2017, 2016b). Two replicates were carried out for each pretreatment. The cooking liquor has a $Ca(HSO_3)_2$ concentration of 34.41 g/kg. The solid to liquid ratio is 1:4. Therefore, 25 ml cooking liquor and 15 ml water were mixed to achieve 6.6% sulfur dioxide loading. 100 ml Parr reactor (No. 4845) was employed for treatments. It took about 30 min to reach 145 °C and 15 min to cool it to room temperature. After treatment, the slurry was separated through vacuum filtration. The spent liquor was collected for sugars, LS, acetic acid and sugar degradation products (furfural and HMF) analysis. Pretreated solids were washed and stored in 4 °C refrigerator before use.

2.3. Spent liquor analysis

The spent liquors were analyzed for sugar, furfural, HMF, acetic acid, and LS content. Two replicates were tested for each sample. The monomeric and oligomer sugars in spent liquor were measured according to NREL standard (Sluiter et al., 2006). The monomeric sugar concentration was measured by anion exchange chromatography (Dionex ICS-3000, CarboPac PA20 Guard column: 4 50 mm and IonPac AS11-HC analytical column: 4 250 mm, ED40 electrochemical detector, AS40 auto sampler, degassed E-pure water, 50 mM and 200 mM NaOH solution as eluent with a flow rate 0.5 ml/min). Oligomer sugar in spent liquor need to be hydrolyzed into monomeric sugar firstly and then measured by Dionex ICS-3000.

The concentration of degradation products was measured by high-performance liquid chromatography (HPLC) using a Waters 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 H_2SO_4 with a flow rate of 0.5 ml/min at 65 °C.

The concentration of LS was measured with UV–Visible spectrophotometer with a scan range from 200 to 400 nm at a 0.5 nm interval (Perkin Elmer Lambda 35, PerkinElmer, Inc. Waltham, MA) and 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).

2.4. X-ray diffractometer (XRD) analysis

XRD analysis of the BM wood powders and control samples were conducted with an X-ray diffractometer (Miniflex 600, Rigaku, Japan) operated at 40 V and 15 mA with Ni-filtered Cu-Ka radiation. Two replicates were tested for each sample. The samples were scanned with the 2θ range of 10–40° at a scan speed of 2° min ^{1} and step size 0.005. The crystallinity index (CrI) of each sample was analyzed using the following equation.

$$CrI = \frac{I_{002} \quad I_{am}}{I_{002}}$$

where I_{002} is the intensity of the 002 peak at $2\theta = 22.4°$, and I_{am} is the intensity of the background scatter at $2\theta = 18.5°$, which is the characteristic peak of cellulose-I.

2.5. Fourier transform infrared (FTIR) analysis

The pretreated samples for Fourier transform infrared analysis were prepared by drying in convection Oven at 45 °C for 24 h. Two replicates were tested for each sample. These tests were carried out with a Thermo Nicolet Avatar 370 spectrometer operating in the attenuated total reflection (ATR) mode (Smart performance, ZnSe crystal performance, ZnSe crystal). The spectra were used to analyze chemical bands difference among various treatment conditions (Liu et al., 2013; Sun et al., 2005).

2.6. Enzymatic hydrolysis

Cellic® CTec2 and HTec2 was complimentarily provided by Novozymes North America (Franklinton, NC). The pretreated solids from acid bisulfite cooking were hydrolyzed for 72 h with a 4% (w/w) CTec2 and 0.4% (w/w) HTec2 (based on oven dry wood basis) enzyme product loading. The solid loading was 2% (w/v) of a total 50 ml volume. 0.5 ml 2% (w/w) sodium azide were used for

antibacterial function in hydrolysis slurry. The supernatant was collected after 72 hours' hydrolysis and diluted 500-fold for analysis of monomeric sugars. Two replicates were carried out for the enzymatic hydrolysis of each pretreated sample. Total sugar yield is the overall sugar recovery from both acid bisulfite treatment process and enzymatic hydrolysis.

2.7. Modeling theory

Acid bisulfite treatment is a heterogeneous 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 the current kinetic modeling are 1) understanding this reaction kinetics of multiple products in the chemical treatment process, 2) establishing an index (reaction rate constants) for multiple products yield evaluation.

