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Influence of sulfur dioxide-ethanol-water pretreatment on the physicochemical properties and enzymatic digestibility of bamboo residues

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ABSTRACT

SO_2-ethanol-water (SEW) is a promising pretreatment for improving enzymatic digestibility of biomass through simultaneously removing hemicellulose and lignin. In this work, SEW pretreatment was performed at different cooking times (10 min–60 min) and different SO_2 concentrations (0.5%–2%) to produce pretreated bamboo residues for enzymatic hydrolysis. Meanwhile, physicochemical features of the residual cellulose and lignin were analyzed to better understand how SEW improves enzymatic digestibility. Under optimized SEW pretreatment condition (1% SO_2 concentration, 150 °C, 60 min), 81.7% of xylan and 80.3% of lignin were solubilized, along with 89.1% of cellulose preserved in pretreated solid. A good enzymatic digestibility (80.4%) was achieved at optimum SEW condition. Several compelling correlations ($R^2 > 0.7$) were observable between enzymatic digestibility and physicochemical features, demonstrating the importance of SEW pretreatment abilities of hemicellulose and lignin removal, reducing cellulose's degree of polymerization, and improving the amount of sulfonyl groups imparted to the original lignin structure.

1. Introduction

Lignocellulosic biomass, such as forest residues and agricultural wastes, are considered as the sustainable resources for producing green products like bioethanol and other biologically-derived chemicals (Narron et al., 2017). Most biologically-derived chemicals are produced from fermentable sugars, which are liberated from lignocellulosic carbohydrates by a variety of means (Huang et al., 2017). One particularly enticing biomass for sustainable chemical production in Asia is bamboo, because about 46 million tons of bamboo processing residues are produced annually in China (Chand et al., 2006). Bamboo residues are rich in structural polysaccharides capable of being converted into fermentable sugars. On average, bamboo residues are comprised of ~30–50% cellulose and ~20–30% hemicellulose on a dry mass basis (Xin et al., 2015). For these two reasons, bamboo residues are an ideal raw material for the production of biologically-derived chemicals.

Within bamboo plant cell walls, cellulose is amalgamated with hemicellulose and lignin. The extent of biopolymer association within this biological composite is regarded as one of the major obstacles preventing monosaccharide production from structural polysaccharides, resulting in hindered cellulose availability to natural cellulolytic ("cellulase") enzyme systems (Shen et al., 2018). To overcome this hindrance, a pretreatment must be performed, which is intended to disrupt the plant cell wall structure for improving cellulose accessibility to cellulolytic enzymes. A wide variety of pretreatments have been developed, with examples including various configurations of acidic or alkaline pretreatments (Huang et al., 2015a; Shen et al., 2018), hydrothermal (autohydrolysis) pretreatment (Huang et al., 2016; Narron et al., 2017), or organosolv pretreatment (Zhang et al., 2018). With regards to utilizing bamboo, it has been revealed that alkaline pretreatments can improve cellulose accessibility in bamboo plant cell walls for enzymatic saccharification. It was found that acid pretreatment of bamboo is not capable of inducing acceptable amounts of disruption to the bamboo cell wall, hypothesized to be due to a limited extent of lignin degradation (Huang et al., 2015a). Alkaline pretreatment is a better pretreatment because it appears capable of disrupting bamboo cell wall to enable cellulase accessibility based on recently published findings. However, acid-based pretreatments are more practical industry utilization due to the prehydrolyzate can be directly use for fermentation after some treatments. Therefore, developing a practical acid-based pretreatment to accomplish the intensive removal of hemicellulose and lignin is of great importance for sugars

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production from bamboo residues.

SO_2-ethanol-water (SEW) pretreatment has recently developed into a considerable acid-based pretreatment option for practically creating enzyme accessibility to cellulose. SO_2 in the pretreatment liquid phase results in formation of sulfurous acid (H_2SO_3), which catalyzes both hemicellulose hydrolysis and lignin solubilization through sulfonation (Iakovlev and van Heiningen 2012a; You et al., 2016). The ethanol in the SEW pretreatment liquid promotes SO_2 delivery to the biomass as well as serve as a solvent for removing lignin by sulfonation. The effect of this chemical action is nearly simultaneous removal of hemicellulose and lignin, rendering a cellulose-rich solid that has undergone sufficient cellulose degradation to enable effective cellulolytic hydrolysis in downstream operations (Pylkkanen et al., 2015). After SEW pretreatment, cellulose is retained at a near-full yield in the pretreated lignocellulosic biomass, while a reduction of cellulose degree of polymerization (DP) is achieved (Iakovlev and van Heiningen, 2012a). For the reduction of cellulose DP, it can enhance the enzymatic digestibility of pretreated biomass, which have been found in acid pretreated spruce and pine (Martínez et al., 2007), alkaline pretreated *Miscanthus* (Zhang et al., 2013b), steam explosion pretreated cotton stalks (Huang et al., 2015b) and alkaline pretreated corn stalk (Tian et al., 2017). Technically, SEW pretreatment can be considered to be a hybrid pretreatment consisting of the primary elements of both acid sulfite and organosolv pulping (or pretreatment in the case of this work). Advantageous to either of the aforementioned pretreatment methods, SEW results in lower amounts of aldonic acids being produced (a negative of acid sulfite) and the absence of a catalyst requirement (a negative of organosolv). Congruent with the lessened extent of aldonic acid production, SEW pretreatment renders a greater degree of carbohydrates preserved in the spent pretreatment liquor, which improves overall carbohydrate recovery and downstream conversion (Iakovlev and van Heiningen, 2012b). Industrial application of SEW treatment of biomass is currently practiced and termed “American Value Added Pulping (AVAP)” by American Process Inc. AVAP technology is currently demonstrated on a 3 tons per day scale in Thomaston, GA. Although SEW technology has showed strong capability for producing relatively pure cellulosic fibers and monosaccharides from spruce and wheat straw (Iakovlev et al., 2014). To our knowledge, no work has evaluated if SEW pretreatment is suitable for bamboo residues in a biorefinery-type process applying sequential pretreatment and enzymatic hydrolysis operations.

