Wet Oxidation Pretreatment of Wood Pulp Waste for Enhancing Enzymatic Saccharification

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Effective pretreatment of wood pulp waste is important for enhancing enzymatic saccharification. For this reason, wet oxidation process conditions were considered with the hypothesis that the alkaline oxygen conditions would favor delignification and hydrolysis of lignocellulose. Enzymatic saccharification was greatly improved to 42.9% in terms of reducing sugar yield under the conditions of pH = 10, oxygen pressure = 1.2 MPa, time = 15 min, and temperature = 195 °C. A total of 39% of lignin and 73% of hemicellulose were removed and dissolved into the hydrolyzate. Furthermore, the chemical structure, crystallinity, and morphology of the treated substrate were characterized by Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD), and scanning electron microscopy (SEM).

Keywords: Wet oxidation; Wood pulp waste; Enzymatic saccharification; Reducing sugar yield; Delignification; Hydrolysis

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INTRODUCTION

Because of the scarcity of global fossil resources, alternative energy resources are the dominant platform for stable and sustainable development, especially for developing countries (Ragauskas *et al.* 2006). With the advantages of being easily available, less expensive, and renewable, lignocellulose, wood pulp waste in particular, is a potential candidate to produce bio-energy and bio-chemicals through the conversion process (Dodds and Gross 2007; Hubbe 2015).

Typically, the conversion process includes acid/enzymatic hydrolysis of polymeric carbohydrates, fermentation of monomer sugars, and distillation of products. However, the complex structural and chemical mechanisms of lignocellulose (known as recalcitrance) hamper the conversion process (Himmel *et al*. 2007). Therefore, pretreatment of lignocellulose has been considered a prerequisite to deconstruct the compact cell wall and liberate the cellulose. To date, various pretreatment technologies have been developed (Eggeman and Elander 2005; Zhu *et al*. 2010; Wyman *et al*. 2011; Ioelovich and Morag 2012; Chen *et al*. 2014), such as dilute acid, hot water, ammonia fiber explosion (AFEX), and ammonia recycle percolation (ARP).

Wet oxidation pretreatment is a new technology using oxygen and alkali as the reaction media. It is known that alkali is favorable for the removal of lignin and xylan side chains, leading to a dramatic increase in enzymatic saccharification. On the other hand, the addition of oxygen could further improve the delignification of lignocellulose. Therefore, the combination of alkali and oxygen could enhance the enzymatic reaction. In one study, rape straw was pretreated by wet oxidation for ethanol production, and it was observed that the highest ethanol yield of 67% was obtained under conditions of 205 ºC for 3 min with 12 bar of oxygen gas pressure (Arvaniti *et al.* 2012). In another study, the superior overall glucose yields was also provided from wet oxidation pretreatment of wheat straw in comparison of alkaline hydrolysis, steaming, and ammonia pretreatment (Klinke *et al.* 2012). Furthermore, the scale-up potential of wet oxidation pretreatment is promising because it uses similar equipment to that required in the oxygen delignification process, which has been used extensively in pulp bleaching.

In this study, wood pulp waste was used to produce fermentable sugars. The effect of wet oxidation was evaluated in terms of reducing sugar yield (RSY) and solids recovery. The chemical composition and structure of the treated substrate were analyzed to illustrate the mechanism of wet oxidation. X-ray diffraction (XRD) and scanning electron microscopy (SEM) were also included to study the crystallinity and morphology changes of the substrate.

EXPERIMENTAL

Materials

Wood pulp waste was provided by Shandong Chenming Paper Co., Ltd. (China) from the preconditioning refiner chemical alkaline peroxide mechanical pulp (P-PC APMP) production process. It was used as received without grinding. Cellulase L-10 was supplied by KDN Biotech Group Co., Ltd. (Qingdao, China) with an activity of 150 filter paper units (FPU)/mL.