The Saeman model (Saeman, 1945) was first proposed to investigate sulfuric acid hydrolysis of Douglas-fir wood. This model was established under the assumption that the reactions happened during acid hydrolysis are irreversible pseudo-homogeneous first order reactions. The process can be expressed as follows:

$$\text{Polymer} \xrightarrow{k_1} \text{Monomeric sugar} \xrightarrow{k_2} \text{Decomposition products}$$

$$\frac{dP}{dt} = -k_1 P \tag{1}$$

$$\frac{dM}{dt} = k_1 P \quad k_2 C \tag{2}$$

where

k_1 = The rate constant of polymer to monomer, min ^{1}.
k_2 = The rate constant of monomer to decomposition products, min ^{1}.
P, C = Concentration of polymer and monomer sugar, g L ^{1}.

Polymeric sugars are glucan, xylan, mannan, galactan, and arabinan. From this reaction mode and solving differential Eqs. (1) and (2), the monomeric sugar 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):

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

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

where

C_m = Monomeric sugar concentration, g L ^{1}.
P_0 = Initial Polymer concentration, g L ^{1}.
Y = Monomeric sugar yield in percent, %.

After obtaining k_1 and k_2, the time at which the maximum sugar yield was reached can be calculated as:

$$t = \frac{lnk_2 \quad lnk_1}{k_2 \quad k_1} \tag{5}$$

In order to investigate the effects on sugar degradation products yield, a kinetic modeling was also employed.

$$C_i = C_{i0}(1 \quad e^{\ k_i t}) \tag{6}$$

where

C_i = Acetic acid or HMF & Furfural concentration, g L ^{1}.
C_{i0} = Regression parameters, potential concentrations, g L ^{1}.
k_i = The rate constants of reactions, min ^{1}.

3. Results and discussion

3.1. Particle size and crystallinity characterization

Original micronized wood powder and BM wood powders were characterized by particle size distribution and cellulose crystallinity. Fig. 1 shows the median particle size decreased from 32 to 17 μm and 18 μm after 30 and 60 min ball milling, respectively. The peak of size distribution curve shifted to a small size after ball milling. However, the BM 60 wood powders had a larger size compared to the particle size of BM 30 wood powders, which may be attributed to wood particles agglomeration (Gharpuray et al., 1983; Yu and Wu, 2011; Zhang et al., 2007).

XRD analysis demonstrates that the cellulose crystallinity of wood powders decreased along with an increase of the milling time. The peak at 18.5° disappeared for ball milled materials, which indicated the transition of crystalline structure to amorphous structure. The CrI decreased from 0.41 to 0.20 and 0.10 after 30 and 60 min ball milling, respectively.

SEM analysis showed that the cell wall structure of wood powder was destroyed after ball milling. The wood powders became smaller after 30 min ball milling. However, the particles obtained from 60 min ball milling become larger than the particles from 30 min. This finding is consistent with the above results of particle size analysis.

3.2. Monomeric sugars production and degradation

Per this modeling theory, we measured monomeric sugars' concentration in spent liquor. Initial polymer concentration can be obtained per the compositional analysis of raw materials (as shown in Table 1). Then, k_1 and k_2 can be obtained through least squares fitting using Eq. (3) and t can be calculated by Eq. (5). These parameters were summarized in Table 1.

Ball milling process accelerated monomeric sugars production process since the parameter k_1 for various sugars increased with 30 min BM wood powders. Further ball milling did not increase k_1 significantly. As to k_2, ball milling did not show influence on it, which indicates degradation products formation was not influenced by ultrastructural changes. Also, this result was verified by measuring sugar degradation products in spent liquor as shown in Table 2.

The treatment time required for achieving maximum sugar in the spent liquor reduced to 45 min for xylan/mannan after 30 min ball milling. Especially, the time required for glucan

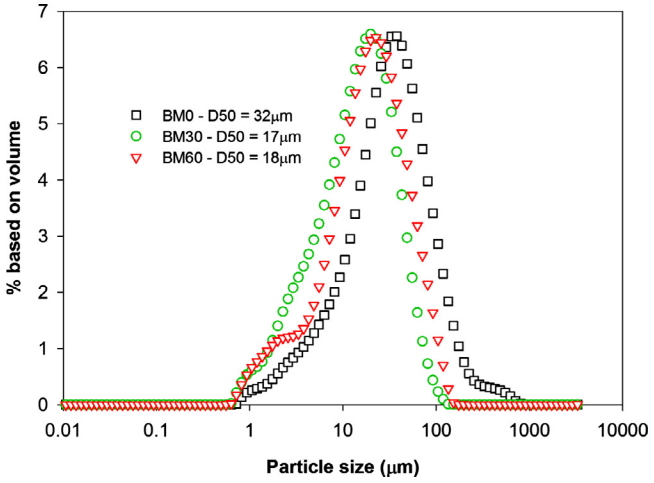

Fig. 1. Particle size distribution of 0, 30 and 60 min ball milled materials.