In attempt to investigate bamboo residue’s response to SEW pretreatment in a biorefinery system, we performed a systematic study of the effects of SEW pretreatment at different cooking times (10 min–60 min) and SO_2 concentration (0.5%–2%) on removal of hemicellulose and lignin. Following pretreatment, subsequent enzymatic hydrolysis of pretreated substrate was performed in order to gauge the effects of SEW upon cellulose digestibility. Moreover, the properties of both lignin and cellulose in the pretreated solid residues were investigated. Residual solid lignin was characterized for sulfur content and overall hydrophobicity, while the cellulose’s DP was quantified. With these results in hand, correlations were drawn between all the studied variables in order to better understand the elements of SEW pretreatment that are most beneficial towards obtaining fermentable monosaccharides from bamboo residues in a biorefinery process.

2. Materials and methods

2.1. Materials

Bamboo residues used in this work was provided by the He Qi Cang Bamboo Processing Factory, located in Fujian, China. The chemical composition of the bamboo residues was 39.2% glucan, 17.3% xylan, and 32.8% total lignin (on a dry weight basis). Cellulase (Cellic CTec2) enzymes and hemicellulase (Cellic HTec2) enzymes were provided by Novozymes NA, located in Franklinton, USA.

2.2. SEW pretreatment

The liquid phase for SEW pretreatment was prepared prior to pretreatment by injecting gaseous sulfur dioxide into an ethanol-water (55:45, v/v) solution, and the SO_2 concentration was adjusted based on the increase in the weight of the solution (Iakovlev and van Heiningen, 2012a). The reported SO_2 concentrations is based on the amount of SO_2 dosed into the initial ethanol-water solution.

SEW pretreatment was carried out in a 1.0 L Parr reactor. 50 g (oven-dry) of the bamboo residue was weighed and added to the reaction vessel. Next, the pre-prepared SEW liquor was poured into the reactor, with a final liquor-to-dry bamboo ratio of 8:1. SEW liquors with different SO_2 concentration (0.5%, 1%, and 2%) were investigated. Pretreatment was performed at 130 °C and 150 °C for specific time allotments time (10 min, 20 min, 40 min and 60 min). The heat-up time for reaching the target temperature approximately 8–9 min. After pretreatment, the reactor was immediately removed from the heater and cooled in an ice water bath. The pretreatment liquor (prehydrolyzate) was separated from pretreated solids by vacuum filtration. The prehydrolyzate was collected for characterization. Pretreated bamboo residue was washed five times with fresh ethanol-water (55:45, v/v) solution (no SO_2) at 60 °C, followed by five washings with deionized water at room temperature. After washing, the solids were collected and stored at 4 °C prior to further analysis.

2.3. Analysis of sugars and organic byproducts in prehydrolyzate

An aliquot of liquid prehydrolyzate was taken to determine the amount of monosaccharides in SEW prehydrolyzate by an HPLC system. Following monosaccharide quantification, another aliquot of hydrolyzate was hydrolyzed by adding sulfuric acid to obtain a 4% acid concentration and then cooked at 121 °C for 60 min. Monomeric sugar totals after 4% acid hydrolysis were then determined by the same HPLC system used to initially monitor monosaccharides. The amounts of oligosaccharides (glucooligosaccharides and xylooligosaccharides) in the SEW prehydrolyzate were then estimated by taking the difference in monosaccharide concentrations measured in original prehydrolyzate and sulfuric acid hydrolyzed prehydrolyzate. Beyond saccharide quantification, concentrations of acetic acid, formic acid, 5-hydroxymethylfurfural (HMF), and furfural in SEW prehydrolyzate were measured by HPLC system.

2.4. Enzymatic hydrolysis

Enzymatic hydrolysis of the pretreated bamboo residue was conducted at a substrate loading of 5% (w/v) in 50 mM acetate buffer (pH 4.8), and shaken at 180 rpm for 48 h at 50 °C. The enzymes used, a mixture of Cellic CTec2 cellulase and 1/9 v/v Cellic HTec2 hemicellulase, was dosed at the rate of 5 FPU/g OD substrate. After enzymatic hydrolysis, solid and liquid were separated via micro centrifugation for 10 min at 13,000 rpm. The resultant supernatant was next filtered through a 0.22 μm syringe filter and analyzed for monosaccharides content by the previously detailed HPLC system for quantifying sugar concentrations. From these results, enzymatic digestibility of pretreated bamboo was calculated as the following equation:

$$\text{Enzymatic digestibility}\,(\%) = \frac{\text{glucose in enzymatic hydrolyzate}\,(\text{g})}{\text{initial glucan in pretreated solid}\,(\text{g}) * 1.11} \times 100\%$$

2.5. Determining the degree of polymerization of cellulose in pretreated bamboo residue

The degree of polymerization (DP) of cellulose in pretreated bamboo residue was calculated as its intrinsic viscosity in

