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A Preliminary Study of Isolation and Characterization of Nanocrystalline Cellulose from Microcrystalline Cellulose by Acid Hydrolysis Process

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Abstract. This research focuses on the isolation of MCC (microcrystalline cellulose) into NCC (nanocrystalline cellulose) by acid hydrolysis process. The sulfuric acid hydrolysis (44 wt.% H₂SO₄) aims to fibrillation from MCC into NCC material. NCC has good properties such as high-surface-area, high-aspect-ratio, weight light, and reactive materials. The morphology of NCC was characterized by SEM (Scanning Electron Microscope) and TEM. The physical characterization was tested using FTIR, XRD, and TGA. The morphological result showed that the particle size of NCC was more homogeneous with a diameter size of 25±3 nm with 310±5 nm in length. The physical properties of NCC better slightly than MCC, indicated by the increasing crystallinity index value from 74.8 to 76.4%, and it has a high thermal resistance of 330°C.

Introduction

In the last decade, the use of renewable material in nanosized was implemented by researchers as a filler composite material. The acid hydrolysis method is a common method used to extract nanocellulose from lignocellulose resources. The hydrolysis process using sulfuric acid has been done by isolated Agave cantala fiber using sulfuric acid with a concentration of 44 wt.% [1], isolated hemp fiber with variations of H₂SO₄ concentration from 41-50 wt.% [2], and isolated Mulberry pulp with a 47 wt.% [3].

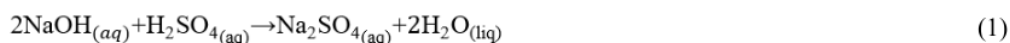
The hydrolysis process using sulfuric acid has been done by Yudhanto et al. (2018) isolated Agave cantala fiber using sulfuric acid with a concentration of 44 wt.%, Listyanda et al., (2020) isolated hemp fiber with variations of H₂SO₄ concentration from 41-50 wt.%, and Reddy et al., (2014) isolated Mulberry pulp with a 47 wt.% [1-3]. The isolation aims to degrade microcellulose into nanocellulose through the fibrillation process. Nanocrystalline cellulose has a 10-25 nm diameter and a length of 100-500 nm [4]. Nano-sized NCC causes them to have a high aspect ratio and surface area values, so it is widely applied to nanocomposite films as reinforcement or filler [5, 6]. Another physical property is the high crystallinity index of NCC (nanocrystalline cellulose), which is an essential factor for the increased mechanical strength when applied as reinforcement or filler to nanocomposite films and nano-membranes with PVA (Polyvinyl Alcohol) matrix [7, 8].

The MCC is a purified, partially depolymerized cellulose from fibrous plant material with long crystalline polymer chains (C₆H₁₀O₅)_n. The NCC was successfully extracted from commercial MCC (microcrystalline cellulose) powder by sulfuric acid hydrolysis in the present study. The main goal of this work focus on the morphological and physical properties of NCC. The raw material starts from MCC powder for shortening the purification process of lignocellulosic fiber, which takes a long time. The use of MCC is expected to speed up the process of obtaining the NCC.

Experimental

Materials. The materials used for this study include MCC commercial was obtained with code 1.02330.0500, H₂SO₄ (analytical purity), NaOH (analytical purity) obtained from Merck and Co., Inc. The commercial MCC has a diameter range of 10-20 μm. The morphological MCC and NCC were observed by SEM (Scanning Electron Microscope) dan TEM (Transmission Electron Microscope) to compare their size and shape. The Physical characterization was analyzed by FTIR (Fourier Transform Infrared), XRD (X-ray Diffraction), and TGA (Thermal Gravimetric Analysis) test.

Methods. The MCC powder was weighed and put in a glass beaker filled with distilled water in a ratio of 1:50 by weight volume. Next, sulfuric acid (H₂SO₄) is dripped into a burette tube with a concentration of 44 wt.%, slowly rotated using a magnetic stirrer with a constant rotation speed of 100 rpm for 1 hour with a preheat temperature of 60°C [9]. After the hydrolysis process, the NCC was then neutralized by adding Sodium hydroxide (NaOH) in an ice bath (5°C), namely salt-hydrolysis and continued neutralization using the centrifugation method was conducted at 4000 rpm for 15 minutes. Finally, the NCC suspension was rinsed with distilled water until pH neutral. The chemical reaction for stopping sulfuric acid is shown in equation 1.