Table 1
Rate constants of monomeric sugar production and degradation obtained by Saeman model fitting.

Sugar type	Composition (%)	BM 0				BM 30				BM 60			
		k_1	k_2	Time (min)	R^2	k_1	k_2	Time (min)	R^2	k_1	k_2	Time (min)	R^2
Arabinan	1.16	0.1680	0.0121	17	0.97	0.4371	0.0098	9	0.90	0.4609	0.0132	8	0.93
Galactan	2.97	0.0272	0.0062	70	0.95	0.0345	0.0100	51	0.99	0.0445	0.0142	38	0.99
Glucan	38.12	0.0015	0.0087	247	0.96	0.0045	0.0075	170	0.97	0.0045	0.0084	160	0.99
Xylan/mannan	13.00	0.0208	0.0079	75	0.99	0.0362	0.0127	45	0.99	0.0405	0.0117	43	0.99

Table 2
The concentrations of LS, acetic acid, HMF, and furfural in the spent liquors.

Ball mill time (min)	Cooking time (min)	LS (g/L)		Acetic acid (g/L)		HMF (g/L)		Furfural (g/L)	
		Ave	SD	Ave	SD	Ave	SD	Ave	SD
0	20	27.18	2.38	0.83	0.11	0.08	0.00	0.25	0.00
	40	32.29	0.26	1.21	0.02	0.21	0.00	0.43	0.08
	60	37.00	2.11	1.33	0.12	0.38	0.03	0.92	0.29
	120	46.69	1.76	1.69	0.05	1.45	0.07	1.68	0.05
	180	49.57	1.59	2.72	0.22	2.30	0.06	2.37	0.32
30	20	32.5	0.17	1.02	0.12	0.09	0.00	0.22	0.00
	40	41.4	2.18	1.59	0.02	0.19	0.00	0.50	0.05
	60	45.5	0.13	1.66	0.11	0.25	0.01	0.75	0.08
	80	45.4	0.31	2.71	0.14	0.30	0.01	1.27	0.10
60	20	33.2	0.10	1.50	0.07	0.09	0.00	0.26	0.00
	40	46.8	0.27	2.17	0.09	0.22	0.00	0.57	0.02
	60	50.2	0.02	2.68	0.10	0.25	0.00	0.85	0.05
	80	51.5	0.33	3.25	0.20	0.35	0.02	1.38	0.13

hydrolysis dropped more than one hour. Also, it can be found that arabinan has quickest reaction rate followed by galactan and xylan/mannan. Glucan is the hardest one to be hydrolyzed in this process, which been significantly improved due to crystalline structure change in cellulose.

Table 2 shows the concentration of sugar degradation products, acetic acid, and LS in the spent liquor. The concentration of these products increased with an increase of treatment time. The increase of ball milling time increased the yield of LS and acetic acid without laying effects on the yields of HMF and furfural. These results are quite approving in pursuit of co-products production boost and high enzymatic hydrolysis yield. In order to quantify the influence of ultrastructural changes (induced by ball milling) on these co-products yield, kinetic modeling of acetic acid and sugar degradation products was carried out according to Eq. (6) (Aguilar et al., 2002). The rate constants were summarized in Table 5.

3.3. Total sugar yields

After obtaining monomeric sugars and other chemicals from the chemical treatment process, pretreated solids were utilized for cellulosic sugar production with enzymes. Most carbohydrates can be hydrolyzed into monomeric sugars depending on chemical treatment efficiency. The residues from enzymatic hydrolysis are rich in lignin (95.1% lignin and 4.9% extractives for BM 60 residues under pretreatment condition: 145 °C and 40 min) and can be further used for other applications such as active carbon production or lignin based polymers (Suhas et al., 2007). Therefore, high carbohydrate hydrolysis yields will result in pure lignin residues, which will be beneficial for utilizing them.

