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Preparation of nano-sized particles from bacterial cellulose using ultrasonication and their characterization

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ABSTRACT

As more applications for nano-sized natural particles are discovered, simple, environmentally friendly ways to produce these particles become more important. This work prepares and characterizes nano-size bacterial cellulose particles using ultrasonication. Pellicle from *nata de coco* containing nanofibers was broken up in an electrical blender, then sonicated using an ultrasonic probe at 20 kHz and 3 W/mL for 30, 60 and 90 min. Transmission electron microscope observations indicate the particles become nano-sized after 60 min ultrasound. The maximum decomposition temperature before sonication was 373 °C, decreased to 357 °C after 60 min ultrasonication. Moisture absorption of the 90 min sonicated particles film is 60% slower compared to non-sonicated particles. After ultrasonication, the crystallinity index of the cellulose decreases. The functional groups of non-sonicated and sonicated cellulose remain the same. This study promotes a potential method of fabrication of nano-sized particles from pure bacterial cellulose.

1. Introduction

Cellulose nanoparticles or nanocrystals from renewable sources are being developed with increasingly high specific strength and modulus, large specific surface area, high aspect ratio, environmental benefits and low cost (Ng et al., 2015). Cellulose nanocrystals were previously isolated from natural sources such as cotton (Farahbakhsh, Venditti, & Jur, 2014), bamboo, *Populus Tremula* (Tka, Jabli, Saleh, & Salman, 2017), wood (Siddiqui, Mills, Gardner, & Bousfield, 2012), and hemp fibers (Fan, Dai, & Yang, 2011). These contain cellulose along with hemicellulose, lignin, and other impurities. Pure nano-sized cellulose can also be obtained from bacterial cellulose (BC).

Acetobacter Xylinum is a bacterium commonly used to produce a BC with a porous tridimensional network immersed in a liquid matrix (Figueiredo, Figueiredo et al., 2015). Fabrication of the nanocellulose from BC is cheaper and simpler than isolation from natural fibers with their more mixed chemical composition. Due to its superior high mechanical strength, high crystallinity, nanofibrillar network structure, purity, and biocompatibility, BC has many potential applications. These have been extensively investigated in the biomedical field (Svensson et al., 2005), for component of audio membranes (Thi, Sugiyama, & Bulone, 2010), electronic paper (Ashjaran, Yazdanshenas, Rashidi, Khajavi, & Rezaee, 2013) and flexible organic light emitting diode displays (Ummartyotin, Juntaro, Sain, & Manuspiya, 2012). Some previous studies have characterized BC tridimensional networks containing the addition of Fe_3O_4 nanoparticles (Zheng et al., 2013), aliphatic polyester (Panaitescu, Frone, & Chiulan, 2016), polycaprolactone (Figueiredo, Silvestre, Neto, & Freire, 2015) and acrylic (Trovatti et al., 2010). BC also hold promise as fillers in composites. With so many potential uses of this material, finding suitable ways to produce nano-sized BC particles becomes a priority.

Many extraction methods have been developed to isolate nano-sized crystals from a variety of natural fibers (Johar, Ahmad, & Dufresne, 2012; Ng et al., 2015; Zhao et al., 2013). Acid hydrolysis methods are widely used for isolation of nanocrystalline cellulose (NCC) from wood (Zhao et al., 2013), cotton (Pandey, Takagi, Nakagaito, & Kim, 2015), bacterial cellulose (Wong, Kasapis, & Tan, 2009), *Nerium oleander* (Jabli, Tka, Ramzi, & Saleh, 2018) and wheat straw (Alemdar & Sain, 2008). However, acid hydrolysis uses chemicals that have the potential to harm health and damage the environment. Hence there is a demand for the development of environmentally friendly alternatives for fabrication of nano-sized particles. Ultrasonication is one widely accepted mechanical method for fabrication of NCC (Asrofi, Abral, Kasim, & Pratoto, 2017; Chowdhury & Sharifah Bee, 2016). This process has potential to isolate nanoparticles effectively (Sun, Fan, & Xiong, 2014). Sound energy produced by ultrasonication provides acoustic cavitation:

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the formation, growth, and collapse of bubbles in a liquid (Li, Yue, & Liu, 2012). The violent collapse of the bubbles produces microjets and shock waves on the surfaces of natural fibers suspended in a liquid, causing scission. The impact can breakup the relatively weak interfaces between the nanofibers, which are bonded to each other mainly by relatively weak forces such as van der Waals forces (Zhao & Feng, 2007). Thus the ultrasonic energy gradually disintegrates micron-sized natural fibers into nanofibers. The strong forces on celluloses resulting from ultrasonication results in depolymerization leading to molecular weight reduction (Wong, Kasapis, & Huang, 2012). Individual cellulose nanofibers have been successfully isolated from wood using high-intensity ultrasonication which resulted in alteration of fiber properties (Chen, Yu, Liu, Chen et al. 2011). Decreases in dimension of pure cellulose fibers change some of their properties, for example the crystallinity index of BC decreases after using a 1500 W ultrasonic probe for 5, 10 and 15 min (Li et al., 2012).

