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Preparation and Characterization of Nano Crystalline Cellulose from Bamboo Fibers by Controlled Cellulase Hydrolysis

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Abstract

The extracellular cellulase enzyme produced by *Trichoderma reesei* was used to prepare Nanocrystalline Cellulose (NCC) by controlled hydrolysis of bamboo fibers. The morphology of the prepared bamboo cellulose nanocrystals was characterized by field emission scanning electron microscopy and the crystallinity was measured by X-ray diffraction. The degree of polymerization was tested by automatic viscosimeter. The surface charge in suspension was estimated by Zeta-potential. The results showed that all NCC from bamboo fibers presented a rod-like shape, an average diameter of 24.7 nm and length of 286 nm, with an aspect ratio of around 12. The zeta potential of cellulase hydrolyzed NCC was 4 times lower than that of NCC prepared by acid hydrolysis process.

Keywords: Nanocrystalline Cellulose; Bamboo Fibers; *Trichoderma Reesei*; Cellulase; Characterization

1 Introduction

Cellulose, the most abundant biopolymer on earth, is a homopolymer of β-1,4-D-glucose molecules linked in a linear chain [1]. Cellulose microfibrils can be found as intertwined microfibrils in the cell wall (containing 500–15 000 glucose units depending on it source) [2]. As well as these microfibrils, there exist Nano Crystalline Cellulose (NCC) (also composed by cellulose) which are elongated and flat, a few hundreds of nanometers long, 10-20 nm wide and a few nm thick [3]. The NCC, also called as cellulose nanowhiskers, cellulose crystallites or crystals in literatures, are mainly produced by concentrated sulfuric acid hydrolysis of Microcrystalline Cellulose (MCC) whereby

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the presence of amorphous region is completely hydrolyzed to yield highly crystalline NCC. These NCC have received increasing attention due to their extraordinary mechanical properties such as high Young's modulus and tensile strength [4]. The Young's modulus of NCC is as high as 134 GPa while the tensile strength of the crystal structure was estimated in the range of 0.8–10 GPa [5].

The filamentous fungus *Trichoderma reesei* is one of the most efficient producers of extracellular cellulose enzyme. Cellulases are produced as multi component enzyme system comprised usually of three components that act synergistically in the hydrolysis of cellulose: endoglucanases (EC 3.2.1.4), cellobiohydrolase (EC 3.2.1.91) and cellobiase (β-glucosidase, EC 3.2.1.91). The extracellular cellulolytic system of *Trichoderma reesei* is composed of 60–80% of cellobiohydrolases, 20–36% of endoglucanases and 1% of β-glucosidases [6]. The first two components act directly on cellulose yielding mainly cellobiose, cellotriose or cellotetraose as the reaction products. The cellobiose is then hydrolyzed to glucose by cellobiase. Though endoglucanases and cellobiohydrolases degrade soluble cellodextrins and amorphous cellulose, cellobiohydrolases degrade crystalline cellulose more efficiently. The oligosaccharides formed during the cellulose hydrolysis are believed to play important roles in the natural cellulase induction. So solid cellulose itself is often used as both the substrate and the source of inducers in fermentation process for cellulose production [7]. Besides, it is commonly understood that bamboo is a plant widely growing all over the world, whose lignocellulosic fibers have a great industrial potential [8].

During the traditional preparation process of NCC, the hydrolyzing agent, sulfuric acid introduces bulky ester groups onto the hydroxyl groups and stabilizing the NCC in solution by preventing its agglomeration [9]. However, the use of sulfuric acid has a number of important drawbacks such as corrosivity, surface modification of cellulose and environmental incompatibility. Apart from use in composites, NCC finds applications in health care like personal hygiene products, biomedicines, cosmetics and so on. NCC in its pure form is safe and biocompatible, but the traditional acid hydrolysis process inserts sulfate groups on the surface of NCC.

Similarly, the sono-chemical assisted hydrolysis of cellulosic materials for the production of NCC is highly energy intensive due to the predominating hydrogen bonding between the cellulose microfibrils. This interfibrillar hydrogen bonding energy ($\sim$20 MJ/kg mol) has to be overcome in order to hydrolyze the cellulose [10].

The surface modifications in traditional acid hydrolysis process pose bio-compatibility problem, fungal degraded cellulose retains its original chemical nature. Cellulase enzyme produced by various microbes, with its proven biotechnological advances in various fields, will be of immense use in the production of NCC. Meanwhile, as a biological catalyst, action of microbial enzymes reduces the energy requirement for cellulose hydrolysis. Earlier work showed a significant reduction in energy consumption during refining process when cellulose is subjected to fungal pretreatment [11, 12]. Therefore, the present study was attempted to produce NCC by controlled hydrolysis of bamboo fibers using the extracellular cellulase enzyme of fungus *Trichoderma reesei* under submerged fermentation process. The obtained NCC was then characterized by Field Emission Scanning Electron Microscopy (FESEM), X-ray Diffraction (XRD), Degree of Polymerization (DP) and Zeta-potential.

The main outcome of this work provided a feasible way of producing NCC from bamboo fibers through enzymatic hydrolysis. This aspect is new as it offers a novel route to reduce energy consumption and increase environmental compatibility in producing these NCC materials.