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Deconstruction of cellulosic fibers to fibrils based on enzymatic pretreatment

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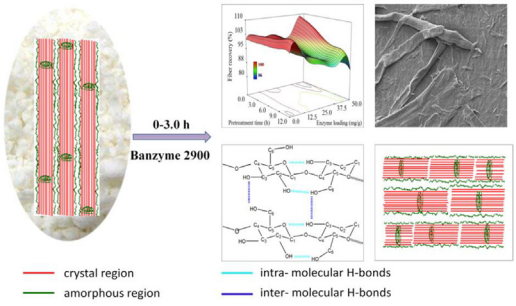
GRAPHICAL ABSTRACT



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ABSTRACT

Enzymatic pretreatment has shown great potential in making the disintegration of cellulosic fibers to fibrils cost-effectively and environmental-friendly. In this study, an extensive commercial endoglucanase was used to pretreat cellulosic fibers for fibrillation. The pretreatment time and the enzyme dosage were optimized using response surface methodology. A 100% fiber recovery was obtained at endoglucanase usage of 9.0 mg/g (substrate) and pretreatment time of less than 3.0 h. A highly fibrillated and fractured surface of pretreated fibers was observed after 0.5 h of pretreatment compared to native fibers. Meanwhile, the progressive deconstruction of cellulosic fibers was occurred with the enzymatic pretreatment time increasing. The degree of deconstruction of fibers was evidenced by changes of the fiber microstructure, such as the inter-/intra-molecular H-bonds, the β-1,4-glucosidic linkages, crystallinity and crystallite size. These discoveries provide new insights into a more efficient and economic pretreatment methods for the disintegration of fibrils from cellulosic fibers.

1. Introduction

Cellulosic fibers are considered as a sustainable raw material used for the production of novel biomass-based products, such as nano-/micro-cellulose materials, bio-fuels, chemicals, etc. (Zhu et al., 2011). Among those bio-products, nano/micro-fibrillated cellulose (NFC/MFC) isolated from cellulosic fibers has obtained increasing attention owing to its high value applications in many fields, such as reinforcing materials for composites, transparent bio-materials, green electronics, flexible displays, drug releases, etc. (Zhu et al., 2016; Xiang et al., 2016;

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Wang et al., 2018). The potential applications of NFC/MFC demand for large amounts of NFC/MFC. However, the large scale production of NFC/MFC haven't been commercialized owing to the high energy consumption during mechanical refining processes of NFC/MFC production (Eriksen et al., 2008).

Due to the condensation of surface hydroxyl groups in cellulose, the rigid intra-molecular H-bonds (from O(6) to O(2)H and from O(3)H to the ring O(5) in cellulose chains) and inter-molecular H-bonds (from O (3) to O(6)H between cellulose chains) are formed, which provide cellulose with a stable crystal structure and a high crystallinity (Zhu et al., 2016). Therefore, the mechanical fibrillation of cellulosic fibers is highly energy intensive (Alemdar and Sain 2008; Daud et al., 2015). To overcome this problem, pre-treatments on cellulosic fibers prior to the mechanical treatment have been studied. For instance, a chemical/biological pretreatment step has been proposed to "unlock/loose" the intrinsic recalcitrance of cellulosic fibers, thus decreasing the energy needed for the fibrillation (Alemdar and Sain 2008; Spence et al., 2010). A number of chemical pretreatments may assist to achieve this goal, such as TEMPO-mediated oxidation, acetylation, carboxymethylation, sulfonation, etc. (Saito et al., 2006; Wang et al., 2017; Liimatainen et al., 2013). However, the high capital costs of chemical pretreatments and the difficulties to recycle of these chemicals result in a negative environmental impact, which have limited their further applications (Lavoine et al., 2012; Kumar et al., 2016). Comparing among the chemical pretreatment approaches, biological pretreatment is considered to be a very promising method for industrial applications due to its low chemical loading, environmentally friendly reaction conditions, and the high selectivity of enzymatic reaction (Pääkkö et al., 2007; Henriksson et al., 2007; Beltramino et al., 2015; Wang et al., 2016a; Long et al., 2017; Guo et al., 2017; Bian et al., 2018). Several types of enzymes have been investigated to facilitate NFC/MFC disintegration from cellulosic fibers (Henriksson et al., 2007; Hu et al., 2015; Spence et al., 2010; Long et al., 2017; Nie, et al., 2018). These studies showed that the enzymatic pretreatment of cellulosic fibers based on *endo*-glucanase (EG) randomly cleaves the celluloseβ-1,4 glucosidic linkages, and thus to facilitate the fibrillation of cellulosic fibers. Therefore, an extensive commercial cellulase (Banzyme 2900) has been claimed consisting mainly of endoglucanase (EG) and may have great potential for facilitating the downstream disintegration of cellulosic fibers.

