EFFECT OF NEUTRALIZATION ON STRUCTURE AND PROPERTIES OF CELLULOSE NANOWHISKERS DERIVED FROM BACTERIAL CELLULOSE

Nunin Aksornthong, Nattaya Tawichai, and Nattakan Soykeabkaew*

School of Science, Mae Fah Luang University, Thasud, Chiang Rai 57100, Thailand
*e-mail: n.soykeabkaew@sci.mfu.ac.th

Abstract

Bacterial cellulose nanowhiskers (BCNWs) were prepared by acid hydrolysis of bacterial cellulose (BC) using 50% w/v sulfuric acid at 50°C for 48 hours. Transmission electron microscope (TEM) revealed that the continuous BC fiber network transformed into the isolated rod-like nanocrystals of the BCNWs with a diameter and length of averaged 28.18±1.99 nm and 637.61±147.10 nm, respectively, after acid hydrolysis. Both X-ray diffraction (XRD) patterns of native BC and BCNWs showed the cellulose I characteristic peaks. It was found that the acid hydrolyzed BCNWs possessed the higher perfection of the crystal lattice or crystallinity index as compared to the native BC. This indicates higher mechanical properties, hence, a higher reinforcing potential of the BCNWs as reinforcement in nanocomposite materials. The resulted BCNWs, however, had a substantial reduction in thermal stability confirmed by thermogravimetric analysis (TGA). With further neutralization step, the thermal stability of BCNWs can be greatly recovered. This allows the effective use of the neutralized BCNWs in processing of nanocomposite materials.

Keywords: bacterial cellulose, acid hydrolysis, TEM, TGA, XRD

Introduction

Cellulose is the most abundant biopolymer found in nature, as it is the major cell-wall component of plants. Plant-derived cellulose has been widely used as reinforcement in polymeric matrices (Zhao et al. 2007). Besides cellulose from plants, cellulose is also secreted extracellularly by some bacterial species known as bacterial cellulose (BC). Plant cellulose and BC have the same chemical structure although they have different structural organization and mechanical properties (Martínez-Sanz et al. 2010). Bacterial cellulose has found many applications in paper, textile, food industry as well as a biomaterial in cosmetics and medicines due to its unique structure and properties, i.e. high purity, high crystallinity, high mechanical strength and good biocompatibility (Rosa et al. 2010). The excellent mechanical properties of BC have also led to its use as reinforcing agents in composite materials (Gea et al. 2011).

Nanometer-sized single crystals of cellulose commonly referred to as whiskers, nanowhiskers or nanofibrils can be obtained from various natural fibers and some sea animals. The extraction of cellulose whiskers from renewable sources has gained more attention in recent years due to their exceptional mechanical properties (high specific strength and modulus), large specific surface area, high aspect ratio, environmental benefits and low cost (Rosa et al. 2010). Different approaches have been applied to prepare cellulose nanowhiskers. All of them lead to different types of nanofibrillar materials, depending on the cellulose raw material and its pre-treatment, as well as the disintegration process itself. Sulfuric acid

hydrolysis of cellulose is a well-known process used to remove amorphous regions (Marta et al. 2010). However, it significantly decreases the thermal stability of cellulose nanowhiskers. Since typical processing temperatures for thermoplastics rise above 200°C, the thermal stability of these crystals is a key factor for them to be used as effective reinforcing materials. After acid hydrolysis, thermal stability of cellulose nanowhiskers can be recovered by neutralizing with strong bases such as sodium hydroxide (Rosa et al. 2010).
The objective of this work was to study the effect of neutralization on structure and properties of cellulose nanowhiskers derived from bacterial cellulose. The resulting cellulose nanowhiskers were characterized by TEM, TGA, and XRD.

