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Morphology and Crystallinity of Sisal Nanocellulose after Sonication

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Abstract. Different preparation methods on the natural fibers resulted in different morphology. However, the relationships between type of natural fibers, preparation methods and the morphology of produced nanocellulose could not be exactly defined. The sisal nanocellulose was presently prepared by alkalization and bleaching followed by sonication to verify changes in the morphology and crystallinity of nanocellulose related to the formation mechanism. The extracted microcellulose was subjected to scanning electron microscopy (SEM) and x-ray ⁶ fraction (XRD) analysis. The isolated cellulose nanospheres were examined with respect to morphology by SEM and transmission electron microscopy (TEM) and, to crystallinity by electron diffraction analysis. Bleaching after alkalization made the microfibrils clearly separated from each other to the individual fiber whose width of the single fiber was ranging from 6 to 13 μm . The XRD crystallinity index (C_I) of microcellulose gradually increased after the chemical treatments; 83.12% for raw sisal fiber, 88.57% for alkali treated fiber and 94.03% for bleached fibers. The ultrasonic agitation after bleaching that was carried out at 750 Watt, 20 kHz and amplitude of 39% for 2 h produces homogeneous cellulose nanospheres less than 50 nm in diameter with relatively low crystallinity. The electron diffraction analysis confirmed that the low crystallinity of produced nncellulose is related to the effect of chemical treatment done before sonication.

Keywords: Sisal Nanocellulose, Crystallinity, Sonication

INTRODUCTION

Natural plant fibers are abundantly available in tropical regions in this earth. They are low cost, biodegradable, renewable and highly potential use for reinforcing the polymer composite. Up to date, green composite technology is being developed on a large scale in order to support an international issue in terms of environmental concern. Besides, natural plant cell wall is the main source of cellulose [1] and the isolated nanocrystalline cellulose has played a crucial role in the development of nanotechnology. These have a motivated research interest in the field of nanotechnology cellulose.

Numerous efforts to prepare cellulose nanocrystals (CNC) from natural fiber have been investigated by various methods since last few decades and resulted in some different morphologies in nano-scale (less than 100 nm) such as whisker or needle-like structure [2-5], sphere [6-10] and rice-like structure [11]. They are classified as the nanomaterial.

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Cellulose nanowhisker with length ranging from 100 to 500 nm and less than 10 nm diameter has been isolated from kenaf fiber by acid hydrolysis after alkalization and bleaching followed by homogenizing in an Ultra Turax homogenizer [2]. A similar chemical treatment to Ref. [2] without homogenizing subjected to kenaf fiber has also produced cellulose nanowhisker with 100 to 1400 nm length and 7 to 80 nm diameter [3]. Again, extraction nanocellulose from kenaf fiber by the chemical treatment revealed few hundred nanometer rod or sphere. The formation of cellulose nanospherical is caused by longer stirring during hydrolysis [4]. Besides, our previous study has reported the isolated cellulose nanocrystalline from sisal fiber by the chemical method; repeated alkalization and bleaching followed by acid hydrolysis produce homogeneous nanowhiskers (~50 nm width and ~500 nm length) of cellulose nanocrystals. But, the unrepeated ones produce double morphologies; cellulose nanowhisker and nanosphere [5].

Moreover, uniformity of particle size (~ 50 nm) of spherical nanocrystalline cellulose obtained from natural cotton fiber strongly depends on the ratio of acid hydrolysis solutions H₂SO₄ and HCl, acid concentration and reaction temperature, and also ultrasonic agitation condition [6]. Ultrasonic acid hydrolysis (H₂SO₄ and HCl) with mechanical stirring has been used to develop the synthesizing cellulose nanospheres with the size ranging from 60 to over 570 nm [7]. Preparation of cellulose nanospheres (200 – 500 nm) has also been developed by Nadanathangam *et al.* [8] by hydrolyzing microcrystalline cellulose (MCC) prepared from cotton using anaerobic microbial consortium. In addition, microcrystal cellulose (MCC, ~ 50 µm) dissolved in concentrated sulfuric acid (50-70 wt. %) with the ratio of acid/MCC: 10/1 by continuous stirring for 1 h and followed by sonication and hydrolysis in cellulolytic enzymes has produced amorphous cellulose nanospheres whose diameter is ranging from 50 to 200 nm [9]. Enzymatic hydrolysis followed by sonication has been developed to prepare cellulose nanospheres with less than 100 nm diameter from waste cotton [10].