Table 1
Effects of SEW pretreatment on chemical composition of bamboo residues.*

Temp. (°C)	Time (min)	SO_2 concentration (%, w/v)	Recovery yield (%)		Removal yield (%)	
			Solid	Glucan	Xylan	Lignin
130	60	0	90.8 ± 1.2	99.3 ± 0.2	28.2 ± 0.4	19.6 ± 0.5
	60	0.5	58.6 ± 0.5	97.9 ± 0.5	49.2 ± 0.3	72.5 ± 08
	60	1.0	56.1 ± 0.2	96.3 ± 0.9	56.3 ± 0.8	73.5 ± 0.1
	60	2.0	50.4 ± 2.3	94.9 ± 0.4	67.4 ± 0.5	83.1 ± 1.5
	10	1.0	77.2 ± 3.5	98.4 ± 0.2	17.3 ± 1.5	38.4 ± 0.8
	20	1.0	66.1 ± 1.8	97.9 ± 0.5	34.6 ± 2.3	58.3 ± 2.5
	40	1.0	60.4 ± 1.1	97.4 ± 0.1	48.0 ± 1.5	65.6 ± 1.3
	60	1.0	56.1 ± 0.2	96.3 ± 0.9	56.3 ± 0.8	73.5 ± 0.1
150	60	0	89.0 ± 2.8	96.3 ± 0.9	33.9 ± 1.2	14.9 ± 1.6
	60	0.5	50.9 ± 1.1	89.6 ± 1.5	74.8 ± 0.9	78.5 ± 1.5
	60	1.0	48.3 ± 0.1	89.1 ± 0.2	81.7 ± 0.0	80.3 ± 0.4
	60	2.0	43.7 ± 1.7	86.8 ± 1.3	85.4 ± 1.8	89.2 ± 1.3
	10	1.0	60.6 ± 0.4	97.6 ± 0.5	58.9 ± 0.1	64.2 ± 0.2
	20	1.0	56.0 ± 1.3	97.1 ± 0.3	72.3 ± 0.3	70.2 ± 1.1
	40	1.0	50.1 ± 2.6	91.8 ± 2.0	78.8 ± 1.6	77.4 ± 0.9
	60	1.0	48.3 ± 0.1	89.1 ± 0.2	80.3 ± 0.0	80.3 ± 0.4

* The mean value and deviation of duplicate experiments are report.

cupriethylenediamine (CED) solution based on the following equation (da Silva Perez and van Heiningen, 2002):

$$DP = \left(\frac{1.65[\eta] - 116[Xyl]solid}{[Glu]solid}\right)^{1.111}$$

Where $[\eta]$ is the intrinsic viscosity of pretreated bamboo residue in CED solution, ml/g, $[Xyl]_{solid}$ is the xylan content of pretreated bamboo residue, unit fraction, and $[Glu]_{solid}$ is the glucan content of pretreated bamboo residue, unit fraction.

2.6. Measuring degree of sulfonation in pretreated bamboo's residual lignin

To determine the degree of sulfonation within the pretreated solid lignin, the sulfur content (wt %) of pretreated bamboo residues was analyzed using a Perkin Elmer 2400 CHNS/O Elemental Analyzer with a method in accordance with Novozamsky et al. (1986). From this information, the degree of sulfonation was calculated based on following equation (Rydholm, 1965):

$$\text{Lignin degree of sulfonation} = \left(\frac{\text{Sulfur content \%}}{\text{Lignin content \%}} \times \frac{190\,\text{g/mol}}{32\,\text{g/mol}}\right) \times 100\%$$

Where lignin content is the amount of lignin (wt %) in pretreated bamboo residue, 190 g/mol is the assumed molecular weight of one lignin unit, and 32 g/mol is the molecular weight of elemental sulfur.

2.7. Estimating the hydrophobicity of pretreated bamboo residues by Rose Bengal

The hydrophobicity of the pretreated bamboo residue was estimated by measuring the distribution of Rose Bengal reagent (hydrophobic dye) in solution and adsorbed to the substrate. A range of substrate (pretreated bamboo residue) concentration (2–10 g/L) was added into 50 mM citrate buffer (pH 4.8) with a constant concentration of Rose Bengal (40 mg/L). The mixture suspension was incubated at 50 °C and 150 rpm for 2 h for the hydrophobic dye to adsorb on substrate's hydrophobic surfaces. After incubation, the substrate and supernatant were separated by centrifugation. The free dye content in the supernatant was measured by UV–Vis spectrometer at 543 nm. The adsorbed dye in the substrate was calculated by the difference between the initial dye content and the free dye content in the supernatant. Partition quotient (PQ) was calculated from the ratio of the adsorbed dye over the free dye. Finally, the obtained PQ was plotted against substrate content, and the slope from the linear plotting was taken as the surface hydrophobicity of pretreated bamboo residue (L/g).

2.8. Analytical methods

The composition of bamboo residue and pretreated samples were determined based on the procedure developed by the National Renewable Energy Laboratory for analyzing biomass materials (Sluiter et al. 2011).

The sugars in the composition analysis, prehydrolyzate, and enzymatic saccharification were measured using high-performance liquid chromatography (HPLC) system (Agilent, 1200 Series) equipped with a Shodex SP-0810 column (300 × 8 mm), degasser, pump, and refractive index (RI) detector. HPLC grade milli-Q water was used as the eluent at a flow rate of 0.5 mL/min at a column temperature of 80 °C. The acetic acid, formic acid, hydroxymethylfurfural (HMF), and furfural in prehydrolyzate were measured using an HPLC system (Dionex, U-3000 system) equipped with Aminex HPX-87H column (300 × 7.8 mm) and refractive index (RI) detector. The separation was performed with 5 mM H_2SO_4 as the eluent at a flow rate of 0.6 mL/min.

$$\text{Recovery of glucan (\%)} = \frac{\text{glucan in pretreated bamboo residue (g)}}{\text{glucan in the raw bamboo residue (g)}} \times 100\%$$

$$\text{Removal of lignin or xylan (\%)} = 1 - \frac{\text{lignin or xylan in pretreated bamboo residue (g)}}{\text{lignin or xylan in raw bamboo residue (g)}} \times 100\%$$

3. Results and discussion

3.1. Influence of SEW on the composition of bamboo residues

To investigate bamboo residue's susceptibility to SEW pretreatment, bamboo residues were pretreated by SEW at different SO_2 concentration (0.5%, 1%, and 2%) under various temperatures (130 °C and 150 °C), and for particular lengths of time (10, 20, 40, and 60 min). The effects of these parameters on solid recovery and chemical composition of pretreated bamboo residues are investigated and shown in Table 1.