Methodology

Preparation of bacterial cellulose nanowhiskers by acid hydrolysis of bacterial cellulose
Firstly, a bacterial cellulose sheet was prepared by compressing a bacterial cellulose pellicle which was sandwiched between woven metal sheets (325 mesh) at 115°C for 5 min using compression machine. This dried bacterial cellulose sheet (BC sheet) was used as a raw material for preparation of bacterial cellulose nanowhiskers (BCNWs). The acid hydrolysis was performed using 50% (w/v) sulfuric acid, at a cellulose/acid ratio of approximately 8 g/L, at 50°C for 48 hours. The BCNWs were obtained as a precipitate collected from 15 centrifugation cycles at 12,500 rpm and 15°C for 20 min. The pH of the samples was measured after the centrifugation, being around pH 2. In order to adjust pH of BCNWs to neutral, all the samples were re-suspended in deionized water and neutralized using 0.5% and 5% (w/v) NaOH solution until neutral pH and subsequently centrifuged to obtain the final product as a partially hydrated precipitate.

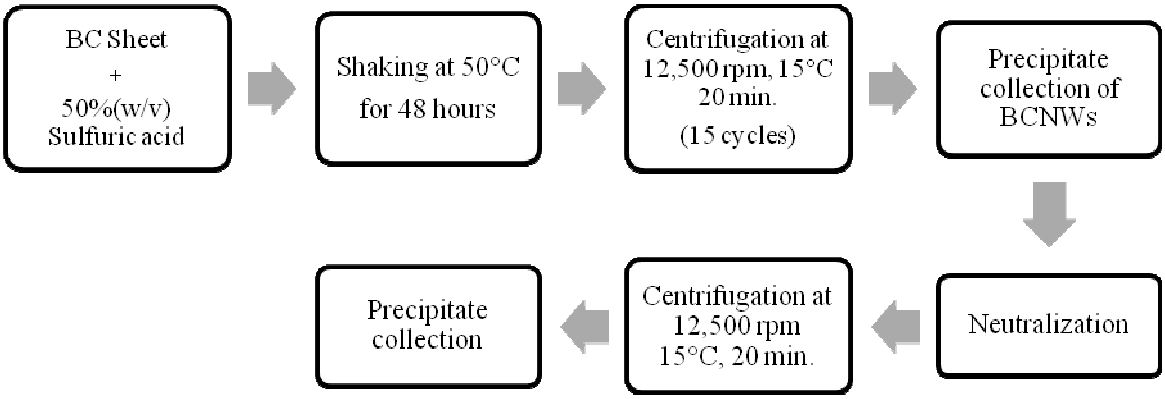

Figure 1 Flow chart of bacterial cellulose nanowhiskers (BCNWs) preparation

Transmission Electron Microscopy (TEM)
One drop (8 µL) of 0.002% aqueous suspension of BCNWs was allowed to dry on a carbon coated grid (200 mesh). The nanocrystals were stained with uranyl acetate. TEM was performed using a JEOL, model JEM-2010, equipped with a digital Bioscan (Gatan) image acquisition system at 80 kV. Lengths and diameters of BCNWs were measured from several TEM micrographs. The reported dimension was averaged from measurements of 10 BCNWS.

Thermal Gravimetric Analysis (TGA)
Thermal analysis was carried out using a Mettler Toledo (851e). Samples of approximately 5 mg were used. All the experiments were conducted using the constant heating rate of 5°C/min, from 25 to 600°C, under a nitrogen atmosphere. The onset degradation temperatures of all samples were determined.

X-ray diffraction (XRD)
X-ray diffraction patterns were detected using Cu Kα radiation, generated with X'pertPro MPD (Philips, Netherlands) at 40 kV, 20 mA. The X-ray beam was operated in reflection mode and the samples were examined over the angular range (2θ) of 5° to 35° with a step size of 0.02° and a count time of 4s per point. The crystallinity index (CI) was determined by the method reported by Wang et al. (2007):

$$\mathrm{CI} = \frac{\sum A_{Crystal}}{A_{Total}} \times 100$$

where A_{Total} is the sum of the areas under all the diffraction peaks and $\sum A_{Crystal}$ is the sum of the areas corresponding to crystalline peaks.