Methods

Pretreatment

Wet oxidation was performed in a 3-L stainless reactor with heating and rotation. Sixty grams of raw material (oven dried basis) were mixed into 1 L of distilled water in the reactor. Four factors, *i.e.*, pH (7, 8, and 10, adjusted by Na_2CO_3), oxygen pressure (0.4, 0.8, 1.0, and 1.2 MPa), reaction time (5, 10, 15, and 20 min), and temperature (165, 175, 185 and 195 °C), were selected to optimize the pretreatment. Once the reaction was completed, the reactor was removed immediately and cooled to room temperature. Then, the substrate was collected after vacuum filtration; the hydrolyzate was also collected for further analysis.

Enzymatic saccharification

A shaking water bath (Zhicheng Co., Shanhai) was used for the enzymatic saccharification. Based on a previous study (Liu *et al*. 2011), the following conditions were selected: substrate concentration of 2% (w/v), pH of 4.8, cellulase dosage of 35 FPU/g, 50 °C, and 48 h. At the end of the reaction, the hydrolyzate and residuals were collected though vacuum filtration.

Chemical analysis

Solids recovery was calculated based on the division of collected pretreated substrate and input raw material.

Reducing sugar released during enzymatic hydrolysis was determined using the DNS method at a wavelength of 540 nm (Pala *et al*. 2001). Reducing sugar yield (RSY) was calculated using Eq. 1,

$$\mathrm{RSY} = \frac{CV}{m(1-\omega/100)} \times n \times 100\% \tag{1}$$

where m is the weight of the substrate (g), ω is the moisture content (%), C is the concentration of reducing sugar (g/L), V is the total volume of the hydrolyzate (L), and n is the number of dilutions.

The chemical composition of the hydrolyzate was analyzed using high-performance liquid chromatography (HPLC; Agilent 1100, USA) by two-stage acid hydrolysis, as described elsewhere (Yang *et al*. 2013).

Characterization methods

Fourier transform infrared (FTIR) spectroscopy was conducted on a MAGNA-IR 550 (Thermo Nicolet, USA) at wavelengths of 500 to 4000 cm^{-1} using the KBr disc method.

X-ray diffraction (XRD) was performed on a D8-FOCUS (Bruker, Germany) to determine the crystallinity of the pretreated substrate with Cu Kα radiation (λ = 0.154 nm) at 10 kV. The XRD data was collected from 2θ = 10 to 60° at a scan rate of 2°/min. The crystallinity was calculated by the division of the crystalline phase and total phase (Huo *et al*. 2013).

The morphology of the substrate was determined using a scanning electron microscope (SEM; S-3400, Toshiba, Japan). The accelerating voltage was 10 kV, and the samples were sputter-coated with platinum for good conductivity.

RESULTS AND DISCUSSION

Optimization of Wet Oxidation

Table 1 shows the effect of various parameters on the wet oxidation pretreatment of wood pulp waste. The RSY of untreated wood pulp waste was 5.12%, as indicated by a previous study (Liu *et al*. 2014). As expected, pH and oxygen pressure had important impacts on the enzymatic saccharification of the pretreated substrate in terms of RSY. Alkaline conditions and high oxygen pressure further decreased solids recovery through delignification and the hydrolysis reaction, thus improving RSY. This can be explained by the increased pressure, which resulted in an increase of oxygen solubility and concentration, thus accelerating the delignification process. The pretreatment time and temperature also had effects on enzymatic saccharification. Longer time and higher temperature contributed to the enhancement of RSY. On the other hand, it was clearly shown that the RSY has a negative correlation with solids recovery; for example, a lower RSY occurred at a higher solids yield. This confirmed that better enzymatic saccharification occurred when more of the cell wall structure was destroyed. The optimized conditions for wet oxidation were a pH of 10, oxygen pressure of 1.2 MPa, time of 15 min, and temperature of 195 °C. Martin *et al.* (2007) also concluded that an alkaline pH is better than an acidic pH because of the decreased degradation and formation of by-products. Kallioinen *et al*. (2013) observed that alkaline oxidation pretreatment could decrease enzyme dosage required to achieve comparable results from 10 to 4 FPU/g.