According to Table 1, the recovery of solid residues decreased from 90.8% to 50.4% from 0% to 2% SO_2 dosage at 130 °C for 60 min. Demonstrating the effects of increased temperature, solid recovery ranged from 89.0% to 43.7% at 0% to 2% SO_2 when pretreatment was performed at 150 °C for 60 min. The clear decreases to solid recoveries as a function of SO_2 dosages and pretreatment temperatures can be attributed to the intended solubilization of hemicellulose and lignin

degradation, also displayed in Table 1. For example, when the SO_2 concentration of the SEW liquor increased from 0% to 2% (150 °C), the degree of delignification increased from 14.9% to 89.2% and xylan removal increased from 33.9% to 85.4%. In addition, prolonging pretreatment time (10 min to 60 min) also resulted in greater extents of lignin and xylan removal. It was found that the removal of lignin and xylan are 38.4% and 17.3% (respectively) when bamboo residues are pretreated at 130 °C with 1% SO_2 for 10 min. However, when the pretreatment time was increased from 10 min to 60 min, lignin and xylan removal was seen to increase to 73.5% and 56.3%, respectively. For the xylan removal, it is due to the acid degradation of hemicellulose during SEW pretreatment. The behavior of delignification by SEW pretreatment is attributed to the sulfonation of lignin during pretreatment, which can solubilize lignin in the prehydrolyzate and result the residual lignin in pretreated substrate within some degree of sulfonation (Iakovlev and van Heiningen, 2012a,b). The cumulative effect of these results is demonstration that SEW pretreatment exhibits excellent performance upon bamboo residues with respect to lignin and xylan removal, intended to improve cellulose accessibility to hydrolyzing enzyme systems (Subhedar and Gogate, 2013).

3.2. Effect of SEW pretreatment on the sugars and by-products in SEW prehydrolyzate

Quantities of carbohydrates and other organic byproducts in SEW prehydrolyzate is presented in Table 2. All results are reported in the units of grams of product per 100 g of raw oven dry bamboo residues. Firstly, it can be observed that the quantity of monosaccharides (glucose and xylose) increase with increasing pretreatment times and SO_2 charges. For the pretreatments performed using 0% SO_2 at 130 °C and 150 °C for 60 min, only 0.4 g (0.1 g + 0.3 g) and 1.2 g (0.3 g + 0.9 g) of total monosaccharides are produced. This amount is much lower compared to the monosaccharide quantities produced in pretreatment with 2% SO_2 charge at the same time and temperature regime. Specifically, the total monosaccharide quantities are 6.1 g (0.8 g + 5.3 g) and 7.4 g (0.2 g + 7.2 g), respectively. We believe this is related to the higher acidity of SEW liquors containing higher amounts of SO_2, leads to extended hydrolysis of oligosaccharides to form monosaccharide products (Iakovlev and van Heiningen, 2012b). In addition, it can be observed in Table 2 that xylan (monomeric and oligomeric) are the dominant carbohydrate product found in SEW prehydrolyzates. This demonstrates that SEW is effective in depolymerizing hemicellulose. The glucose found in solution may come from partial cellulose degradation, but it is more likely to come from hemicelluloses presenting in bamboo. Exact delineation between hemicellulosic and cellulosic glucose is not able to be surmised from the data obtains.

The total quantity of monomeric (glucose and xylose) and oligomeric (glucooligosaccharides and xylooligosaccharides) carbohydrates in prehydrolyzate are in the range of 0.4 g–7.5 g and 1.4 g–9.5 g, respectively. These results demonstrate that SEW pretreatment is capable of producing ~30% monomeric sugars from the dissolved cellulose and hemicellulose, suggesting that SEW prehydrolyzate alone may be an excellent feedstock for fermentation schemes (Sklavounos et al., 2011). For the oligomeric carbohydrates, they are widely used as a food additive for the selective stimulation of the growth of intestinal bacteria that contributes to human health, especially oligomeric carbohydrates with low degree of polymerization (Moure et al., 2006). Hence, it is important to note that the production of monomeric and oligomeric carbohydrates comes in addition to the beneficial effect for the SEW pretreatment.

In addition to sugars, SEW pretreatment also results in the formation of the organic byproducts acetic acid, formic acid, HMF, and furfural. Production of acetic acid is due to hydrolysis of hemicellulosic acetyl esters, resulting in acetic acid being transferred to the liquid phase. Formic acid, furfural, and HMF are byproducts most associated with acid-catalyzed carbohydrate dehydration. Furfural is formed when pentoses dehydrate, and HMF is formed from hexose dehydration (Girisuta et al., 2006; Iakovlev and van Heiningen, 2012b). As shown in Table 2, furfural and formic acid are the dominant byproducts found in the prehydrolyzate. In addition, it can be observed that generation of these compounds is more pronounced with increasing pretreatment times and SO_2 concentrations. These byproducts are considered to be negative towards the effort to produce high quantities of monosaccharides for two reasons: 1) their existence alone indicates that potent monosaccharide products were lost and therefore maximum monosaccharide production cannot be achieved, and 2) it is reported that these byproducts can be inhibitory towards both downstream enzyme hydrolysis or fermentation (Bellido et al., 2011). The presence of these byproducts in high quantities in SEW prehydrolyzate suggests that detoxification of prehydrolyzate will be necessary to promote optimal fermentation yields using monosaccharides (Lai et al., 2016).

