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Green preparation and characterization of size-controlled nanocrystalline cellulose via ultrasonic-assisted enzymatic hydrolysis

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ABSTRACT

Cellulose is the most abundant biopolymer on earth and the main component of crop by-products, but its development is inadequate. Preparation and application of nanocrystalline cellulose (NCC) are drawing much attention from both the academia and the industry because of its unique and exceptional physicochemical properties. For the first time, rod-like NCC was successfully prepared through an environmentally friendly ultrasonic-assisted enzymatic hydrolysis process from wheat microcrystalline cellulose. NCC yield reached 22.57% under the optimal condition of hydrolysis time of 120 h combined with ultrasonic treatment of 10 times each for 60 min, whereas the NCC yield was only 15.76% in the absence of ultrasonic treatment. Images of transmission electron microscopy showed that the NCC samples exhibited a rod-like structure with a width of less than 10 nm and a length of 200–500 nm, 100–200 nm, and 40–50 nm when the ultrasonic treatment was 0, 30, and 60 min, respectively. Dynamic light scattering analysis demonstrated that as-obtained NCC exhibited a smaller value in particle size than that prepared in the absence of ultrasonic treatment. X-ray diffraction results revealed that the NCC sample exhibited higher crystallinity as ultrasonic time increased. NCC fabricated by this facile, safe, and eco-friendly ultrasonic-assisted enzymolysis method can have great potential in applications in bionanocomposites, drug delivery, agriculture, and cosmetic industry.

1. Introduction

Cellulose is the most abundant renewable natural resource on earth, but the use of waste or by-products of crops is inadequate. In recent years, the extraction/preparation of nanocellulose from various plant or bacteria sources has seen increasing interest. The reason behind this rapid progress lies in the promising properties of nanocellulose and its products. Nanocrystalline cellulose (NCC) not only has the inherent characteristics of natural cellulose (Fatehi et al., 2010; Qian et al., 2009), but it also exhibits such appealing properties as low density, renewability, biodegradability, high reinforcing capability, and low production cost compared with glass and carbon nanofibers (Mariano et al., 2014). Owing to these features, NCC has attracted great interest in various fields, including regenerative medicine (Klemm et al., 2001), printing applications (Torvinen et al., 2012), optical applications (Okahisa et al., 2011), and composite materials (Zaman et al., 2012).

Native cellulose has both crystalline and amorphous regions, with the former being able to be isolated by chemical or mechanical methods to produce a series of products in the form of crystalline cellulose, such as microcrystalline cellulose (MCC), NCC, microfibrillated cellulose, nanofibrillated cellulose, or bacterial NCC, depending on the preparation methods and sources (Habibi et al., 2010). Recently, various chemical and mechanical methods have been adopted for the preparation of NCC. Chemical methods include acid/alkaline hydrolysis (Johar et al., 2012) or enzyme-assisted hydrolysis (Henriksson et al., 2007). Mechanical methods include steam explosion treatment (Deepa et al., 2011), high-pressure homogenization (Li et al., 2012a), ultrasonic technique (Li et al., 2012b), and the combination of these processes. Among these methods, acid hydrolysis produced NCC with a rod-like structure with a width and length of 10–20 nm and 50–150 nm, respectively (Tang et al., 2014). Acid hydrolysis has the advantage of convenience and moderate operation conditions. However, acid hydrolysis needs to consume a large quantity of acid, and therefore it easily contributes to environmental pollution. Conversely, mechanical methods need to consume large amounts of energy. To

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overcome these drawbacks, a green, facile, and promising method that has been developed recently to fabricate nanoparticles is the enzymatic hydrolysis method that has the advantage of security, no pollution, high efficiency, and low consumption of energy (Eslahi et al., 2013; Meyabadi and Dadashian, 2012). Ultrasonic energy has homogenization and heating effects (Eslahi et al., 2013). Ultrasonic treatment can enhance the contact area of enzyme and substrate, as well as the temperature, which may accelerate the reaction between enzyme and substrate. The effect of ultrasonic-assisted sulfuric acid hydrolysis conditions and properties on the resultant NCCs was determined (Zhang et al., 2012). The results showed that low-intensity ultrasonic treatment could improve the yield of NCC through sulfuric acid hydrolysis. Ultrasonic-assisted enzymolysis is a combined extraction method that has the advantages of two extraction methods, such as mild extraction conditions, low investment costs and energy requirements, and simplified manipulation (Chen et al., 2014). However, the preparation of nanoparticles by ultrasonic-assisted enzymatic hydrolysis has rarely been reported. In this context, we submitted MCC from wheat fiber to ultrasonic-assisted enzymatic hydrolysis to fabricate NCC and examined their characteristics. The shape and size distribution of the resultant NCC were determined by transmission electron microscopy (TEM) and dynamic light scattering (DLS). Fourier transform infrared (FTIR) spectrum and X-ray diffraction (XRD) experiments were used to describe the structure and crystallinity of NCC. Thermogravimetric analysis (TGA) was conducted to determine their thermal stability.