Based on the previous studies, the difference in morphology of nanocellulose is related to the differences in preparation methods for the isolation of nanocellulose from natural fibers. However, the relationship between type of natural fiber, isolation (preparation) method and the morphology of produced nanocellulose could not yet be exactly defined. Thus, the formation mechanism, especially for morphology of cellulose nanosphere still remains unclear. In this work, we have prepared nanocellulose from sisal fiber by alkalization and bleaching coupled with sonication. Morphology and crystallinity of produced nanocellulose were investigated and compared to the other previous results to verify changes in morphology of the nanocellulose related to the formation mechanism.

EXPERIMENTAL

Sisal fibers (*Agave sisalana*) purchased from Balittas, Malang, Indonesia were cut into about 5 cm. Approximately 5 g sisal fibers were soaked in 300 ml 6% NaOH and heated at 100 °C for 3 h by continuous magnetic stirring. The alkalinized fibers were neutralized with 1% CH₃COOH and washed in running distilled water. Fiber treatment was continued by bleaching in (1% NaOH + 3% H₂O₂) at 70°C for 2 h also by continuous stirring. Alkalization and bleaching were repeated in 3 times. Few amounts of bleached fibers were soaked in ethanol and subjected to sonication at 750 Watt, 20 kHz and amplitude of 39% for 2 h.

The extracted microcellulose (bleached fiber) was subjected to scanning electron microscopy (SEM, JSM-6510LA) and x-ray diffraction (Philips X'Pert MPD). The nanocellulose resulted from sonication was examined with respect to morphology by SEM and transmission electron microscopy (TEM, JEM-1400) and, to crystallinity by electron diffraction analysis. For both of SEM and TEM observations, the specimen was prepared by dispersing a small amount of the nanocellulose in ethanol and dropped onto a C-tape substrate for SEM specimen and C-coated Cu-grid substrate for TEM specimen. They should be left in air for about 15 min. Prior to SEM observation, the specimen was metallic coated (Pt coater, JEC-3000FC) to increase the electrical conductivity of the non-conductive specimen, but it was not required for TEM specimen.

RESULTS AND DISCUSSION

Morphology of micro- and nano-cellulose

Almost all previous works have used cotton fiber for preparing the crystalline cellulose nanosphere [6, 7, 9, 10]. No report that use sisal or kenaf fiber was found. It may be due to cellulose contained in cotton (85-90%) is higher than that in sisal (66-78%) and kenaf (45-57%) fibers [1].

The natural fibers obtained from plant fibers mainly comprise of cellulose, hemicellulose, lignin, pectins and waxes. Cellulose is present in crystalline and amorphous phases in which the concentration distribution of crystalline phase is higher than that of the amorphous phase. In the fiber structure cellulose is embedded in a hemicellulose-lignin matrix with varying composition which differs from one fiber type to another. This is often called a natural composite [1].

Lignin is amorphous in nature and soluble in hot alkali, but cellulose is resistant to strong alkali [1, 12]. When the sisal fibers were treated in 6% NaOH at 100 °C for 3 h the microcellulose or cellulose microfibrils were still tightly bound with each other. Repeated alkalization for 3 times led to the partial separation of the microfibrils from the fiber bundles, indicating most of lignin dissolved in alkali solution. (Fig. 1a, see arrows). The microfibrils seem to completely separate from fiber bundle to be individual fiber after repeated bleaching for 3 times (Fig. 1b) with an average width of microfibril ranging from 6 to 13 μm. However, after exposed to the air and dry, the bleached fibers form aggregate cellulose microfibrils in which the individual fiber is difficult to be separated from each other (Fig. 1c), because the existence of hydroxyl groups in cellulose causes a reaction with water by hydrogen bonding either