3.3. Enzymatic hydrolysis of SEW pretreated bamboo residues

Fig. 1 displays the effects of each conducted SEW pretreatment on the subsequent enzymatic digestibility of the pretreated bamboo residues. As shown in Fig. 1a, enzymatic conversion of residual cellulose to glucose increases with increasing SO_2 concentrations. When SEW

Table 2
Sugars and by-products in the prehydrolyzate of bamboo residues pretreated by SEW.*

Temp.(°C)	Time (min)	SO_2 concentration (%, w/v)	Sugar in prehydrolyzate (g/100 g raw material)				By-products in prehydrolyzate (g/L)			
			Glucose mono.	Glucose oligo.	Xylose mono.	Xylose oligo.	Acetic acid	HMF	Furfural	Formic acid
130	60	0	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	1.3 ± 0.2	0.0 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
	60	0.5	0.2 ± 0.0	0.5 ± 0.1	2.5 ± 0.2	3.8 ± 0.6	0.4 ± 0.0	0.3 ± 0.0	0.7 ± 0.1	1.6 ± 0.9
	60	1.0	0.6 ± 0.1	0.8 ± 0.1	4.5 ± 0.5	5.2 ± 0.0	0.6 ± 0.1	0.5 ± 0.1	0.8 ± 0.2	1.9 ± 0.1
	60	2.0	0.8 ± 0.1	0.7 ± 0.2	5.3 ± 0.1	4.8 ± 0.1	0.5 ± 0.2	0.5 ± 0.2	0.9 ± 0.0	2.0 ± 0.0
	10	1.0	0.2 ± 0.1	0.4 ± 0.0	0.6 ± 0.0	1.8 ± 0.2	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.9 ± 0.1
	20	1.0	0.3 ± 0.0	0.4 ± 0.0	2.4 ± 0.1	2.9 ± 0.4	0.5 ± 0.1	0.2 ± 0.0	0.6 ± 0.1	1.0 ± 0.0
	40	1.0	0.4 ± 0.0	0.6 ± 0.1	3.3 ± 0.3	4.2 ± 0.4	0.8 ± 0.0	0.4 ± 0.3	1.1 ± 0.1	1.3 ± 0.2
	60	1.0	0.6 ± 0.1	0.8 ± 0.1	4.5 ± 0.5	5.2 ± 0.0	0.6 ± 0.1	0.5 ± 0.1	1.0 ± 0.2	1.9 ± 0.1
150	60	0	0.3 ± 0.0	0.9 ± 0.0	0.9 ± 0.1	1.4 ± 0.2	0.1 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.2
	60	0.5	1.2 ± 0.1	2.2 ± 0.2	5.4 ± 0.5	4.8 ± 1.1	0.5 ± 0.2	0.7 ± 0.2	2.0 ± 0.2	2.0 ± 0.1
	60	1.0	1.2 ± 0.3	2.4 ± 0.2	6.3 ± 0.2	7.1 ± 0.9	1.0 ± 0.0	0.9 ± 0.0	2.5 ± 0.7	2.3 ± 0.4
	60	2.0	0.2 ± 0.0	2.1 ± 0.0	7.2 ± 0.6	5.9 ± 1.2	0.8 ± 0.4	1.0 ± 0.1	2.9 ± 0.1	2.6 ± 0.1
	10	1.0	0.2 ± 0.0	0.6 ± 0.0	2.6 ± 0.2	4.1 ± 0.2	0.5 ± 0.0	0.4 ± 0.0	1.0 ± 0.1	1.1 ± 0.1
	20	1.0	0.4 ± 0.0	0.6 ± 0.1	3.2 ± 0.1	4.8 ± 0.4	0.7 ± 0.1	0.5 ± 0.2	1.6 ± 0.0	1.5 ± 0.3
	40	1.0	0.8 ± 0.1	2.0 ± 0.2	3.7 ± 0.0	5.9 ± 0.8	1.0 ± 0.2	0.6 ± 0.0	2.5 ± 0.9	1.9 ± 0.1
	60	1.0	1.2 ± 0.3	2.4 ± 0.2	6.3 ± 0.2	7.1 ± 0.0	1.2 ± 0.0	0.8 ± 0.0	2.5 ± 0.7	2.3 ± 0.1

* The mean value and deviation of duplicate experiments are reported.

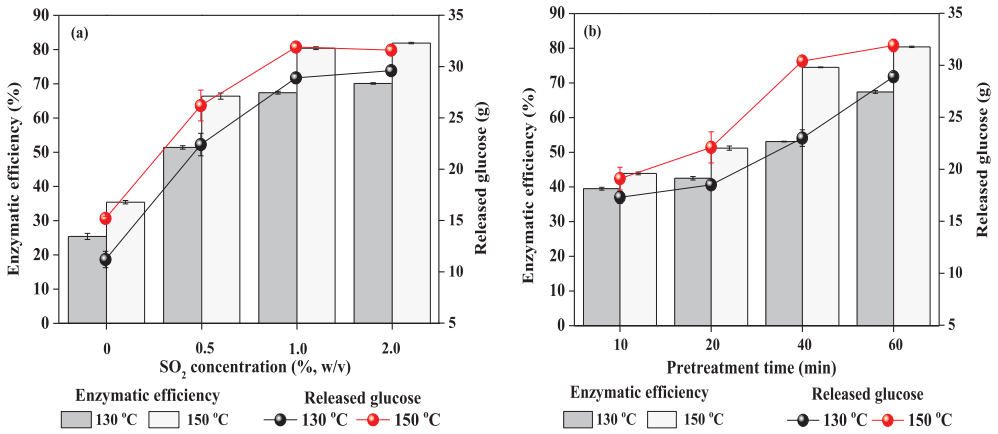

Fig. 1. Effects of SEW pretreatment on enzymatic digestibility and released glucose from 100 g original bamboo residues (combining SEW pretreatment and enzymatic hydrolysis processes).