Several studies have focused on the de-polymerization of the BC molecular structure (Gao, Chen, Yang, Yang, & Han, 2010; Wong et al., 2009). The ultrasound sonication process was capable of yielding small-sized particles within 30 min of treatment but these were not yet nano-sized (Wong et al., 2009). As far as the authors are aware there are no published studies describing the production of nanoparticles from BC pellicles. In the present paper, nano-sized BC particles were successfully fabricated from pellicles using ultrasonication, without chemical pre-treatment. The BC morphology before and after ultrasonication were observed using both scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The non-sonicated and sonicated-BC properties measured are moisture absorption, thermal resistance, X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), and chemical structure.

2. Materials and methods

2.1. Materials

The original BC pellicle, a sheet of about 250 × 150 × 20 mm, was obtained from a small-scale industry in Padang, Indonesia that manufactures in the form of *nata de coco*, a popular sweet produced from the fermentation of a proprietary mixture of glucose, acetic acid and coconut water with *Acetobacter xylinum*. The mixture is heated to boiling point then cooled to room temperature over the space of a day. The mixture is then inoculated with the bacteria in a static closed container for one week. The resulting wet pellicle forms on the surface as a white jelly.

2.2. Preparation of BC nanoparticle

The wet pellicle was repeatedly pressed between two metal plates then soaked in distilled water until pH 7 was achieved. The clean pellicle that still contained water was cut with a knife into 10 mm^2 squares, with thickness of about 12 mm, then mixed with an equal weight of water. This mixture was then disintegrated using an electrical blender at 12000 rpm for 5 min. Then, the 200 mL of this mixture was sonicated using a 20 mm diameter SJIA 1200 W ultrasonic probe at 600 W (a 3 mL/watt ratio) at 100% amplitude and 20 kHz. The mixture was sonicated for durations of 30, 60 and 90 min. These time intervals were chosen as a previous study demonstrated that 30 min sonication at 150 W was not sufficient to break the cellulose down into nano-sized particles so it is hypothesized that this higher power and longer durations will be required to produce this effect (Wong et al., 2009). Temperature increase due to ultrasound was limited, using the built-in temperature control, so the mixture did not exceed 60 °C. The sonicated-BC was dried in a drying oven (Memert Germany, Model UN 55) at 50 °C for 20 h.

2.3. Characterization

2.3.1. SEM and TEM observation

The BC film samples were placed on the SEM sample stub. The sheet was coated with carbon followed by gold to reduce the electron charge. A model JFIB 4610 SEM from JEOL was used with an accelerating current of 15 kV and probe current of 8 mA to optimize observation of the surface morphology of the sample. The TEM sample was prepared by mixing BC powder with ethanol using an ultrasonic bath for 10 min. The sample was dropped onto a Holley carbon grid and air dried for 4 h. A Tecnai G2Twin model TEM from FEI with accelerating voltage of 200 kV was used to characterize the microstructure of the sample.

2.3.2. FTIR

The aim of FTIR characterization was to determine the functional groups of the bacterial celluloses. It was performed by using PerkinElmer Frontier equipment. The dried samples were formed into a sheet film and scanned at a frequency range of 4000–600 cm^{-1}.

2.3.3. X-ray diffraction

PANalytical Xpert PRO at 25 °C, 40 kV and 30 mA was used to perform X-ray diffraction testing. The samples were scanned from 2θ = 3° to 40°. The crystallinity index (CI) percentage was measured using Eq. (1) (Segal, Creely, Martin, & Conrad, 1958):

$$CI\,(\%) = \frac{(I_{200} - I_{am})}{I_{200}} \times 100 \quad (1)$$

where I_{200} is the intensity of the peak corresponding to cellulose I, and I_{am} is the intensity of the peak of the amorphous fraction.

2.3.4. Thermogravimetry analysis (TGA) and derivative (DTG) and differential thermal analysis (DTA)

TGA, DTG and DTA of samples were measured using a thermal analysis instrument DTG-60 from Shimadzu. The sample was input into the instrument which was set up with a nitrogen flow rate of 50 mL/min. The heating rate was 20 °C/min.

2.3.5. Moisture absorption

The BC films were dried in an oven (Universal Oven Memmert UN-55) until constant weight. The dried samples were stored in a closed chamber with 99% relative humidity (RH) at 25 °C for 10 h. The samples were taken out and weighed for each 30 min using a precision balance to the nearest 0.1 mg (Kenko). Percentage of moisture absorption in the sample was calculated using Eq. (2) (Jabli et al., 2018):

$$Moisture\ absorption\ (\%) = \frac{(w_h - w_o)}{w_o} \times 100 \quad (2)$$

where:

w_h is final weight and w_o initial weight of sample.

3. Results and discussions

3.1. Physical appearance of BC particles

Fig. 1 displays the appearance of BC suspension after scission with electrical blender and ultrasonic crusher for 30, 60, 90 min respectively. Transparency of the suspension increased with ultrasonic duration. No BC sediment was observed at the base of the suspension indicating good dispersion. The suspension containing the sample which had not received ultrasound treatment was less translucent (Fig. 1a) that the others due to scattering of light by macro- and micro-sized BC bundles. Fig. 1b also shows this; the black coloring of the TEM non-sonicated-BC is due to the lack of transmitted electrons through this BC sample.

Meanwhile Fig. 1d displays a SEM of a non-sonicated-BC film in which nano-porosity is evident but not scission of the fiber. These nano- and micro-sized porosities resulted from a three-dimensional network