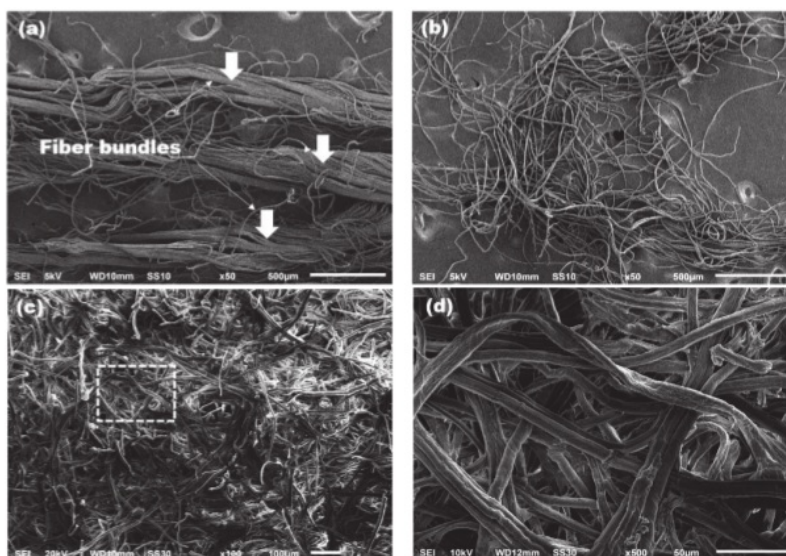


FIGURE 1. SEM micrographs of repeated alkalization for 3 times of sisal fibers in 6% NaOH at 100 °C for 3 h (a), repeated bleaching for 3 times after alkalization showing complete fibrillation of sisal microfibrils (b), aggregate microcellulose after drying (c) and magnified image of a rectangular area in Fig. 1c (d).

at the surface or in the bulk of the microfibrils. Molecule chains of crystalline cellulose were connected to form a larger unit of microfibrils through hydrogen bonding as well [13]. Morphology of aggregate cellulose microfibrils is clearly seen in a magnified image (Fig. 1d) of a rectangular area in Fig. 1c. Besides, SEM-energy dispersive x-ray spectroscopy (SEM-EDS) analysis results confirmed that no residual alkali and other elements on the cellulose microfibrils were identified, suggesting that the microfibrils surface was free from residual elements.

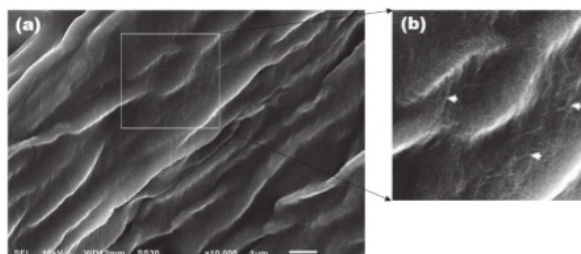


FIGURE 2. (a) SEM image of a microfibril surface after repeated bleaching and (b) a magnified image of a rectangular area in (a) showing very fine nonfibrils.

In general, a fiber was formed by three layers of cell wall which are formed and arranged by semi-crystalline cellulose microfibrils reinforced hemicellulose-lignin matrix with varying compositions [1]. According to magnified SEM images (Fig. 2) taken from a single microfibril of repeated bleaching fibers, surface morphology clearly shows very fine nanofibrils with a typical diameter of around few tens of nanometers (Fig. 2b, see arrows). This is in accordance with what have been described in the literature [1]; i.e. such microfibrils/nanofibrils have a diameter of about 10-30 nm typically and are made up of 30-100 cellulose molecules in extended chain conformation and provide mechanical strength to the fiber.

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Sonication after alkalization and bleaching at the condition as mentioned in the previous section resulted in spherical morphology in nano scale < 50 nm in diameter as represented in SEM (Fig. 3a) and TEM (Fig. 3b and 3d) micrographs. Confirmation of spherical morphology was done by taking some TEM images of the nanocellulose from the entire specimen areas. However, some regions showed the nanoparticles of cellulose nanosphere are distributes within the amorphous matrix (Fig. 3d) which is considered as residual amorphous cellulose. It is known that cellulose contained in the natural fibers actually consists of crystalline and amorphous phases. Agglomeration of cellulose nanospheres (Fig. 3b) was due to naturally hydrophilic of cellulose as reported by other research groups [6, 7, 9]. An electron diffraction pattern (Fig. 3c) obtained from a circle in Fig. 3b reveals relatively weak diffraction rings due to the existence of residual amorphous cellulose.