Results and discussion

Bacterial cellulose (BC) fibers contain crystalline cellulosic domains but also noncrystalline (amorphous) domains located at the surface and form weak spots along their main axis (Eichhorn et al. 2010). Since the hydrolysis rate of amorphous region is much higher than that of the crystalline region in cellulose, under controlled acid condition, hydrolysis may remove the amorphous region of BC fibers and leave the crystalline region almost untouched in a rod-like nanocrystal form (Zhao et al. 2007). In this present work, bacterial cellulose nanowhiskers (BCNWs) were prepared by acid hydrolysis of bacterial cellulose (BC) sheet using 50% w/v sulfuric acid at 50°C for 48 hours. The obtained rod-like nanocrystals of BCNWs after acid hydrolysis were confirmed by transmission electron microscope (TEM) analysis as displayed in Figure 2b. The acid hydrolysis transformed the continuous BC fiber network (native BC) (Figure 2a) into the isolated BCNWs with a smaller diameter and length approximately 28.18±1.99 nm and 637.61±147.10 nm, respectively (estimated from several measurements on TEM micrographs).

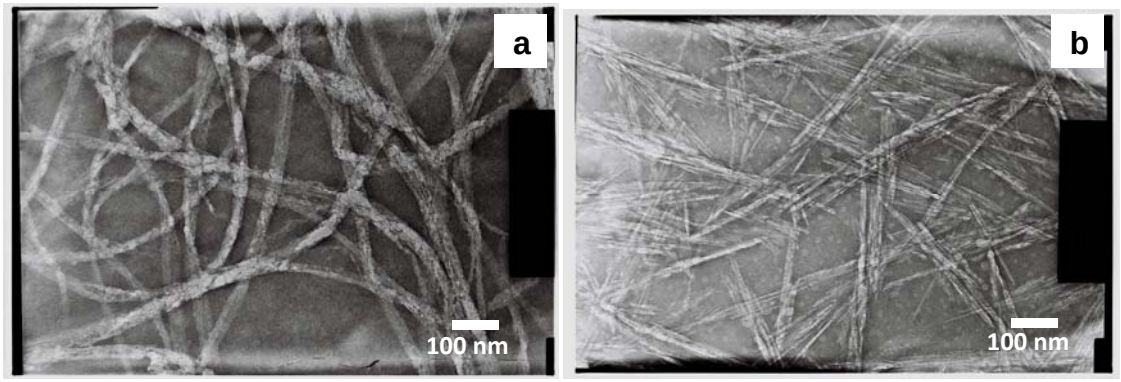

Figure 2 Transmission electron micrographs of (a) bacterial cellulose nanofibres (native BC) and (b) bacterial cellulose nanowhiskers (BCNWs) after acid hydrolysis of native BC with 50% v/v sulfuric acid for 48 hours

For reaction mechanism, Lu and Hsieh (2010) described that acid hydrolysis of cellulose in sulfuric acid involves rapid protonation of glucosidic oxygen or cyclic oxygen by protons from the acid, followed by a slow splitting of glucosidic bonds induced by the addition of water. Besides chain scission, hydrolyzing cellulose with sulfuric acid also involved esterification of the hydroxyl groups and yielded acid half-ester or the so-called 'cellulose sulfate'. The presence of the induced sulfate groups on the nanocrystal surfaces can greatly improve the separation of nanocrystals by charge repulsion. Due to their strong hydrogen bonding, cellulose nanocrystals normally have high tendency to form bundles or aggregates. Therefore, this is the major challenge in developing the cellulose nanocrystals as a reinforcement in effective nanocomposites regarding to the difficulty in their homogeneous dispersion within the polymer matrix (Eichhorn et al., 2010).

With the induced sulfate groups on the surfaces, however, the thermal stability of BCNWs was considerably decreased after acid hydrolysis as shown in Figure 3. From the TGA curves, onset degradation temperature of the native BC decreased from 294°C to 122°C for the acid hydrolyzed BCNWs. Conversely, with the additional neutralization step, the thermal stability of BCNWsN can be significantly improved and the onset degradation temperature increased to 271°C. Since typical processing temperatures for thermoplastics are normally above 200°C, this BCNWsN with improved thermal stability could be effectively used in the nanocomposite materials as a reinforcement.