The ultrasonication was used for the next fibrillation of the MCC with a power of 240 watt for 60 minutes to obtain NCC suspension. This NCC was centrifuged to obtain NCC suspension, the scheme of this research was shown in Fig. 1.

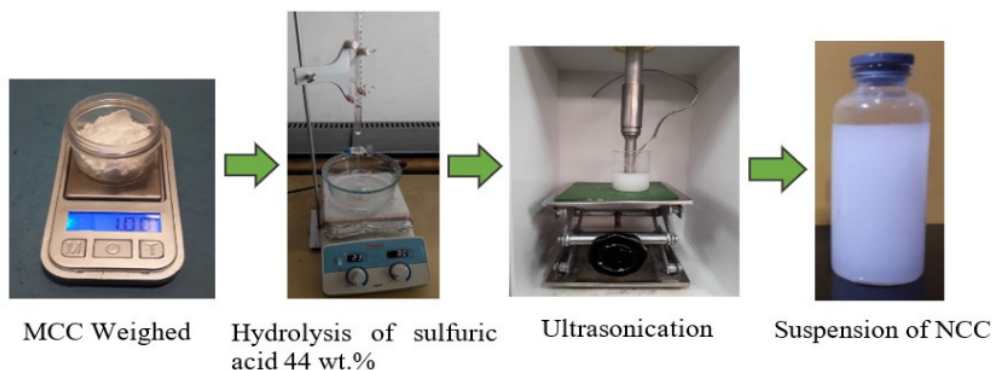


Figure 1. Schematic flow of MCC isolation into NCC with sulfuric acid hydrolysis-ultrasonic process

Characterization

Morphology of MCC and NCC. Morphology of MCC was observe by SEM JEOL type JSM 6510 with operating voltage range of 5-40 kV. The sample were coated with gold using sputtering technique. The nanosized of NCC was confirm by TEM (JEOL JEM-1400 series).

FTIR Test. FTIR analysis aims to detect possible changes in the functional group of the sample (MCC and NCC materials) in the wavenumber range of 500 to 4000 cm⁻¹. The test equipment model is IR Prestige-2 with a thin pellet-shaped test sample. The samples were mixed with KBr (1:100, w/w), after the sample was mixture then pressed into thin pellets that were analyzed.

XRD Test. XRD test is performed to analyze the CI (Crystallinity Index) of the materials. XRD patterns were collected by an X-ray diffractometer model Rigaku-miniflex 600 (40 kV, 15 mA). The instrument was operated at scanning rate of 2°/min from 2θ = 3-40° with Cu Kα radiation

($\lambda=1540$ nm). Calculation of the CI value following the Segal's empirical method [10], with the equation 2 as follows:

$$CI = (I_{002} - I_{\text{amorphous}}) / I_{002} \quad (2)$$

The maximum intensity of crystalline from plane 002 at $2\theta = 22.5^\circ$ was noted I_{002} and $I_{\text{amorphous}}$ is the intensity of diffraction attributed to amorphous cellulose. The higher CI was indicated that cellulose crystallinity is better.

TGA Test. The TGA test aims to degradation of MCC and NCC materials because of high temperature, the analysis obtained in the form of T_{onset} (the initial temperature of degradation) and T_{max} (maximum temperature of degradation). Thermalgravimetric analysis was carry out using a Mettler Toledo. Each sample (MCC and NCC) was heated from temperature of 30°C to 600°C under nitrogen atmosphere at heating rate of $10^\circ\text{C}/\text{min}$.

