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# Effect of depth beating on the fiber properties and enzymatic saccharification efficiency of softwood kraft pulp

Wenhua Gao [a], Zhouyang Xiang [b], Kefu Chen [a], Rendang Yang [a], Fei Yang [a,*]

[a] State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, Guangzhou 510640, China
[b] Department of Biological Systems Engineering, University of Wisconsin-Madison, Madison, WI 53706, USA

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ABSTRACT

Commercial bleached softwood kraft pulp was mechanically fibrillated by a PFI-mill with beating revolution from 5000 to 30,000 r. The extent of fibrillating on the pulp was evaluated by beating degree, fiber morphological properties (fiber length, width, coarseness and curls index), water retention value (WRV) and physical properties of paper made from the pulp. Depth beating process significantly affected the pulp fibrillations as showed by the decreased fiber length and width as well as the SEM analysis, but the effects were limited after beating revolution of 15,000. Depth beating process also improved the total internal pore and inter-fibril surface areas as shown by the increased WRV values. Substrate enzymatic digestibility (SED) of beaten pulp at 5000 revolutions could reach 95% at cellulase loading of 15 FPU/g of glucan. After the enzymatic hydrolysis, the size of the pulp residues was reduced to micro-scale, and a relative uniform size distribution of the residues appeared at 10,000 r beating revolution.



## 1. Introduction

With the increasing environmental issues and energy security concerns, the interests in alternative nonpetroleum-based sources of energy, chemicals and materials have strengthened. Lignocellulosic biomass is considered as a suitable and renewable primary resource, which can potentially provide us with renewable transportation fuels, polymer or fibrous materials, and synthesized chemicals (Alvira, Tomás-Pejó, Ballesteros, & Negro, 2010; Gupta, Khasa, & Kuhad, 2011). The main structure of lignocellulosic materials is composed of cellulose, hemicellulose, and lignin (Xiang et al., 2015). Cellulose is a linear homogeneous biopolymer composed exclusively of D-glucose (Xiang, Watson, Tobimatsu, & Runge, 2014a), which is the most abundant biopolymers on earth (Siró & Plackett, 2010) and has the most values for the biorefinery process due to its uniform structure. However, cellulose is covered or interlaced with hemicelluloses and lignin limiting its efficient utilization (Fratzl, Burgert, & Keckes, 2004). In order to exploit cellulose from lignocellulose to make fuels or high value biomaterials, physical, chemical and enzymatic pretreatment methods are widely studied (Klemm et al., 2011; Zhu & Pan, 2010). For example, alkaline or dilute acid pretreatments can effectively remove hemicelluloses (Xiang & Runge, 2014; Xiang, Anthony, Tobimatsu, & Runge, 2014b; Hendriks & Zeeman, 2009), and green liquor pretreatment can effectively remove lignin (Xiang et al., 2015; Yamashita, Shono, Sasaki, & Nakamura, 2010). However, those pretreatment methods could cause environmental problems due to the chemical used. The mechanical pretreatment might eliminate this problem but is energy intensive (Hendriks & Zeeman, 2009).

In recent years, the preparation of cellulose nanofibrils (CNF) or microfibrillated cellulose (MFC) from lignocellulosic biomass has gained increasing attention due to its potential application in nano-composite materials (Siró & Plackett, 2010). MFC is usually produced by refining or high-pressure homogenization via fibrillation of wood fibers (Nakagaito & Yano, 2005). The mechanical refining is highly energy intense, and about 12,000–70,000 kW h/t of energy was necessary to make gel-like MFC from kraft pulp suspension (Wang et al., 2012; Klemm et al., 2011). Additionally, the mechanical refining usually results in low quality of MFC by either under disintegrating (the fiber size is still large) or over disintegrating (cellulose degree of polymerization is low) the fibers (Henriksson, Henriksson, Berglund, & Lindström, 2007). For reducing the energy demand or improving quality for the MFC production, mild enzymatic hydrolysis combined with less intense mechanical treatments is introduced in the preparation process (Zhu, Sabo, & Luo, 2011; Pääkkö et al., 2007). The accessibility of enzyme to cellulose is a main factor affecting the enzymatic hydrolysis efficiency (Leu & Zhu, 2013). Thus the mechanical treatment

*Abbreviations:* BSKP, bleached softwood kraft pulp; CNF, cellulose nanfibrills; SED, substrate enzymatic digestibility; WRV, water retention value.

* Corresponding author. Tel.: +1 020 87110084.
*E-mail address:* xzyocean@hotmail.com (F. Yang).

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before enzymatic hydrolysis is generally performed to increase fiber surface area by disintegrating it into fine fragments and deconstruct the compact structure of cellulose (Zhu et al., 2011; Zhu, Wang, Pan, & Gleisner, 2009). After the enzymatic hydrolysis process, the size of the fiber is reduced and the crystallized structure of cellulose is loosened facilitating the following second mechanical treatment for preparing MFC (Henriksson et al., 2007).