Table 1. Optimization of Wet Oxidation Pretreatment of Wood Pulp Waste

pH	O_2 pressure (MPa)	Time (min)	Temperature (°C)	Solid Recovery (%)	RSY (%)
7	1.2	15	195	52.0±0.3	38.4±0.1
8				50.7±0.2	39.6±0.2
10				50.3±0.4	42.9±0.2
10	0.4	15	195	57.0±0.2	34.5±0.1
	0.8			55.1±0.1	37.4±0.3
	1.0			53.0±0.2	39.3±0.1
	1.2			50.3±0.3	42.9±0.1
10	1.2	5	195	57.4±0.2	33.6±0.2
		10		54.6±0.1	36.9±0.1
		15		50.3±0.1	42.9±0.3
		20		49.6±0.2	41.8±0.1
10	1.2	15	165	74.9±0.3	15.9±0.2
			175	62.1±0.3	28.5±0.3
			185	54.9±0.4	39.1±0.1
			195	50.3±0.1	42.9±0.2

Mass Balance of Optimized Wet Oxidation

The mass balance of optimized wet oxidation pretreatment is summarized in Fig. 1. As can be seen, 39% of lignin and 73% of xylose were dissolved into the hydrolyzate by the wet oxidation pretreatment though delignification and the hydrolysis reaction. The degraded compounds, such as acetic acid and furfural, remained at low levels.

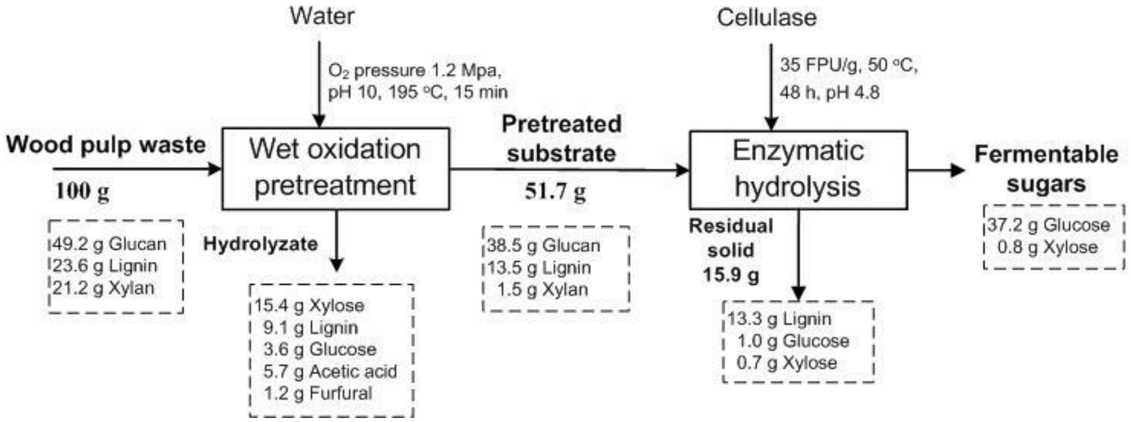

Fig. 1. Mass balance of optimized wet oxidation pretreatment of wood pulp waste

A total of 78% of glucan was preserved in the recovered substrate, suggesting the good performance of wet oxidation pretreatment. After enzymatic hydrolysis, 37.2 g of glucose was recovered as fermentable sugars, representing 76% of the total glucose in the raw material.

Characterization of Treated Substrates

Structural features such as linkages, crystallinity, and defibrillation of the pretreated substrates are shown in Figs. 2a, 2b, and 2c. The diminished peak at 1732 cm^{-1} belonged to the lignin-hemicellulose ester bond (Zhang *et al*. 2013a,b), suggesting that the bond between lignin and xylan was cleaved through wet oxidation pretreatment. The peaks at 1605 and 1515 cm^{-1} could be attributed to the aromatic skeleton vibration of lignin (Liu *et al*. 2009), which still existed in the treated substrate, indicating that a certain amount of lignin remained. This agreed with the chemical composition analysis results (Fig. 1). The peak at 1105 cm^{-1} corresponded to crystalline cellulose (Li *et al*. 2010), as the increases in intensity of the treated substrate indicated increasing crystallinity. These linkage changes would minimize non-productive adsorption of cellulase and therefore promote enzyme performance.