Results and Discussion

Morphology of MCC and NCC. The morphology of MCC powder was shown in Fig. 2a and 2b, an irregular length and shape with a range diameter of $10\text{-}20\ \mu\text{m}$. The combination of chemical (acid hydrolysis) and mechanical (ultrasonication) treatments aims to change the dimension from micro into nano scale. The NCC has shaped like a long needle shape. NCC looks like a homogeneous single crystal with a higher aspect ratio value than MCC. The average diameter and length obtained were of $25\pm 3\ \text{nm}$ and $310\pm 5\ \text{nm}$, respectively. The comparison of length and diameter is called the aspect ratio (L/D), which is 12.4. These results were observed from TEM photos, as shown in Fig. 3a. The results of the diameter and length distribution of NCC were then calculated using image-J software, as shown in Fig. 3b. This homogenous nano-size increases bind with nanocellulose as reinforcement with the polymer matrix. The addition of NCC into thermoplastic polymers such as PLA (Polylactic acid) or PVA is expected to increases the mechanical strength of bio-plastic films.

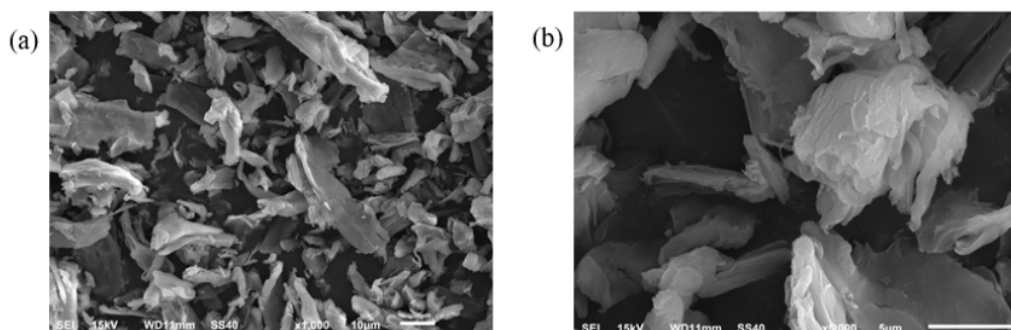


Figure 2. SEM Photo of MCC of (a) 1000 and (b) 5000 x magnification

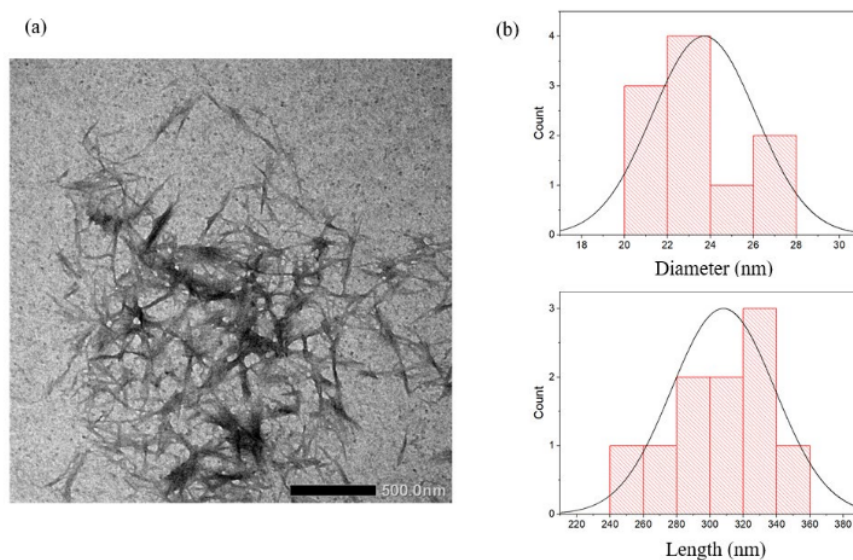


Figure 3. Nanocrystalline Cellulose (a) TEM photo, (b) Distribution of diameter (D) and length (L)

