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Fabrication of bacterial cellulose thin films self-assembled from sonochemically prepared nanofibrils and its characterization

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ABSTRACT

Bacterial cellulose (BC) film formation could be a critical issue in nanotechnology applications such as biomedical or smart materials products. In this research, purified pretreated BC was subjected to high intensity ultrasound (HIUS) and was investigated for the development of BC films. The morphological, structural and thermal properties of the obtained films were studied by using FE-SEM, AFM, FT-IR, XRD, TGA and DSC characterizations. Results showed that the most favorable purification treatment was the 0.01 M NaOH at 70 °C for 2 h under continuous stirring. The most suitable ultrasound operating conditions were found to be, 1 cm distance of ultrasonic probe from the bottom of the beaker, submerged in cold water bath cooling around 12 ± 2 °C. The power (25 W/cm^2), time (30 min), BC concentration (0.1% w/w), amplitude (20 μm) and frequency (20 kHz) were maintained constant.

1. Introduction

Bacterial cellulose (BC), also known as microbial cellulose, is a promising natural polymer synthesized by certain bacteria such as *Gluconacetobacter xylinus*. Even though it is chemically identical to plant cellulose, its supramolecular structure and high purity cellulose content demonstrates unique properties such as high crystallinity (63–71%), high water holding capacity (up to 200 times of its dry mass) and excellent mechanical strength. Young's modulus of BC sheets is in the range of 15–40 GPa, while that of a BC single fibril up to 114 GPa [1,2]. BC binds large amounts of water – up to 99 wt% during its biosynthesis in the aqueous culture media [3]. Several studies have focused on the utilization of BC as reinforcement material, in biomedical applications [4,5] or cellulose based smart material devices [6,7].

The isolation of cellulose nanoparticles without serious degradation, at low costs and using an environmentally friendly method is constantly being sought. Recently the application of ultrasound assisted extraction of plant polysaccharides [8–10], ultrasound assisted delignification [11,12], ultrasound assisted size reduction of cellulose [13] or intensification of enzymatic hydrolysis [14,15] has gained much interest.

Wang and Cheng [16] examined the use of high intensity ultrasound to isolate fibrils from four cellulose sources: regenerated cellulose (lyocell), pure cellulose fiber, microcrystalline cellulose and pulp fiber. Wong et al. [17] investigated the effect of ultrasound irradiation time on the depolymerization of plant and bacterial cellulose. Tischer et al. [18] subjected BC pellicles to a high power ultrasonic treatment for 15, 30, 60 and 75 min; these were carried out in an ice bath for tissue engineering applications.

The aim of the present study was to examine the effect of two main ultrasound operating conditions, i.e. the effect of temperature and distance of ultrasonic probe from the bottom of the beaker on morphological, structural and thermal properties of ultrasound defibrillated BC films. BC was previously pretreated in chemically mild conditions in order to: (i) maintain its native cellulose I structure, (ii) remove bacterial cell debris and (iii) to emphasize the subsequent ultrasound defibrillation treatment. The overall purpose of this research was to develop a method of obtaining highly crystalline and thermally more stable BC films suitable for energy harvesting devices, such as piezoelectric strain sensors.

2. Experimental section

2.1. Purification of nata de coco

Nata de coco cubes (PT. Cocomas, Indonesia) were washed and soaked in distilled water (water purification, WP) until the pH was

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neutral (pH 5–7) to remove the citric acid and other components of syrup added for preservation. In order to improve purity of BC, nata de coco was further purified by alkaline treatment to remove any remaining bacterial cell debris, microorganisms and other soluble polysaccharides. After being water purified, the nata de coco cubes were immersed in 2.5 wt% NaOH (6×10^{-3} M) overnight. This process will be hereafter referred as one step purification (OSP). Another sample was prepared in the same way and successively treated with 2.5 wt% NaOCl (3.4×10^{-3} M); hereafter referred to as two step purification (TSP). OSP and TSP treatments were carried out by adopting the methodology as reported by Gea et al. [19]. A third sample was prepared by warming nata de coco in 0.01 M NaOH at 70 °C for 2 h under continuous stirring; this will be called as 0.01 M NaOH purification.

Subsequently, nata de coco cubes were rinsed under distilled water at room temperature (RT) until the pH of the water became neutral. Once neutral pH was reached, BC was mechanically ground and homogenized in a 400 W blender for 10 min (medium speed, 5 times × 2 min with 5 min intervals). Afterwards, blended BC was poured into confined space silicon trays, and dried via solvent evaporation in an oven at 50 °C, for two to three days.