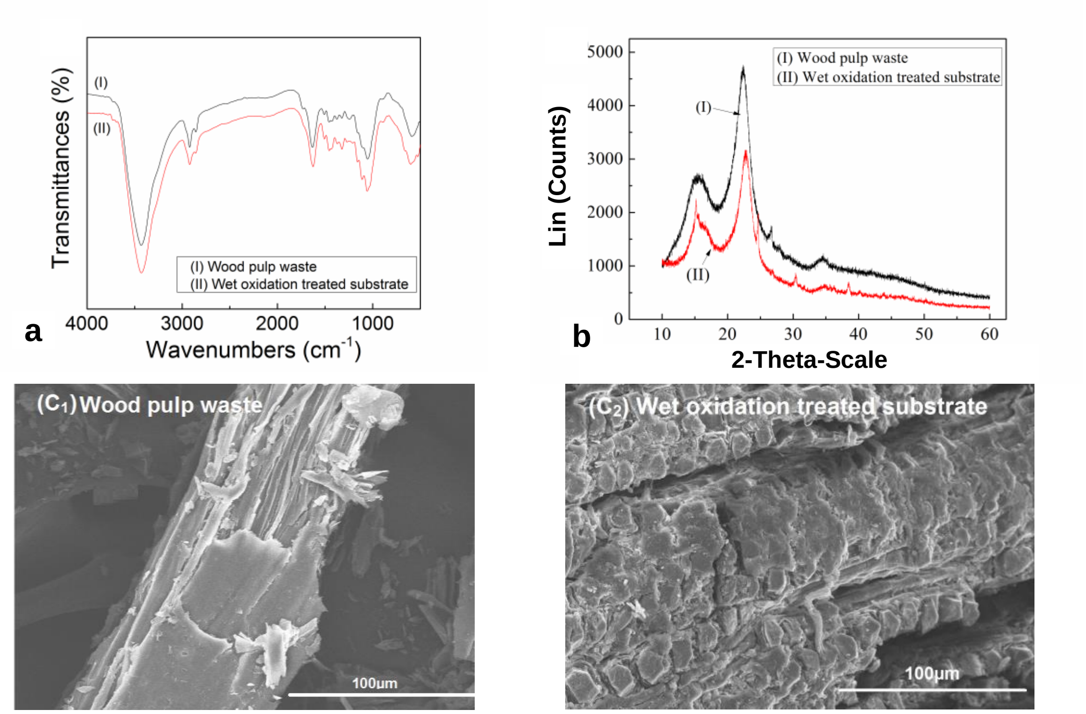

Fig. 2. Spectra of untreated and wet oxidation-treated substrates: (a) FTIR; (b) XRD; and (c$_1$ and c$_2$) SEM

As shown in Fig. 2b, significant peaks at 15° and 22° corresponded to crystalline cellulose (Tang *et al*. 2014). The estimated crystallinity index increased from 55.4% to 63.7% for the treated substrate. This increase in crystallinity might be attributed to the removal of amorphous components such as hemicellulose, lignin, and amorphous cellulose.

Figure 2c shows the morphological changes of the treated substrate. The untreated sample has a very smooth and compact surface. After wet oxidation treatment, the surface was full of cracks and trenches, confirming that extensive delignification and hydrolysis reactions occurred. The open structure made the substrate more accessible to cellulase, thus enhancing enzymatic saccharification.

CONCLUSIONS

1. Wet oxidation pretreatment was performed on wood pulp waste from the P-RC APMP production process to enhance enzymatic saccharification. The optimal conditions were a pH of 10, oxygen pressure of 1.2 MPa, time of 15 min, and temperature of 195 °C. The enzymatic saccharification was enhanced from 5.12% to 42.9% in terms of reducing sugar yield.
2. A 39% of lignin and 73% of xylose were dissolved into the hydrolyzate under optimized wet oxidation pretreatment, which resulted in a 76% glucose recovery from subsequent enzymatic hydrolysis process.

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