Table 3 shows total sugar yield from both chemical treatment and enzymatic hydrolysis under various ball milling and cooking time conditions. Total sugar yield all firstly increased and then decreased along with an increase of cooking time. Maximum total sugar yields: 65%, 81%, and 92% were achieved at 120, 40 and

Table 3
Total sugar yields from spent liquor and enzymatic hydrolysis under various conditions.

Ball mill time (min)	Cooking time (min)	Arabinan (%)	Galactan (%)	Glucan (%)	Xylan/mannan (%)	Total (%)	
						Ave.	SD
0	0	0.0	0.0	14.9	8.7	12.3	0.1
	60	53.7	82.4	48.2	81.5	58.0	1.5
	120	40.4	71.1	66.2	64.2	65.5	0.1
	180	19.1	41.7	58.2	33.8	50.7	0.1
30	0	10.3	0.0	51.4	23.2	41.2	0.6
	20	56.3	69.1	71.8	69.3	70.7	2.4
	40	43.1	74.3	85.2	73.9	81.0	0.4
	60	34.4	63.7	76.5	64.9	72.2	0.2
	80	31.5	58.1	74.4	63.1	69.9	0.2
60	0	12.6	0.0	64.9	26.4	51.3	0.3
	20	61.8	66.1	90.0	74.8	84.6	0.6
	40	54.6	73.9	98.1	81.7	92.0	0.3
	60	49.4	70.1	88.3	70.2	82.2	3.5
	80	32.2	57.4	79.4	61.6	73.0	2.6

Table 4
FTIR bands intensity normalization by 1511 cm $^{-1}$.

Wave number (cm $^{-1}$)	Band origin	Cooking time (min)				
		0	20	40	60	80
1731	C=O stretch in hemi/lignin	0.70	0.19	0.29	0.32	0.33
1267	Guaiacyl unit of lignin	1.50	1.33	1.28	1.28	1.24
1158	C—O—C vibration cellulose	1.47	1.80	1.96	1.95	1.81
1030	C-O vibration in C—rbohydrate	4.21	4.06	4.01	3.89	3.67

Table 5
Comprehensive analysis between ultrastructural properties and total sugar yields, rate constants of multiple products.

Ball mill time (min)	Ultrastructure properties		Optimal treatment time (min)	Rate constants k_1 of		Rate constants k_i of		Total sugar yield (%)
	CrI	Median size (μm)		Xylan/mannan	Glucan	Acetic acid	HMF& furfural	
0	0.41	32	120	0.0208	0.0015	0.0111	0.00036	65
30	0.20	17	40	0.0362	0.0045	0.0130	0.00033	81
60	0.10	18	40	0.0405	0.0045	0.0222	0.00036	92

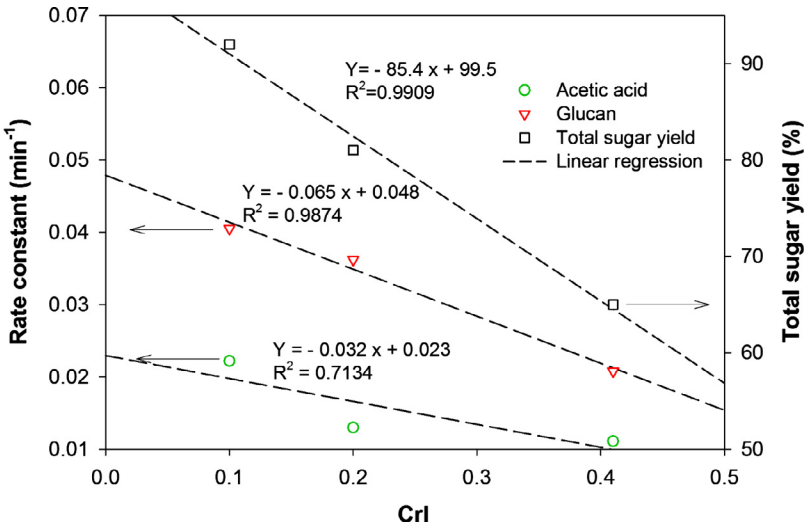

Fig. 2. Linear fitting between CrI and rate constants, total sugar yield.

40 min cooking for 0, 30 and 60 min BM wood powders, respectively. 30 min ball milling decreased cooking time and increased total sugar yield. However, further ball milling did not have effects on decreasing chemical treatment time but still increased total sugar yield by 11% compared to 30 min ball milling.