2.2. Ultrasonication of bacterial cellulose films

After drying, the BC films were cut and were redispersed (0.1% w/w, immersed in 80 mL distilled water) and subjected to further grinding, this time with a hand blender for 20 s, prior to ultrasonication. Sonication was directly applied at low frequency (20 kHz) using an ultrasonic horn (Tesla 150 WS) with a tip diameter of 18 mm immersed in the suspension. HIUS treatment of BC performed using three levels of temperature; room temperature or no water bath (NoW), cold water bath (CW) and ice water bath (IW) and two levels of ultrasonic probe distance from the bottom of a beaker; 1 cm and 4 cm respectively to evaluate the effect of cavitation active zones, local circulation and ultrasonic intensity distribution on BC microfibrils. The ultrasonic probe was placed close to the surface (4 cm distance) and close to the bottom (1 cm distance) of a 100 mL cylindrical beaker. A 7.4 cm distance (1 wavelength of ultrasound in water) was not possible to be examined, owing to the height of the beaker. When cold water bath was used for cooling, the temperature was about 12 ± 2 °C, whereas it was around 5 ± 1 °C when ice bath was used. Frequency (20 kHz), amplitude (20 μm), power (25 W/cm^2) and ultrasonication time (30 min) were kept constant.

2.3. Preparation of bacterial cellulose films

Resulting ultrasound colloid dispersions were left to stand overnight. Thereafter, the liquid supernatant phase (around 40 ml) was collected from ultrasound treated BC, poured again into silicon trays and dried similarly through solvent evaporation, for a second time. The dried, ultrasound reconstituted BC films were carefully removed and stored in plastic bags until further analysis. Due to the drying method, BC micro/nanofibrils were randomly oriented, which assumes isotropic characteristics for the BC film.

2.4. Characterizations

2.4.1. Field Emission Scanning Electron Microscopy (FE-SEM)

FE-SEM micrographs were obtained using a Zeiss ULTRA Plus (Oberkochen, Germany) instrument at an acceleration voltages of 1 and 2 kV. The suspensions were filtered through a gilded PC membrane and dried for 1 h at room temperature. All samples were coated with a highly conductive film of gold by Bal-Tec SCD 500.

2.4.2. Atomic force microscopy (AFM)

AFM experiments were performed using a MultiMode atomic force microscopy 8 with a Nanoscope Veeco V controller (Bruker Nano Surfaces, Santa Barbara, CA, USA) instrument. Small cut pieces of dried BC films were placed on magnetic slides and the scans were obtained in tapping mode using a V-shape Silicon Nitride cantilever. Prior to the measurements, the tip radius and geometry of the tip were calculated. Two repetitions of imaging (5×5 μm and 1×1 μm) were carried out. These experiments were implemented in an environment with constant relative humidity and temperature. Width was measured by image analysis using ImageJ software (ImageJ 1.46, National Institute of Health (NIH), USA).

2.4.3. Fourier Transform Infrared Spectroscopy (FT-IR)

FTIR spectra of the BC films were obtained using a Jasco FT/IR6300 equipped with an ATR PRO 470-H spectrometer. A total of 50 cumulative scans were taken per sample with a resolution of 4 cm^{-1}, in the frequency range of 4000–400 cm^{-1}, in absorbance mode. ATR correction was applied in each measurement.

2.4.4. X-ray powder diffraction (XRD)

The X-ray diffraction patterns were recorded at room temperature in the 5–80° 2θ angle using an MPD Pro Panalytical diffractometer equipped with an Xcelerator linear detector. Cu-K (1.54056Å) radiation was used with the 0.016° recording step and the 1000 s per step counting time. The samples were powdered before the analysis.

XRD peak height method, developed by Segal and coworkers [20], was used to determine the crystallinity index (Cr.I) by the following equation (Eq. (1))

$$\text{Cr.I} = \frac{I_{200} - I_{\text{am}}}{I_{200}} 100 \tag{1}$$

where I_{200} is the peak intensity at the (200) ($2\theta \approx 22.5°$) plane, and I_{am} is the minimum intensity (amorphous scatter) at the valley between (200) and (110) peaks ($2\theta \approx 18°$).

The interplanar distances of the crystallites (d-spacings) were calculated with Bragg's law,

$$\lambda = 2d \sin\theta \tag{2}$$

where λ is the wavelength of the X-rays, d is the spacing between the crystal planes in the atomic lattice, and θ is the Bragg angle between the incident ray and the scattering planes [21].

The crystallite sizes at d_1, d_2 and d_3, the three main peaks respectively, were determined using the Scherrer equation [22]:

$$\text{Cr.S.} = \frac{0.9\lambda}{H_{hkl} \cos\theta_{hkl}} \tag{3}$$

where Cr.S. is the crystallite size, λ is the wavelength of incident X-rays, H_{hkl} is the full-width at half-maximum (FWHM) and θ_{hkl} is the Bragg angle at the corresponding lattice plane.

2.4.5. Thermal analysis

Thermal analysis techniques, thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) were used to measure the thermal stability behavior of BC films. Thermogravimetric (TG) data were acquired between 0 and 500 °C using a Perkin Elmer Diamond thermal analyzer under nitrogen purging gas (100 cm^3 min^{-1}) at a heating rate of 2 °C min^{-1}. Differential scanning calorimetry (DSC) analysis was carried out on a Netsch DSC204 instrument under nitrogen purging gas (30 cm^3 min^{-1}) at a heating/cooling rate of 2 K min^{-1}. Temperature and enthalpy were calibrated using the melting transition of standard materials (Hg, In, Sn).