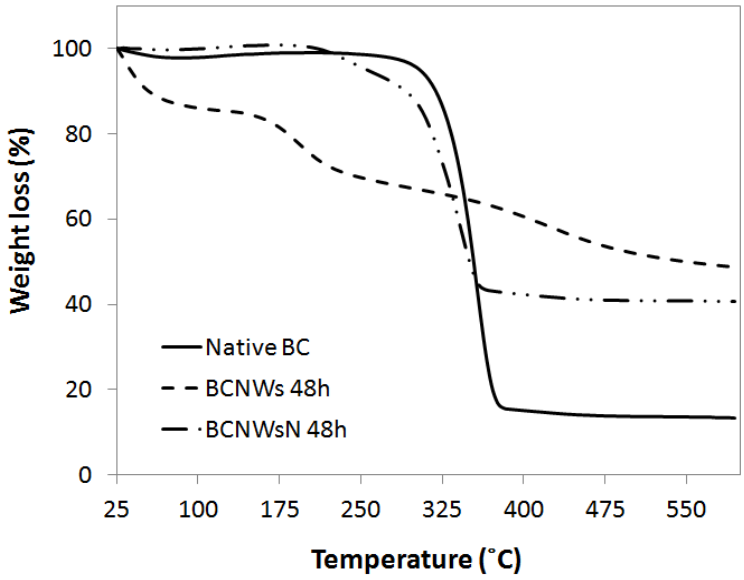

Figure 3 TGA curves of bacterial cellulose nanofibres (native BC), bacterial cellulose nanowhiskers after acid hydrolysis time of 48 hours (BCNWs 48 h) and BCNWs 48h after neutralization (BCNWsN 48h)

Table 1 Crystallinity index (CI) and crystallite sizes ($D_{(101)}$, $D_{(10\bar{1})}$ and $D_{(102)}$) determined from the XRD patterns of bacterial cellulose (native BC) and the obtained nanowhiskers (BCNWsN 48h)

Sample	CI (%)	$D_{(101)}$(nm)	$D_{(10\bar{1})}$(nm)	$D_{(002)}$(nm)
Native BC	78.54	0.35	1.63	0.61
BCNWsN 48h	80.35	1.46	1.53	0.31

In view of crystalline structure, both X-ray diffraction patterns of native BC and BCNWsN (Figure 4) showed three cellulose I characteristic peaks at 2θ = 14.5°, 16.4°, and 22.5° (corresponding to 101, $10\bar{1}$ and 002 crystal planes, respectively) (Martinez-Sanz et al. 2011). The peaks of BCNWsN, particularly at 22.5° of the (002) crystalline plane, becomes sharper, indicating the higher perfection of the crystal lattice or crystallinity of the BCNWsN than the native BC. The crystallinity index (CI) of the native BC and BCNWsN were 78.54% and 80.35%, respectively. This could imply to the higher mechanical properties of the BCNWsN, hence, providing a higher reinforcing effect if incorporated in nanocomposite materials. Interestingly, an increase of the crystallite size in the (101) crystalline plane of the BCNWsN is observed (see Table 1). This probably indicates that the smaller or more defective crystals are being digested by means of the sulfuric acid treatment as would be expected.