was performed without SO_2, enzymatic conversion of bamboo residues pretreated at 130 °C and 150 °C was found to be 25.4% and 35.4%, respectively. Increasing the SO_2 concentration to 2%, the greatest extents of enzymatic digestibility were 70.1% and 81.9% at 130 °C and 150 °C, respectively. Regarding time, increasing pretreatment time significantly enhanced the enzymatic digestibility of all 2% SO_2-pretreated bamboo residues. Fig. 1b shows that prolonging pretreatment time from 10 min to 60 min improved enzymatic digestibility by 22.9% and 36.5% at 130 °C and 150 °C, respectively. Similar to what was discussed for carbohydrates in the prehydrolyzate, we attribute this improvement in digestibility to extended lignin and hemicellulose removal at longer SEW pretreatment times, resulting in a more accessible residual cellulose (Rollin et al., 2011).

Enzymatic digestibilities were correlated with both lignin and xylan removal, shown in Fig. 2a. Firstly, enzymatic digestibility of pretreated bamboo residues was found to positively correlate with extent of xylan removal, with a rough linear correlation ($R^2 = 0.76$). A possible explanation for this is that removal of hemicellulose may result in formation of pores which expose the residual solid cellulose. Zhang et al. (2013a) also reported that xylan removal had a strong positively impact on improving cellulose accessibility to cellulases. In Fig. 2b, it can be seen that the degree of delignification make positive contribution for the enzymatic digestibility of SEW pretreated bamboo residues, which can be approximately described by a sigmoidal function. Specifically, a gentle change in the enzymatic digestibility (25.4%–43.9%) slope was fitted between the degree of delignification (14.9%–64.2%). While, a rapid change in the enzymatic digestibility (51.4%–81.9%) slope was between the degree of delignification from 65.6% to 89.2%. Li et al., (2012) also reported that the lignin removal showed a sigmoidal function relationship with the enzymatic digestibility of pretreated grass. Regarding delignification, it is speculated that removal of lignin discourages non-productive binding of cellulase to lignin-rich surfaces on the substrate (Lou et al. 2013a). It should be noted that increasing SO_2 concentrations from 1% to 2% improved delignification from 73.5% to 83.1% and from 80.3% to 89.2% at 130 °C and 150 °C, respectively. At these conditions, the ~10% increase in delignification did not result in a significant increase in enzymatic digestibility (less than 4%). One plausible explanation for this diminutive increase in digestibility is that the degree of extensive delignification may result in collapse of the newly-formed pores, which lowers substrate surface area and inadvertently harms enzymatic productivity (Yu et al., 2011).

In this work, a maximum yield of enzymatic saccharification (81.9%) could be achieved from the bamboo residues pretreated at 150 °C with 2% SO_2 charge for 60 min. While, an "ideal" pretreatment would not only able to produce a readily digestible substrate (high yield of enzymatic saccharification) with low content of lignin and hemicellulose, but also enable maximum recovery of the cellulose in substrate for releasing glucose by enzymes (Li et al. 2014). Hence, the total amount of released sugars from pretreated substrates after enzymatic hydrolysis should be calculated to evaluate the SEW pretreatment, based on 100 g original bamboo residues. When combining pretreatment and enzymatic hydrolysis, it was found a large difference in enzyme-produced glucose took place when SO_2 concentration increase from 0% to 1% at the same pretreatment time. As shown in Fig. 1a, increasing from 1% to 2% SO_2 charge did not result in a similarly drastic increase. For example, increasing the SO_2 concentration from

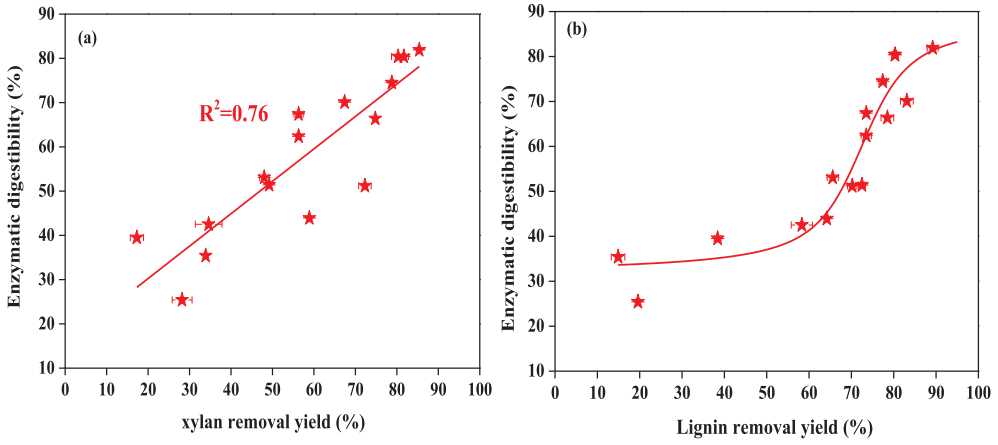

Fig. 2. The respective relationship between removal yield of lignin and xylan and enzymatic digestibility of pretreated bamboo residues.