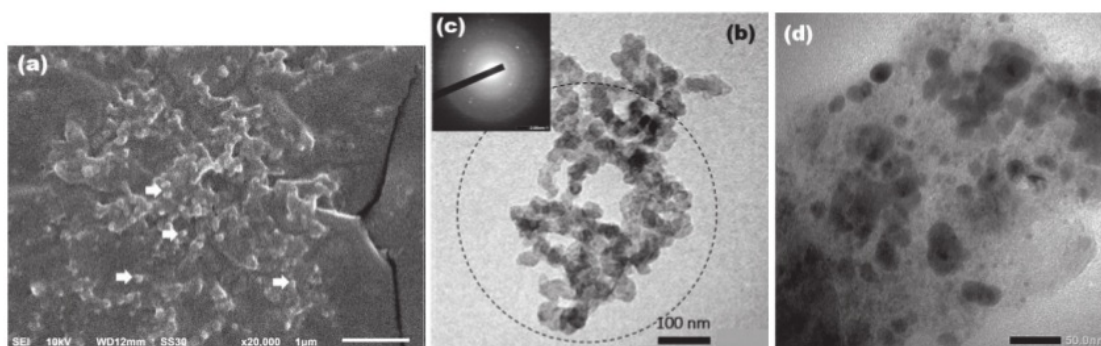


FIGURE 3. (a) SEM and (b, d) bright field (BF)-TEM images of spherical nanocellulose and (c) electron diffraction pattern obtained from a circle area in (b).

Crystallinity

XRD profile of the treated fibers is different with that of untreated fiber (Fig. 4). The position of the main peaks on the treated fibers (peaks 4) slightly shifted to a higher 2 theta. The peak width and the number of identified cellulose crystal planes were smaller and higher than that of untreated fiber, respectively, indicating amorphous constituents contained in untreated fiber were partially removed. Peaks positions of all fiber specimens were identified and summarized in Table 1. Clear identification of the main peak at around 22.6° indicated the characteristic of crystal polymorph I of cellulose [1, 10, 14]. This suggested that the chemical treatments effectively remove lignin, hemicellulose and other non-cellulosic compounds in which they generally exist as an amorphous phase especially lignin, and assured success in extracting the crystalline cellulose phase.

XRD crystallinity index (CI) of those specimens gradually increased after the chemical treatments; i.e. 83.12% for untreated fiber, 88.57% for alkali treated fiber and 94.03% for bleached fiber. The crystallinity index (CI) was estimated using an empirical equation described elsewhere [4]. Related to the effect of the treatment on the degree of crystallinity, it has been reported that hydrolysis after repeated bleaching for sisal fiber reduced the crystallinity index from 94.03% to 90.71% [4]. Sonication at (200 W, 24 kHz) of the hydrolyzed cotton also decreased the crystallinity index from 82.80% to 78.98% [10]. This is inconsistent with the result studied by Chen *et al.* (16); the crystallinity index was relatively constant with increasing the output power of ultrasonication from 400 W to 1200 W. In present work, unfortunately, no XRD data of the produced nanocellulose after sonication was available. It will be discussed based on electron diffraction analysis.

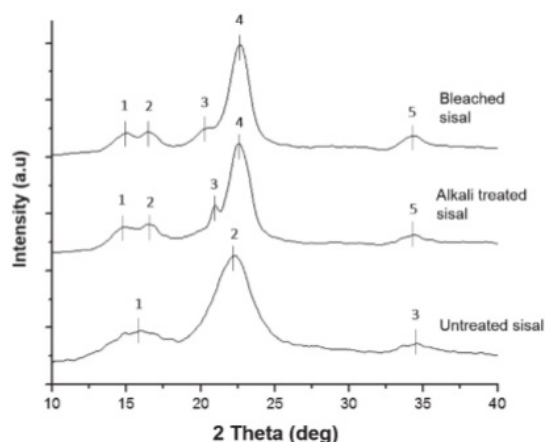


FIGURE 4. XRD profiles of untreated-, alkali treated- and bleached-sisal fibers

TABLE 1. Peak positions on XRD profiles related to plane orientation hkl (PDF# 030289)

| Specimen | Peak position / (hkl) | | | | |
|----------------------|-----------------------|--------------|--------------|-------------|--------------|
| | Peak-1 | Peak-2 | Peak-3 | Peak-4 | Peak-5 |
| Untreated sisal | 16.10/(-111) | 22.40/(022) | 34.60/(-231) | - | - |
| Alkali treated sisal | 15.10/(011) | 16.50/(-111) | 21.10/(021) | 22.60/(002) | 34.55/(-231) |
| Bleached sisal | 15.00/(011) | 16.50/(-111) | 20.05/(021) | 22.60/(002) | 34.60/(-231) |