FTIR analysis. Fig. 4 shows the FTIR spectra obtained from MCC and NCC materials. There are offered three leading region bands, in the range of $3348\text{--}3464\text{ cm}^{-1}$, $2900\text{--}2924\text{ cm}^{-1}$, and $1050\text{--}1111\text{ cm}^{-1}$ [10]. These three areas vibration of the intramolecular bonds stretches such as the O-H compound, C-H bending, and C-O stretching pyranose ring group, a natural cellulose constituent [11]. The absence of wavenumber 1739 cm^{-1} , 1371 cm^{-1} and 1250 cm^{-1} , indicates disappeared residual acetate group elements (hemicellulose and lignin) in the NCC material [12,13]. The change of band located at 1630 cm^{-1} to 1690 cm^{-1} was shows hydrophilicity properties. The water content in cellulose has been reduced due to the loss of hemicellulose content. The band located at $1427\text{--}1466\text{ cm}^{-1}$ was H-C-H bonds or CH_2 symmetric bending indicating cellulose type II. The cellulose type I showed at the peaks of 895 cm^{-1} , 1050 cm^{-1} , and 1111 cm^{-1} ; changes in the peaks of these wavenumbers indicate a difference in the regeneration of crystalline cellulose I to cellulose II [14]. It is also supported by the appearance of new peaks in the spectrum of NCC material.

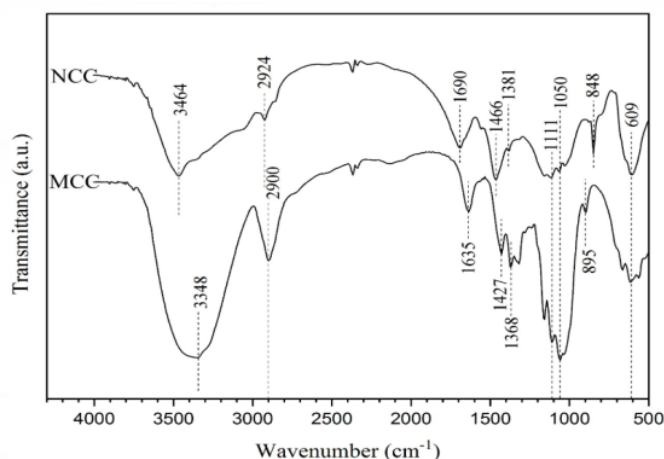


Figure 4. FTIR Spectra of MCC and NCC materials

XRD Analysis. The crystallinity index affects the mechanical strength of the composite when used as a reinforcement in the polymer matrix. The cellulose, which contains impurities such as lignin and hemicellulose, has a CI value of 45-80%. Another crystallinity index of raw materials for several types of plant fiber is shown in Table 1. The higher value of crystallinity index for natural fibers, the crystalline cellulose, indicates a good quality fiber.

Table.1 Crystallinity Index of various raw natural fibers

Raw Fibers	Crystallinity Index [%]
Ramie [2]	79.75
Agave cantala [9]	64.50
Agave sisalana [17]	79.32
Agave angustifolia [18]	59.00
Agave americana [19]	50.10
Bamboo [20]	45.57
White straw [21]	54.42
Salacca midrib [22]	62.40

Fig. 5 shows XRD patterns of MCC and NCC. The crystallinity index calculates using Segal's equation and the data was presented in Table 2. The CI result indicated that NCC increased slightly by 2.2% after the acid hydrolysis process. The diffraction peaks at around $2\theta = 16^\circ$, 22.5° , and 34.2° , from the JCPDS#030289 for native cellulose representing the [111], [002] and [040] crystallographic planes of typical cellulose type I [24]. A high-crystallinity index of NCC increases the intermolecular bond of the OH- (hydroxyl) group in the NCC polymer chain. Thus, it impacts strengthening the intramolecular bond between NCC and the polymer.

The NCC isolated from natural fibers has been widely applied to the manufacture of membranes and nanocomposite films as a reinforcement in the PVA (polyvinyl alcohol) matrix. For example, Rochardjo et al. (2021) used the NCC isolated from ramie fiber as filler to reinforce the PVA matrix to manufacture nano-membranes by the electrospinning process. The addition of NCC in the membrane can increase the tensile strength and elongation break by 112% and 50%, respectively [8].

PVA nanocomposite film material reinforced with NCC isolated from Agave cantala increased tensile strength and elongation break by 76.7% and 138%, respectively [15]. The NCC suspension, which high-crystallinity and high-surfaces area, causes an excellent intramolecular bond with the PVA polymer [16].