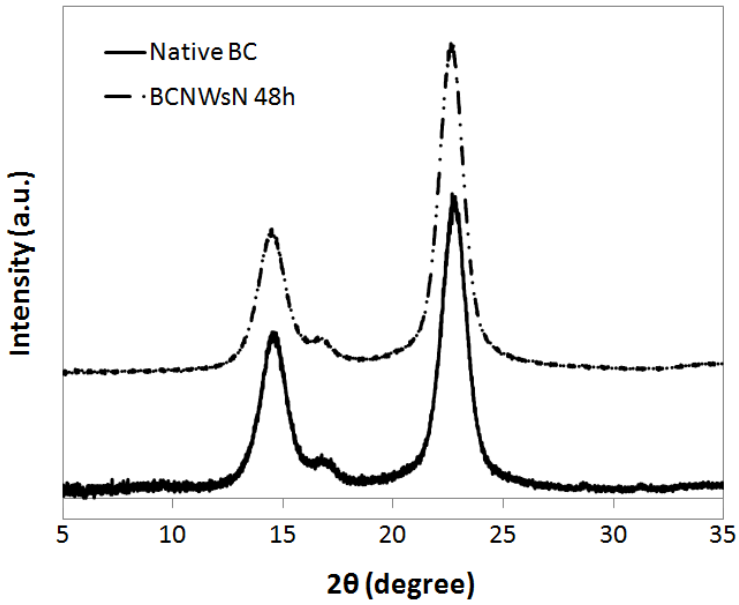

Figure 4 X-ray diffraction patterns of bacterial cellulose nanofibres (native BC) and bacterial cellulose nanowhiskers after acid hydrolysis time of 48 hours and neutralization (BCNWsN 48h)

Conclusions

Bacterial cellulose (BC) was treated with 50% w/v sulfuric acid at 50°C for 48 hours to prepare bacterial cellulose nanowhiskers (BCNWs). The acid hydrolysis transformed the continuous BC fiber network (native BC) into the isolated rod-like nanocrystals (BCNWs) possessing a smaller diameter and shorter length. For crystalline structure, the native BC and BCNWs showed three cellulose I characteristic peaks at 2θ = 14.5°, 16.4°, and 22.5° in their XRD patterns. The XRD peaks of the BCNWs become sharper than the native BC, indicating the higher perfection of the crystal lattice of the acid hydrolyzed BCNWs. This would lead to the higher mechanical properties and reinforcing effect of BCNWs if incorporated in nanocomposite materials. However, a considerable reduction in the thermal stability of the acid hydrolyzed BCNWs was as well observed. Additional neutralization step can significantly improve the thermal stability of the BCNWs. The BCNWs with recovered thermal stability could be further used as an effective reinforcement in the nanocomposite materials.

Acknowledgements

Financial support from Mae Fah Luang University, Thailand is gratefully acknowledged. The authors wish to thank Dr. S. Wongsakul, School of Agro-Industry, Mae Fah Luang University for the *A. xylinum* TISTR 975 and Dr. A. Owatworakit, Mr. N. Yodsuwan, Ms. N. Laosat and Ms. A. Ngaokla for the cultured bacterial cellulose.

References

1. Eichhorn SJ, Dufresne A, Aranguren M, Marcovich NE, Capadona JR, Rowan SJ, Weder C, Thielemans W, Roman M, Renneckar S, Gindl W, Veigel S, Keckes J, Yano H, Abe K, Nogi M, Nakagaito AN, Mangalam A,Simonsen J, Benight AS, Bismarck A, Berglund LA, Peijs T (2010) Review: current international research into cellulose nanofibres and nanocomposites. Journal of Materials Science 45:1-33.
2. Gea S, Reynolds CT, Roohpour N, Wirjosentono B, Soykeabkaew N, Bilotti E, Peijs T (2011) Investigation into the structural, morphological, mechanical and thermal behaviour of bacterial cellulose after a two-step purification process. Bioresource Technology 102:9105-9110.
3. Lu P, Hsieh YL (2010) Preparation and properties of cellulose nanocrystals: Rods, spheres, and network. Carbohydrate Polymers 82:329-336.
4. Martínez-Sanz M, Lopez-Rubio A, Lagaron JM (2011) Optimization of the nanofabrication by acid hydrolysis of bacterial cellulose nanowhiskers. Carbohydrate Polymers 85:228-236.
5. Martínez-Sanz M, Olsson RT, Lopez-Rubio A, Lagaron JM (2010) Development of electrospun EVOH fibers reinforced with bacterial cellulose nanowhiskers. Part1. Characterization and method optimization. Cellulose 18:335–347.
6. Rosa MF, Medeiros ES, Malmonge JA, Gregorski KS, Wood DF, Mattoso LHC, Glenn G, Orts WJ, Imam SH (2010) Cellulose nanowhiskers from coconut husk fibers: Effect of preparation conditions on their thermal and morphological behavior. Carbohydrate Polymers 81:83-92.
7. Zhao H, Kwak JH, Zhang ZC, Brown HM, Arey BW, Holladay JE (2007) Studying cellulose fiber structure by SEM, XRD, NMR and acid hydrolysis. Carbohydrate Polymers 68:235-241.