Electron diffraction patterns taken from the entire areas of the specimen showed two type of ring patterns: i.e. the pattern with very weak or low intensity of diffraction rings (Fig. 5a) and halo pattern (Fig. 5b). Analysis of an electron diffraction pattern in Fig. 5a identified the cellulose crystal planes: i.e. $(-132)_{\text{cellulose}}$ and $(-232)_{\text{cellulose}}$ which were not found by XRD. The high intensity of crystalline planes recorded by XRD profile (Fig. 4) completely disappeared after sonication, indicating strongly decreases of crystallinity of produced spherical nanocellulose. Low intensity of the diffraction rings is also considered due to the larger size of a selected area diffraction (SAD) aperture used for obtaining the electron diffraction pattern than the area covering the nanocellulose (Fig. 5b, see white circle), resulting in some of the diffracted beams are coming from amorphous carbon substrate.

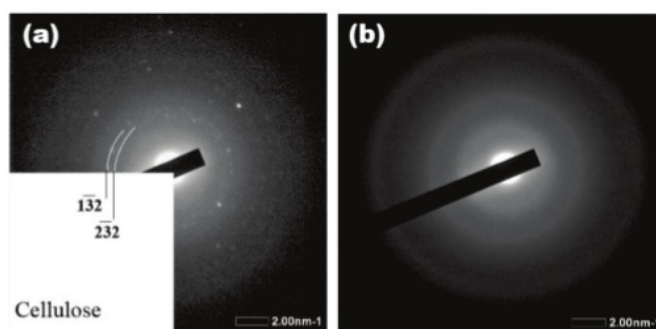


FIGURE 5. Electron diffraction patterns showing very low intensity of cellulose crystal planes (a) and halo pattern (b).

Sonication with the output power of 750 watts in this work is higher than other studies; 200 W [10] and 400 W [6], but lower than that has been used by Chen *et al.* [15]; 800 – 1200 W. Chen and co-worker [15] have reported that ultrasonic treatment at 800 W after chemical-purified cellulose wood fibers (3 wt% and 6 wt% K₆I, 80 °C, 2 h) produces crystalline cellulose nanofibers (5 – 20 nm width). Sonication at 800 W made weaker the hydrogen bonding between the nanofibers with the crystallinity index of 69.72% at 1200 W. Ultrasound energy transferred to the cellulose chains can provide impact to destroy the cellulose microfibrils into nanofibrils gradually.

On the other hand, related to the mechanism of morphological formation of the nanocellulose from cotton fibers, Meyabi *et al.* [10] have proposed the mechanism schematically. At First, the enzymatic hydrolysis attacks the crystalline structure of cellulose and decreases the chain length of cellulose. The degree of polymerization decreases after the relatively long duration of enzymatic hydrolysis. Hydrolyzed fibers convert into cellulose nanosphere less than 100 nm in diameter after sonication with the crystallinity index of 78.98%.

In this study, sonication at (750 W, 20 kHz) combined with chemical pre-treatments of alkalization and bleaching of sisal fibers produces the morphological formation of cellulose nanosphere which tends to be relatively low crystallinity. In this case, alkalization and bleaching before sonication dissolved off the non-cellulosic components as binding agent the micro/nanofibrils which lead to increase the crystallinity index of the treated fibers. However, the amorphous cellulose that exists inside the nanofibrils in the arrangement of fiber layer structure could not dissolve in by the bleaching solution. Therefore, amorphous phase/matrix observed in TEM images (Fig. 3c and 3d) has been verified as amorphous cellulose which made low crystallinity of produced cellulose nanospheres.

Based on the studies, the morphological formation of nanocellulose resulted from a combination of sonication with chemical pre-treatments seems to depend on the type of natural fiber. Sonication can increase and decrease the crystallinity of isolated nanocellulose depending upon the chemical treatment before sonication.

CONCLUSIONS

SEM, TEM, XRD and electron diffraction analysis of sisal fibers have verified that morphological formation resulted from repeated alkalization and bleaching followed by sonication is spherical nanocellulose less than 50 nm in diameter with relatively low crystallinity. The type of fiber may provide an effect on the morphological formation of the isolated nanocellulose after sonication. The low crystallinity of produced nanocellulose is related to the effect of chemical treatment done before sonication. The alkali and bleaching solution in this work could not dissolve the amorphous cellulose as one of the main components of inside the fiber layer structure.

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