Bleached kraft pulp is a feedstock from pulp and paper industry with very high cellulose content and thus widely used for MFC production (Siró & Plackett, 2010). Mechanical refining or beating methods from the papermaking industry can be used for fibrillating and swelling pulp fiber, which increases fiber accessibility to enzyme for the subsequent enzyme treatment (Henriksson & Berglund, 2007; Zhu et al., 2009). A beating machine (e.g. PFI-mill) can achieve internal fibrillation of fibers through replacing intra-fiber hydrogen bonds by fiber-water hydrogen bonds and achieve external fibrillation of fibers through high external forces (Oksanen, Pere, Buchert, & Viikari, 1997). Very few studies have used beating machine before enzymatic hydrolysis of preparing MFC especially at high revolution. Henriksson et al. (2007) obtained well desintegrated MFC through PFI-mill beating and enzymatic hydrolysis; however their revolution was only up to 4000 r. PFI-mill beating revolutions represented the beating intensity, and the structure and properties of fibers varies depending on the extent of beating treatment, which may affect the enzymatic hydrolysis process. Therefore, this study evaluates the relations among depth PFI-mill beating on bleached softwood pulp with high revolutions (up to 30,000 r), variations of fiber structures, and substrate enzymatic digestibility (SED). The structure and property of enzymatic hydrolysis residues were also analyzed to provide a good theoretical basis for the preparation of MFC or CNF from combined mechanical and enzymatic hydrolysis pretreatments. This technology might provide a method to improve the enzymatic hydrolysis efficiency at a relative low cellulase loading and reduce energy consumption in the following homogenizing process for MFC or CNF production. In this study, the PFI-mill comes from the pulp and paper industry, and the results could also provide the opportunity for promoting this industry to produce new biomaterials by using the existing equipment.

## 2. Experimental

### 2.1. Materials

Bleached softwood kraft pulp (BSKP) was obtained from a paper-making plant Yongtai Group, Zhejiang, China. The main chemical compositions of this BSKP were listed in Table 1. PFI-mill (Mark VI NO.621) was manufactured by Hamjerb Maskjin A/S Hamar Company, Norway. Cellulase (C2730, 1,4-(1,3:1,4)-β-D-glucan 4-glucano-hydrolase Celluclast 1.5 L) was obtained from Sigma-Aldrich and produced by submerged fermentation of a selected strain of the fungus *Trichoderma reesei* ATCC 26921, Novozyme Corp., USA.

### 2.2. Beating and paper sheets formation

About 30 g pulp was diluted by DI water to attain 10% (w/w) medium concentrated pulp, and then beaten by PFI-mill according to TAPPI method T248. Beating was performed at different beating revolutions (r): 5000, 10,000, 15,000, 20,000, 25,000 and 30,000. Paper hand-sheets were made by the beaten pulp according to TAPPI method T205. Bulk density and air permeability were tested following ISO 438.1 and TAPPI method T547, respectively. Tensile index, tear index, folding endurance and burst index were tested according to TAPPI method T220.

**Table 1**
Main chemical composition of the bleached softwood kraft pulp (based on % of oven-dried weight).

| % | Arabinan | Glactan | Glucan | Xylan | Mannan | Klason lignin |
|---|---|---|---|---|---|---|
| BSKP | 0.4 | 0.3 | 78.6 | 10.2 | 5.8 | 0.2 |

### 2.3. Enzymatic saccharification

Cellulase solution was added into the beating pulp with the loadings of 3, 5, 10, 15 FPU/g of glucan. Enzymatic saccharification reactions were performed at a substrate solid content of 1% (w/v) in sodium acetate buffer of pH 4.8. The reactions were kept at 50 °C and shaking speed of 200 rpm in a shaker for 48 h. The enzymatic hydrolysis solution and residue were separated by a centrifuge at 10,000 rpm for 5 min. The glucose produced was tested by Ion Chromatography (ICS3000). The substrate enzymatic saccharification (SED) represented saccharification efficiency of beating pulps, and was defined as the percent of glucan in the substrate enzymatically saccharified to glucose.

### 2.4. Fiber characterization

The freeness of pulp was tested by a Riegler YQ-Z-Grenoble 13 beating degree tester, which reflects the pulp drainage property. The characteristics of fibers, such as fiber length, fiber width, fiber coarseness and fiber curl index were tested by a Kajaani FS300 fiber analyzer. For the water retention value (WRV), a modification of the TAPPI method T256 was used (Hoeger et al., 2013). 6.25 g of pulps of 4% (m/m) solid content were centrifuged at 900 × *g* for 30 min. The samples were then dried at 105 °C until reaching a constant weight. The WRV was calculated by the amount of water held in the samples upon centrifugation relative to the oven dry weight. The size of enzymatic hydrolysis residues was tested by Mastersizer 2000, Malvern Instruments Ltd. UK.

### 2.5. X-ray diffraction (XRD)

XRD measurements of enzymatic hydrolysis residues were performed by Rigaku D/Max-III X-ray diffraction analyzer. These residues were obtained from beating pulps at the cellulase loading of 15 FPU/g of glucan after the enzymatic hydrolysis process. They were dried and grinded into powders for XRD test. The diffracted intensity of Cu *K*α radiation (40 kV and 40 mA) was measured in a 2$\theta$ range between 4° and 60°. The crystallinity index ($I_c$) was determined by using the following equation (Mwaikambo & Ansell, 2002):

$$I_c = \frac{(I_{(002)} - I_{(am)})}{I_{(002)}} \times 100$$

where $I_{(002)}$ is the counter reading at peak intensity at a 2$\theta$ angle close to 22° representing crystallized regions, and $I_{(am)}$ is the counter reading at peak intensity at a 2$\theta$ angle close to 18° representing amorphous regions in cellulosic fibers.

### 2.6. Scanning electron microscopy (SEM)

SEM mircographies of fibers surfaces were taken by a scanning electron microscopy (SEM, S3700, Hitachi of Japan). Prior to SEM evaluation, all of samples were coated with a layer Au.