3.4. FTIR analysis

Above analysis showed that excessive cooking can lower total sugar yield. It is crucial to explore the underlying mechanism for this phenomenon to optimize pretreatment condition. FTIR was employed to characterize pretreated solids. The bands of chemical groups in BM 30 and BM 60 under various chemical treatment conditions were investigated. Band 1731 cm $^{-1}$ represents C=O stretch in hemicellulose and lignin. It disappeared after chemical treatment due to hemicellulose and lignin removal. Band 1598/1602 and 1511 cm $^{-1}$ are the characteristic bands of lignin (Li and McDonald, 2014). Band 1511 cm $^{-1}$ can be used for bands intensity normalization (Dizhbite et al., 2011). The bands analyzed in this study were assigned in accordance with (Li et al., 2015b; Marques et al., 2006; Rana et al., 2010; Sun et al., 2002; You et al., 2015): 1646 cm $^{-1}$ for absorbed water, 1367 and 897 cm $^{-1}$ for C—H deformation in cellulose and hemicellulose, 1315 cm $^{-1}$ for C—H vibration in cellulose, 1267 cm $^{-1}$ for guaiacyl unit of lignin, 1158 cm $^{-1}$ for C—O—C vibration in cellulose and hemicellulose, 1104 cm $^{-1}$ for aromatic skeletal vibration or C—O stretch in lignin or carbohydrates, 1056 and 1030 cm $^{-1}$ for C—O vibration in cellulose and hemicellulose.

The intensities of four bands were normalized and compared among various chemical treatment time to determine chemical groups changes of pretreated solids. As shown in Table 4, the intensity of band 1731 cm $^{-1}$ decreased after chemical treatment and then increased along with an increase of treatment time. Since this band was C=O stretch in hemicellulose or lignin, it decreased due to hemicellulose removal and then increased due to C=O stretch from lignin, since lignin content comparatively increased after removing hemicellulose. According to band 1267 cm $^{-1}$, G-unit lignin decreased along with an increase of treatment time and maintained at the same level after 40 min cooking. The intensity of band 1158 cm $^{-1}$ indicated cellulose content in pretreated material firstly increased because of hemicellulose removal and then decreased due to acid hydrolysis of glucan. In general, carbohydrates in raw materials continued to decrease along with an increase of treatment time as proved by the intensity change of band 1030 cm $^{-1}$.

According to above analysis, it can be concluded that 40 min chemical treatment achieved stable lignin removal and high hemicellulose removal with a small amount of cellulose hydrolysis.

With an increase in acid bisulfite pretreatment time, a large amount of cellulose tended to be hydrolyzed with the concomitant of more sugar degradation products, which is harmful to downstream enzymatic hydrolysis. In addition, many studies showed that lignin condensation or pseudo-lignin occurred in hydrothermal treatments (Kumar et al., 2013; Sannigrahi et al., 2008, 2011), which negatively influenced on total sugar yields. Since LS yields maintain the same amount at 60 and 80 min cooking as shown in Table 2, lignin segments may reconstruct with carbohydrate and formed pseudo lignin, which also inhibited enzymatic hydrolysis of carbohydrates.

3.5. Comprehensive analysis

As shown in Table 5, rate constants k_1 of xylan/mannan and acetic acid, as well as total sugar yield all increased along with a decrease of crystallinity. Good linear fitting was obtained between these parameters as shown in Fig. 2, which indicated that the decrease of crystallinity accelerates xylan/mannan hydrolysis and acetic acid production process, as well as increases total sugar yields. Optimal treatment time and rate constants of glucan showed a plateau between 30 and 60 min ball milling, whereas median particle size tended to decrease after 30 min ball milling. Wood powder agglomeration may be responsible for this plateau. Also, the rate constants of HMF and furfural maintained same level indicating ultrastructural changes did not influence on sugar degradation process.

Mechanical treatment (ball milling) altered the crystalline structure of materials thus in alleviating chemical treatment severity (decreasing chemical pretreatment duration time) at achieving higher total sugar yield. Also, the yield of sugar degradation products did not increase when the ultrastructural properties of raw materials changed, which can benefit downstream enzymatic hydrolysis process. In addition, the increasing of LS and acetic acid yield can bring extra profitability for cellulosic sugar production process.