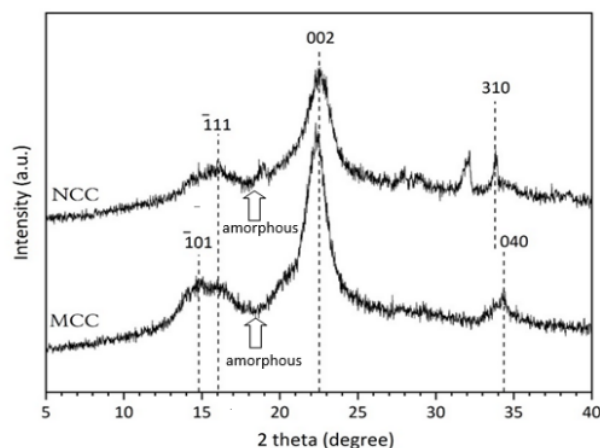
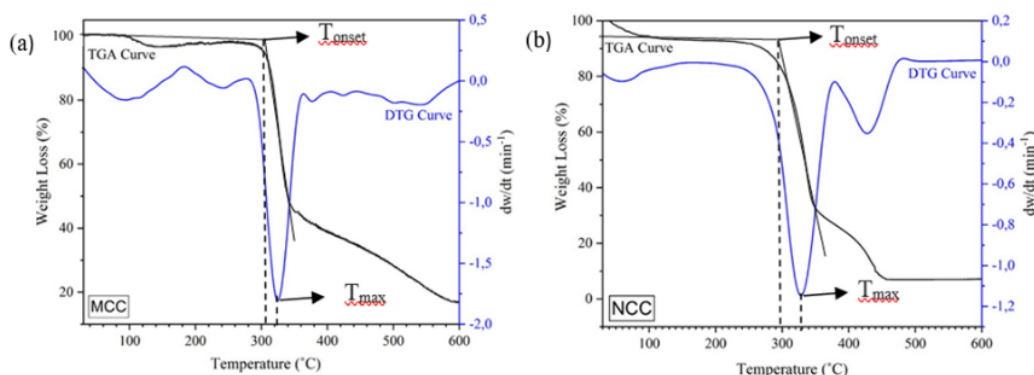


Figure 5. XRD diffraction patterns of MCC and NCC materials on the $2\theta = 5^\circ - 40^\circ$

Table 2. Comparison of crystallinity index of MCC and NCC materials

Material	$I_{\text{amorphous}}$ [cps]	I_{002} [cps]	CI [%]
MCC	786	198	74,8
NCC	573	135	76,4

TGA analysis. Fig. 6a shows the TGA (thermal gravimetric analysis) and DTG (differential thermogravimetric analysis) curves of MCC material. It shows the initial (T_{onset}) and maximum (T_{max}) degradation temperature values. The T_{onset} for NCC decreased about 10°C lower than MCC, and it indicated that a bit of sulfate ion still reacted on the surfaces of NCC. This statement is supported by Listyanda et al. (2020) using ramie fiber as NCC with variation time of hydrolysis process. A more extended interaction between negative sulfate ion and nanocellulose can reduce thermal stability due to dehydration reaction [23]. The T_{max} values between MCC and NCC did not make a significant difference. The T_{max} of NCC show that it is 5°C higher than the MCC material (Fig. 6b). That thermal condition indicated that the sulfate ion in the NCC suspension has been successfully removed by combining salt-hydrolysis and centrifugation processes on the cold temperature. The detailed thermal stability showed in Table 3.

**Figure 6.** TGA and DTG curve of (a) MCC and (b) NCC materials**Table 3.** Initial and maximum degradation temperature of MCC and NCC material.

Material	T_{onset} [°C]	T_{max} [°C]
MCC	305	325
NCC	295	330

Conclusion

Nanocrystal cellulose has been isolated from commercial MCC with acid hydrolysis to distinct the morphology and physical properties. The acid hydrolysis process for a concentration of 44 %wt. for an hour and preheat 60°C successfully decreases the diameter and length of NCC to 25±3 nm, 310±5 nm, respectively. Moreover, the NCC has good physical properties by high-value crystallinity index (76.4%), high-aspect ratio (12.4), and high-thermal resistance (330°C). NCC's properties make this material potentially used as filler to reinforced nanocomposite.

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