Table 3
Physicochemical properties of bamboo residues pretreated by SEW.*

Temp. (°C)	Time (min)	SO_2 concentration (%, w/v)	Cellulose DP	Lignin degree of sulfonation (mol S/mol C_9)	Hydrophobicity (L/g)[b]
130	60	0	/[a]	0.00 ± 0.00	2.1 ± 0.00
	60	0.5	4969 ± 101	0.08 ± 0.01	1.4 ± 0.05
	60	1.0	3686 ± 85	0.10 ± 0.00	1.3 ± 0.04
	60	2.0	3277 ± 284	0.18 ± 0.01	0.9 ± 0.08
	10	1.0	/ [a]	0.01 ± 0.00	1.9 ± 0.00
	20	1.0	5123 ± 105	0.02 ± 0.01	1.5 ± 0.09
	40	1.0	4607 ± 135	0.07 ± 0.01	1.5 ± 0.04
	60	1.0	3686 ± 85	0.10 ± 0.00	1.3 ± 0.04
150	60	0	4547 ± 131	0.00 ± 0.00	1.7 ± 0.03
	60	0.5	3887 ± 103	0.09 ± 0.02	1.1 ± 0.11
	60	1.0	3184 ± 57	0.16 ± 0.02	0.9 ± 0.05
	60	2.0	2590 ± 94	0.20 ± 0.01	0.5 ± 0.05
	10	1.0	4551 ± 112	0.03 ± 0.00	1.6 ± 0.01
	20	1.0	4104 ± 69	0.04 ± 0.01	1.4 ± 0.02
	40	1.0	3506 ± 127	0.09 ± 0.01	1.1 ± 0.00
	60	1.0	3184 ± 57	0.16 ± 0.02	0.9 ± 0.05

[a] Pretreated bamboo residues cannot be dissolved in cupriethylenediamine (CED) solution.
[b] Estimated by measuring the distribution of Rose Bengal reagent.
* The mean value and deviation of duplicate experiments are reported.

0% to 1% improved the released glucose amount from 11.2 g to 28.9 g at 130 °C and from 15.2 g to 31.9 g at 150 °C, representing positive increases of 17.7 g and 16.7 g glucose, respectively. However, increasing the SO_2 concentration from 1% to 2% only raised glucose production by ~ 0.3–0.6 g. Therefore, from this set of experiments, the SEW pretreatment of 1% SO_2 charge, 150 °C and 60 min is considered as optimal for providing the greatest amount of total glucose productions from pretreated bamboo residues, with an enzymatic digestibility of 80.4%.

3.4. The relationship between cellulose degree of polymerization and enzymatic digestibility of SEW pretreated bamboo residues

Cellulose DP is regarded as another major influencer for the enzymatic digestibility of lignocellulosic biomass. For this reason, we have determined the DP of residual solid cellulose in all of the pretreated bamboo residues to better understand the effects of SEW pretreatment on bamboo residues. As can be seen in Table 3 and Fig. 3a, elevated SO_2 concentrations and pretreatment times lead to a predictable decrease in cellulose DP. Overall, cellulose DP in pretreated substrates ranged from ~2600 to 5100. This can be attributed to the acidic conditions generated at severe SEW pretreatment conditions, which leads to disruption of cellulose's crystalline structure and hydrolysis of glycosidic bonds within cellulose (You et al., 2016). However, because the DP of the pretreated cellulose chains is still over 1000, it can be surmised that most of the cellulose is preserved by SEW pretreatment and its chemical degradation resulted in minimal loss of carbohydrates. As shown in Table 1, ~95% of the original glucan was found to be retained in bamboo residues pretreated at 130 °C. This result is in agreement with Iakovlev and van Heiningen (2012a), who claim that decreasing DP of cellulose in spruce biomass did not translate to loss of glucan to glucose to HMF and other associated non-carbohydrate byproducts. Interestingly, increasing pretreatment temperature to 150 °C resulted in lower glucan recovery (~85%), suggesting that cellulose exhibits moderate temperature sensitivity in the SEW pretreatment environment. The results indicate that bamboo cellulose does not appreciably degrade under all applied SEW, despite the significant reduction in cellulose DP.

Another significant observation regarding residual cellulose DP is that a correlation was found relating lowered cellulose DPs to enzymatic digestibility (Fig. 3a) of pretreated bamboo residues. This correlation (R^2 = 0.81) between DP reduction and enzymatic digestibility was observed across all SEW pretreated samples. These results indicate that reduction in cellulose DP is favorable towards enzymatic digestibility, and plays to the idea that pretreatment not only exposes cellulose but also makes its fiber network much less recalcitrant towards enzyme access (Arantes and Saddler, 2010). From this, it can be deduced that reducing cellulose DP is another important factor controlling the enzymatic digestibility of bamboo residues pretreated by SEW.