2. Materials and methods

2.1. Materials

Commercial MCC (95%) power derived from wheat straw was provided by Shanghai Nuoshen Food Trading Co., Ltd. (Shanghai, China). Celluclast 1.5 L (700 EGU/g; EGU means endoglucanase unit) was purchased from Novozymes (China) Biotechnology Co., Ltd. (Beijing, China). Deionized water was used for all experiments. All other chemicals, such as sodium acetate, sulfuric acid, and acetic acid, were of analytical grade.

2.2. Preparation of nanocrystalline cellulose (NCC)

A total of 6 g MCC, 200 mL acetate buffer solution (pH 4.8), and 3 mL cellulase were added into a 250 mL conical flask. Enzymatic hydrolysis was performed at 50 °C under stirring using a choppy water bath pot (DKZ-3, Shanghai Yiheng Instruments Co., Ltd., China) for 72, 96, and 120 h, respectively. The resultant dispersion was treated in a common ultrasonic generator (KQ - 200 VDE, Kunshan ultrasonic instrument Co., Ltd., China) with a constant power of 300 W to promote the enzymolysis hydrolysis. To investigate the effect of ultrasonic time on NCC, ultrasonic treatment was conducted for 30 and 60 min every 12 h, respectively. After the ultrasonic-assisted hydrolysis was run for a desired duration, the resultant dispersion was boiled for 15 min to terminate the hydrolysis. Subsequently, the mixture was initially centrifuged at 1000 g for 15 min to remove particles larger than 1 μm using a centrifuge (TGL-16, Changsha Yingtai Instrument Co., Ltd., China). The resultant supernatant was then centrifuged at 14,000 × *g* for 20 min to separate the NCC. The NCC was washed with deionized water until the wash water was maintained at constant pH. The colloidal suspension of NCC was collected and freeze–dried for testing (Meyabadi and Dadashian, 2012). The NCC samples prepared through ultrasonic-assisted enzymolysis hydrolysis were denoted as NCC-30-72 and NCC-60-72, where 30 and 60 denote the ultrasonic time (min) and 72 denotes the enzymolysis time (h). Other samples were labeled by the same rules. To examine the effects of the ultrasonic treatment, these procedures were repeated under the same conditions but in the absence of ultrasonic treatment. That is, these samples prepared through a pure enzymolysis method were labeled as NCC-0-72, NCC-0-96, and NCC-0-120.

For comparison, NCC was also prepared by a conventional process using 60 wt% of sulfuric acid hydrolysis of MCC at 45 °C for 1.5 h according to the method stated in the literature (Zhang et al., 2012). The acid-hydrolyzed sample was washed with sufficient deionized water by repeating the centrifugation and dilution processes until its pH was neutral, and then the sample was freeze–dried.

2.3. Calculation of the nanocrystalline cellulose (NCC) yield

The samples were weighed with an analytical balance. The final result for each sample was obtained as the average of three runs of measurements. The yield (%) was calculated according to Eq. (1):

$$\text{Yield}(\%) = \frac{m_1 - m_2}{m_3} \times 100 \quad (1)$$

where m_1 is the total mass of vacuum freeze–dried NCC and weight bottle (mg), m_2 is the mass of the weight bottle (mg), and m_3 is the mass of MCC (mg) (Tang et al., 2014).

2.4. The morphology analysis

The morphology of NCC was observed using TEM according to the method reported in the literature (Jia et al., 2014). The NCC sample was ultrasonically dispersed in distilled water to form a 0.01% (w/v) NCC suspension. Then, a small droplet of the diluted NCC suspension was deposited on a 300 mesh copper grid coated with holey carbon film. The excess liquid was removed by filtration. The as-obtained specimen was subsequently dried under the vacuum condition, negatively stained by 2% uranyl acetate for 20 min, and dried under an infrared lamp for 5 min. The TEM analysis was performed using an H-7650 TEM (Hitachi, Japan) at 80 kV.

2.5. Particle size analysis

The particle size distributions of all the NCC samples were analyzed using a Malvern Mastersizer 2000 laser diffraction particle size analyzer (Malvern Instruments Ltd., UK) with Malvern Mastersizer 2000 software (Geissler et al., 2014). The NCC suspensions (0.02% w/v), previously sonificated for 5 min, were prepared and analyzed to determine the length.

2.6. X-ray diffraction analysis (XRD)

The XRD patterns for the MCC and NCC samples were performed on a diffractometer (X′TRA-055, ARL, Switzerland) with Cu Kα radiation (k = 0.154 nm) at 40 kV and 40 mA. XRD data were collected from $2\theta = 5°$–$40°$ by steps of 0.01° (Qua et al., 2011).

The following empirical equation was adopted to estimate the crystallinity index Eq. (2):

$$X_C = \frac{I_{002} - I_{am}}{I_{002}} \times 100 \quad (2)$$

where X_c is the crystallinity index, I_{002} is the scattered intensity at the main peak, and I_{am} is the scattered intensity due to the amorphous portion (Lu and Hsieh, 2010).

2.7. Fourier transform infrared (FTIR) analysis

The NCC structure was determined using the method as described in the literature (Wijesena et al., 2015). FTIR spectra of the NCC and MCC samples were obtained on a Nicolet 5700 spectrometer (Thermo Fisher Scientific, USA). FTIR spectra were recorded