4. Conclusions

Monomeric sugar production process in acid bisulfite treatment was accelerated with a decrease of crystallinity, however, no difference was found for glucan hydrolysis between 30 and 60 min ball milled materials. The yields of HMF and furfural were not affected by ultrastructural changes, whereas the yields of acetic acid and LS increased as a decrease of crystallinity. In all, 60 min ball milling substantially improved total sugar yield from 65% to 92% and significantly decreased chemical treatment time from 120 min to 40 min. When viewed from the total mechanical-chemical pretreatment time, the 27% increase in sugar yield was achieved with 20 min decrease in time.

Acknowledgements

The authors are grateful to the financial support from the Northwest Advanced Renewables Alliance (NARA), supported by the Agriculture and Food Research Initiative Competitive Grant no. 2011-68005-30416 from the USDA National Institute of Food and Agriculture, and the China Scholarship Council (CSC) funding for Yalan Liu's living expenses. Thanks to Dr. Johnway Gao from Weyerhaeuser Co. for providing calcium bisulfite cooking liquor and Dr. Armando McDonald for providing HPLC and FTIR in his lab.

References

Aguilar, R., Ramırez, J.A., Garrote, G., Vazquez, M., 2002. Kinetic study of the acid hydrolysis of sugar cane bagasse. J. Food Eng. 55, 309–318.

Ballesteros, I., Oliva, J.M., Navarro, A.A., Gonzalez, A., Carrasco, J., Ballesteros, M., 2000. Effect of chip size on steam explosion pretreatment of softwood. Appl. Biochem. Biotechnol. 84–6, 97–110.

Dizhbite, T., Telysheva, G., Dobele, G., Arshanitsa, A., Bikovens, O., Andersone, A., Kampars, V., 2011. Py-GC/MS for characterization of non-hydrolyzed residues from bioethanol production from softwood. J. Anal. Appl. Pyrol. 90, 126–132.

Gharpuray, M.M., Lee, Y.H., Fan, L.T., 1983. Structural modification of lignocellulosics by pretreatments to enhance enzymatic-hydrolysis. Biotechnol. Bioeng. 25, 157–172.

Hendriks, A.T.W.M., Zeeman, G., 2009. Pretreatments to enhance the digestibility of lignocellulosic biomass. Bioresour. Technol. 100, 10–18.

Himmel, M.E., Ding, S.Y., Johnson, D.K., Adney, W.S., Nimlos, M.R., Brady, J.W., Foust, T.D., 2007. Biomass recalcitrance: engineering plants and enzymes for biofuels production. Science 315, 804–807.

Inoue, H., Yano, S., Endo, T., Sakaki, T., Sawayama, S., 2008. Combining hot-compressed water and ball milling pretreatments to improve the efficiency of the enzymatic hydrolysis of eucalyptus. Biotechnol. Biofuels 1, 2.

Jeoh, T., Ishizawa, C.I., Davis, M.F., Himmel, M.E., Adney, W.S., Johnson, D.K., 2007. Cellulase digestibility of pretreated biomass is limited by cellulose accessibility. Biotechnol. Bioeng. 98, 112–122.

Jin, S., Chen, H., 2006. Superfine grinding of steam-exploded rice straw and its enzymatic hydrolysis. Biochem. Eng. J. 30, 225–230.

Jönsson, L.J., Alriksson, B., Nilvebrant, N.-O., 2013. Bioconversion of lignocellulose: inhibitors and detoxification. Biotechnol. Biofuels 6, 16.

Kumar, R., Wyman, C.E., 2014. Strong cellulase inhibition by Mannan polysaccharides in cellulose conversion to sugars. Biotechnol. Bioeng. 111, 1341–1353.

Kumar, R., Hu, F., Sannigrahi, P., Jung, S., Ragauskas, A.J., Wyman, C.E., 2013. Carbohydrate derived-pseudo-lignin can retard cellulose biological conversion. Biotechnol. Bioeng. 110, 737–753.

Lenihan, P., Orozco, A., O'Neill, E., Ahmad, M.N.M., Rooney, D.W., Mangwandi, C., Walker, G.M., 2011. Kinetic modelling of dilute acid hydrolysis of lignocellulosic biomass. In: Bernardes, D.M.A.D.S. (Ed.), Biofuel Production-Recent Developments and Prospects.

Li, H., McDonald, A.G., 2014. Fractionation and characterization of industrial lignins. Ind. Crops Prod. 62, 67–76.