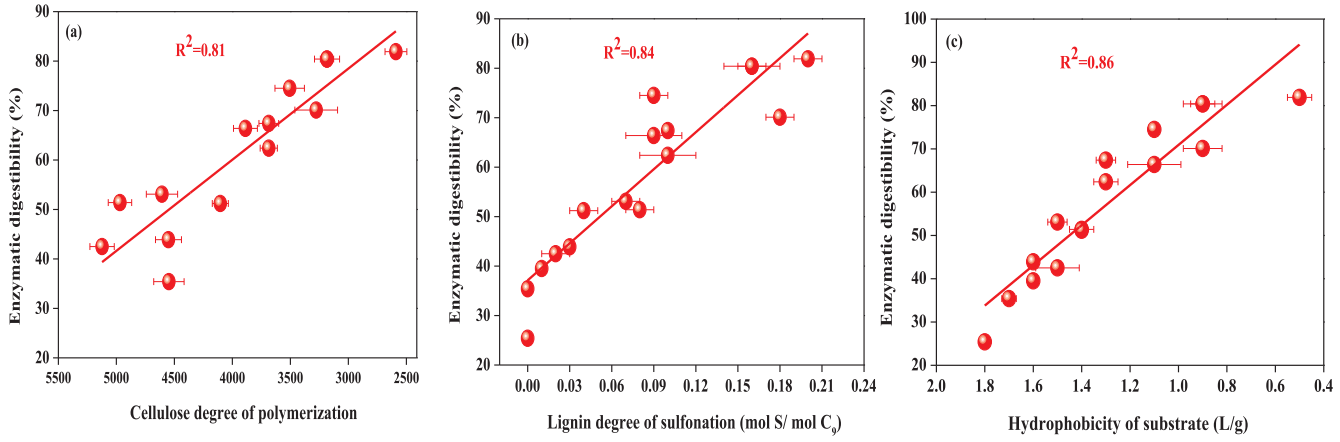

Fig. 3. The relationship between cellulose degree of polymerization (a), lignin degree of sulfonation (b), substrate hydrophobicity (c) and enzymatic digestibility of pretreated bamboo residues.

3.5. The relationship between lignin degree of sulfonation and enzymatic digestibility of SEW pretreated bamboo residues

It is agreed that lignin in pretreated solids can still inhibit enzymatic productivity through the mechanism of non-productively adsorbing enzymes to the pretreated surface (Nakagame et al., 2011). However, Wang et al., (2015) reported that if a hydrophilic moiety can be introduced to the residual lignin (e.g. sulfonyl groups), then this mechanism can be nullified. According to the previous work of You et al., (2016) residual lignin in SEW treated lignocellulosic biomass is partially sulfonated by SO_2. For this reason, we evaluated the residual lignin in pretreated bamboo residue for sulfur content in attempt to relate it to enzymatic digestibility.

It is evident from Table 3 that the degree of sulfonation within the residual lignin in pretreated bamboo residues increases with the increasing SO_2 concentration and pretreatment time. For instance, the degree of sulfonation increases from 0.08 to 0.18 mol S/ mol C_9 when increasing SO_2 concentrations from 0.5% to 2% at 130 °C. A possible explanation for the higher S/C_9 ratio at higher SO_2 concentration is that the rate of removal of sulfonated lignin by diffusion out of the fibre walls affects the amount of lignin sulfonation within the fibre itself, a mechanism previously reported by Iakovlev and van Heiningen (2012a). Overall, Table 3 shows that the degrees of sulfonation across all SEW pretreated samples are from 0.01 mol S/mol C_9 to 0.2 mol S/ mol C_9.

In order to understand the impact of residual lignin sulfonation on enzymatic hydrolysis, a linear fitting was constructed. From the plot (Fig. 3b), it was found that a good correlation between degree of sulfonation and enzymatic digestibility exists (R^2 = 0.84). One potential explanation for this observation is that the sulfonated residual lignin exhibits lessened inhibitory properties towards cellulase systems, and may even improve their kinetics around the substrate surfaces (Wang et al., 2013). Based on these findings, it can be inferred that the residual lignin being sulfonated is also a determinant factor for improving the enzymatic digestibility of bamboo residues pretreated by SEW.

3.6. The relationship between substrate hydrophobicity and enzymatic digestibility of SEW pretreated bamboo residues

To further gauge residual lignin's effect on enzymatic digestibility, the residual lignin was evaluated for hydrophobicity. This was performed in light of previous discussion around hydrophobic lignin inhibiting enzymes through non-productive binding (Wu et al., 2018). In order to understand the hydrophobicity of pretreated bamboo residues, the hydrophobicity of all SEW pretreated samples was determined using an assay involving application of a hydrophobic dye (Rose Bengal). Once evaluated, the relationship between hydrophobicity and enzymatic digestibility of pretreated bamboo residues was probed (Wu et al., 2018).

The results in Table 3 clearly indicate a decrease in hydrophobicity alongside increasing SO_2 concentrations and pretreatment times. It is worth noting that the hydrophobicity of pretreated bamboo residues is also shown to decrease with increasing degrees of residual lignin sulfonation. For example, when the degree of sulfonation increased from 0.08 mol S/mol C_9 to 0.18 mol S/mol C_9 at 130 °C, the hydrophobicity of SEW pretreated substrate decreased from 1.4 L/g to 0.9 L/g. These results indicate that the hydrophobicity of pretreated bamboo residues is indeed changed by sulfonation during SEW pretreatment, in line with discussion in the previous section. Lou et al. (2013b) also reported that introduction of sulfonic groups in lignin significantly reduces the hydrophobicity of a lignocellulosic substrate, causing a reduction in binding strength between lignin and cellulase enzymes that correlated with improvement in enzymatic hydrolysis yields.

As shown in Fig. 3c a favorable correlation (R^2 = 0.86) was able to be fitted between hydrophobicity of variously SEW pretreated bamboo residues and corresponding enzymatic digestibility. This supports the belief that a less hydrophobic lignin will induce a lower extent of non-productive binding to cellulases. Wang et al., (2015) and Zhu et al., (2009) also reported that the residual lignin in sulfite-pretreated substrate was partially sulfonated, resulting in a weakening to non-productive binding of cellulase and the partially sulfonated residual lignin.

4. Conclusion

SEW pretreatment was demonstrated to efficiently pretreat bamboo residues by simultaneously solubilizing hemicellulose and lignin while leaving nearly all of the original cellulose in the pretreated solids. After SEW pretreatment, excellent enzymatic digestibility (over 80%) could be achieved for the bamboo residues, with the optimal pretreatment conditions. After characterizing the physicochemical properties of the pretreated solids, it was found that each of the following correlated with improved enzymatic digestibility: 1) removal of hemicellulose and lignin, 2) decreasing residual cellulose's DP, 3) degree of sulfonation within the residual solid lignin, and 4) hydrophobic character of the residual solid lignin.

Acknowledgements

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