For different applications, the properties of NFC/MFC, e.g. morphology, rheology, average size, size distribution, should be tailored to meet certain requirements, and achieve its industrial-scale production. However, the accurate characterization of the production of NFC/MFC is challenging, owing to its heterogeneous nature, unique arrangement, slender fibrils with a high degree of branching (Kangas et al., 2014). Direct characterization of NFC/MFC depends primarily on microscopic imaging. This method only gives assessment of a small proportion from a large sample, which may not be representative to the entire samples and is time consuming. The properties of NFC/MFC, to a large extent, are affected by the properties of cellulosic fibers, pretreatment approaches, and preprocessing conditions, e.g. reagent dosage, pretreatment time, and pretreatment temperature). During the pretreatment step, cellulosic fibers exhibit "deconstruction" (Wang et al., 2016b). Even though the deconstruction model of cellulosic fibers has been proposed, no systematic studies have been reported on the changes of microstructural properties, such as the binding force (H-bonds), crystal structure, and morphology of cellulosic fibers. Therefore, investigating the effects of enzymatic pretreatment on the microstructural properties of cellulosic fibers is the key in developing energy efficient and economical NFC/MFC production methods.

In this study, bleached softwood kraft pulp (BSKP) was pretreated by commercial endoglucanase, in order to "unlock/loose" the intrinsic recalcitrance of softwood fibers. The enzyme dosage and pretreatment time were optimized using response surface methodology (RSM), by quantifying the recovery yield of pretreated fibers. Meanwhile, the changes in microstructures of fibers undergoing endoglucanase pretreatments were evaluated. This study may provide new insights into developing a more efficient and economical pretreatment technology for disintegrating cellulosic fibers into fibrils.

2. Materials and methods

2.1. Materials

Commercial dried bleached softwood kraft pulp (BSKP) was obtained from a paper mill (Guangdong Province, China). Commercial endoglucanase (Banzyme 2900) was purchased from UPM-kymmene Co., Ltd (Jiangsu, China). The optimum pH and temperature for the Banzyme 2900 were 5.5 and 50 °C, respectively. Enzyme activity (7.3 IU/ml) was assayed by dinitrosalicylic acid (DNS) method using D-glucose as a standard (Sengupta et al., 2000). Bovine serum albumin (BSA) was used as the standard of proteins for measurement the protein content (9.4 mg/ml) of the Banzyme 2900 (Frolund et al., 1995). The cupriethylenediamine hydroxide solution was purchased from the China Pulp and Paper Research Institute (Beijing, China). All others chemicals used were obtained from Macklin Biochemical Co., Ltd (Shanghai, China).

2.2. Enzymatic pretreatment

The enzymatic pretreatment was carried out according to a previous work with some modification (Wang et al., 2016b). Briefly, 200.0 g of BSKP were suspended in 4000 mL of a 50 mM citrate acid-sodium citrate buffer (pH 5.5) with various enzyme loading amounts (1 to 50 mg/g substrate). The mixture of samples was incubation at 50 °C for various retention times. After enzymatic pretreatment, the slurry was centrifuged to separate the solid and liquid phases. The solid phases were stored at 4 °C for measuring. The fiber recovery (Y) was calculated using Eq. (1).

$$Y = \frac{M_2}{M_1} \times 100\% \quad (1)$$

where Y is the fiber recovery yield (%), M_1 is the weight of fiber before pretreatment (g), M_2 is the weight of fiber after pretreatment (g).

2.3. H-bond characteristic analysis

The H-bond characteristic parameters of the enzymatic samples were analyzed by using a Fourier transform infrared (FT-IR) spectroscopy. The absorption spectra (3700 to 3000 cm^{-1}) was studied using Peak-Fit software (4.12.00, Jinan, China) in conjunction with Gaussian distribution function to analyze the H-bonds content (%), energies (E_H), and bond lengths (R) of different H-bonds models. The H-bond characteristic parameters were calculated as follows (Struszczyk and Laine 1986; Wan et al., 2015),

$$E_H = \frac{1}{K}\frac{\nu_0 - \nu}{\nu_0} \quad (2)$$

where ν_0 is the standard free hydroxyl frequency (3650 cm^{-1}), ν is the hydroxyl frequency of pretreatment samples, and k is a constant (6.7×10^{-2} kJ^{-1}).

$$\Delta\nu = 4.43 \times 10^3 \cdot (2.83 - R) \quad (3)$$

In. Eq. (3), $\Delta\nu = \nu_0 - \nu$, ν_0 is the stretch vibrational frequencies of standard free hydroxyl (3600 cm^{-1}), ν is the samples hydroxyl frequency (cm^{-1}), and R is the bond length (Å).

2.4. Swelling property of fibers

The water retention value (WRV) was performed and calculated according to ISO 23714:2007 in two replicates ("Pulps. Determination