Li, H., Sivasankarapillai, G., McDonald, A.G., 2014. Lignin valorization by forming thermally stimulated shape memory copolymeric elastomers-partially crystalline hyperbranched polymer as crosslinks. J. Appl. Polym. Sci. 131, 12050–12060.

Li, H., Sivasankarapillai, G., McDonald, A.G., 2015a. Highly biobased thermally-stimulated shape memory copolymeric elastomers derived from lignin and glycerol-adipic acid based hyperbranched prepolymer. Ind. Crops Prod. 67, 143–154.

Li, H., Sivasankarapillai, G., McDonald, A.G., 2015b. Lignin valorization by forming toughened thermally stimulated shape memory copolymeric elastomers: evaluation of different fractionated industrial lignins. J. Appl. Polym. Sci. 132, 1505–1517.

Li, H.Y., Chen, X., Wang, C.Z., Sun, S.N., Sun, R.C., 2016. Evaluation of the two-step treatment with ionic liquids and alkali for enhancing enzymatic hydrolysis of Eucalyptus: chemical and anatomical changes. Biotechnol. Biofuels 9, 166.

Liu, Z.-H., Qin, L., Pang, F., Jin, M.-J., Li, B.-Z., Kang, Y., Dale, B.E., Yuan, Y.-J., 2013. Effects of biomass particle size on steam explosion pretreatment performance for improving the enzyme digestibility of corn stover. Ind. Crops Prod. 44, 176–184.

Liu, Y., Wang, J., Wolcott, M.P., 2016a. Assessing the specific energy consumption and physical properties of comminuted Douglas-fir chips for bioconversion. Ind. Crops Prod. 94, 394–400.

Liu, Y., Wang, J., Wolcott, M.P., 2016b. Multistep process to produce fermentable sugars and lignosulfonates from softwood enzymolysis residues. ACS Sustainable Chem. Eng. http://dx.doi.org/10.1021/acssuschemeng.6b02328.

Liu, Y., Wang, J., Wolcott, M., 2017. Modeling the production of sugar and byproducts from acid bisulfite pretreatment and enzymatic hydrolysis of Douglas-fir. Bioresour. Technol. 224, 389–396.

Llano, T., Rueda, C., Quijorna, N., Blanco, A., Coz, A., 2012. Study of the delignification of hardwood chips in a pulping process for sugar production. J. Biotechnol. 162, 422–429.

Mais, U., Esteghlalian, A.R., Saddler, J.N., Mansfield, S.D., 2002. Enhancing the enzymatic hydrolysis of cellulosic materials using simultaneous ball milling. Appl. Biochem. Biotechnol. 98–100, 815–832.

Malester, I.A., Green, M., Shelef, G., 1992. Kinetics of dilute acid hydrolysis of cellulose originating from municipal solid wastes. Ind. Eng. Chem. Res. 31, 1998–2003.

Marques, A.V., Pereira, H., Rodrigues, J., Meier, D., Faix, O., 2006. Isolation and comparative characterization of a Bjorkman lignin from the saponified cork of Douglas-fir bark. J. Anal. Appl. Pyrol. 77, 169–176.

Marqués, A.P., Evtuguin, D.V., Magina, S., Amado, F.M.L., Prates, A., 2009. Chemical composition of spent liquors from acidic magnesium-based sulphite pulping of Eucalyptus globulus. J. Wood Chem. Technol. 29, 322–336.

Miura, T., Lee, S.-H., Inoue, S., Endo, T., 2012. Combined pretreatment using ozonolysis and wet-disk milling to improve enzymatic saccharification of Japanese cedar. Bioresour. Technol. 126, 182–186.

Peng, H., Li, H., Luo, H., Xu, J., 2013. A novel combined pretreatment of ball milling and microwave irradiation for enhancing enzymatic hydrolysis of microcrystalline cellulose. Bioresour. Technol. 130, 81–87.

Rahman, S.H.A., Choudhury, J.P., Ahmad, A.L., 2006. Production of xylose from oil palm empty fruit bunch fiber using sulfuric acid. Biochem. Eng. J. 30, 97–103.
Rana, R., Langenfeld-Heyser, R., Finkeldey, R., Polle, A., 2010. FTIR spectroscopy, chemical and histochemical characterisation of wood and lignin of five tropical timber wood species of the family of Dipterocarpaceae. Wood Sci. Technol. 44, 225–242.
Rowell, R.M., Pettersen, R., Han, J.S., Rowell, J.S., Tshabalala, M.A., 2005. Cell wall chemistry. Handbook of Wood Chemistry and Wood Composites, 35–74.
Saeman, J.F., 1945. Kinetics of wood saccharification – hydrolysis of cellulose and decomposition of sugars in dilute acid at high temperature. Ind Eng Chem 37, 43–52.
Sannigrahi, P., Ragauskas, A.J., Miller, S.J., 2008. Effects of two-stage dilute acid pretreatment on the structure and composition of lignin and cellulose in loblolly pine. Bioenergy Res. 1, 205–214.
Sannigrahi, P., Kim, D.H., Jung, S., Ragauskas, A., 2011. Pseudo-lignin and pretreatment chemistry. Energy Environ. Sci. 4, 1306–1310.
Schwanninger, M., Rodrigues, J.C., Pereira, H., Hinterstoisser, B., 2004. Effects of short-time vibratory ball milling on the shape of FT-IR spectra of wood and cellulose. Vib. Spectrosc. 36, 23–40.
Shuai, L., Yang, Q., Zhu, J.Y., Lu, F.C., Weimer, P.J., Ralph, J., Pan, X.J., 2010. Comparative study of SPORL and dilute-acid pretreatments of spruce for cellulosic ethanol production. Bioresour. Technol. 101, 3106–3114.
Silva, G.G.D., Couturier, M., Berrin, J.-G., Buléon, A., Rouau, X., 2012. Effects of grinding processes on enzymatic degradation of wheat straw. Bioresour. Technol. 103, 192–200.
Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., 2006. Determination of sugars byproducts and degradation products in liquid fraction process samples. In: Laboratory Analytical Procedure from the National Renewable Energy Laboratory Biomass Analysis Technology (NREL BAT) Team.
Suhas, Carrott, P.J.M., Ribeiro Carrott, M.M.L., 2007. Lignin – from natural adsorbent to activated carbon: a review. Bioresour. Technol. 98, 2301–2312.
Sun, R.C., Sun, X.F., Fowler, P., Tomkinson, J., 2002. Structural and physico-chemical characterization of lignins solubilized during alkaline peroxide treatment of barley straw. Eur. Polym. J. 38, 1399–1407.
Sun, X.F., Xu, F., Sun, R.C., Fowler, P., Baird, M.S., 2005. Characteristics of degraded cellulose obtained from steam-exploded wheat straw. Carbohyd. Res. 340, 97–106.
Yang, Y., Ji, G., Xiao, W., Han, L., 2014. Changes to the physicochemical characteristics of wheat straw by mechanical ultrafine grinding. Cellulose 21, 3257–3268.
You, T.T., Zhang, L.M., Zhou, S.K., Xu, F., 2015. Structural elucidation of lignin-carbohydrate complex (LCC) preparations and lignin from Arundo donax Linn. Ind. Crops Prod. 71, 65–74.
Yu, Y., Wu, H.W., 2011. Effect of ball milling on the hydrolysis of microcrystalline cellulose in hot-compressed water. AIChE J. 57, 793–800.
Yuan, Z., Long, J., Wang, T., Shu, R., Zhang, Q., Ma, L., 2015. Process intensification effect of ball milling on the hydrothermal pretreatment for corn straw enzymolysis. Energy Convers. Manage. 101, 481–488.
Zakaria, M.R., Hirata, S., Hassan, M.A., 2014. Combined pretreatment using alkaline hydrothermal and ball milling to enhance enzymatic hydrolysis of oil palm mesocarp fiber. Bioresour. Technol. 169, 236–243.
Zhang, W., Liang, M., Lu, C., 2007. Morphological and structural development of hardwood cellulose during mechanochemical pretreatment in solid state through pan-milling. Cellulose 14, 447–456.
Zhou, S., Osman, N.B., Li, H., McDonald, A.G., Mourant, D., Li, C.Z., Garcia-Perez, M., 2013. Effect of sulfuric acid addition on the yield and composition of lignin derived oligomers obtained by the auger and fast pyrolysis of Douglas-fir wood. Fuel 103, 512–523.
Zhuang, X., Yuan, Z., Ma, L., Wu, C., Xu, M., Xu, J., Zhu, S., Qi, W., 2009. Kinetic study of hydrolysis of xylan and agricultural wastes with hot liquid water. Biotechnol. Adv. 27, 578–582.