Nanocellulose prepared by acid hydrolysis of isolated cellulose from sugarcane bagasse

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Abstract. Cellulose in nanometer range or called by nano-cellulose has attracted much attention from researchers because of its unique properties. Nanocellulose can be obtained by acid hydrolysis of cellulose. The cellulose used in this study was isolated from sugarcane bagasse, and then it was hydrolyzed by 50% sulfuric acid at 40 °C for 10 minutes. Nanocellulose has been characterized by Transmission Electron Microscope (TEM), Particle Size Analyzer (PSA), Fourier Transform Infrared Spectroscopy (FTIR) and X-Ray Diffraction (XRD). Analysis of FTIR showed that there were not a new bond which formed during the hydrolysis process. Based on the TEM analysis, nano-cellulose has a spherical morphology with an average diameter of 111 nm and a maximum distribution of 95.9 nm determined by PSA. The XRD analysis showed that the crystallinity degree of nano-cellulose was higher than cellulose in the amount of 76.01%.

1. Introduction
Cellulose is one of the most abundant, natural, renewable and biodegradable polymers [1, 2]. Cellulose can be found in wood, cotton, rice straws, and sugarcane bagasse [2-5]. Sugarcane bagasse is a residue from the sugar and alcohol industry, and it was produced in large quantities every year. The sugar production process utilizes sugarcane bagasse as a fuel for the sugar mill. Some industry uses sugarcane bagasse as a raw material for electric generation, paper production, fermented products, and being developed for biomass [3].

Sugarcane bagasse contains 40-50% cellulose, much of which is in the crystalline structure. Another component in sugarcane bagasse is hemicellulose as much as 25-35% which is an amorphous polymer and mainly composed of xylose, arabinose, galactose, and mannose. The rest is mostly lignin with about 18-24% [3,6].

Cellulose is a polysaccharide composed of D-glucopyranose linked by 1,4-β glycoside bond. There are three hydroxyl groups in cellulose; secondary -OH at the C-2 and at the C-3, primary -OH at the C-6 position. [3,7,8].These hydroxyl groups in cellulose have an important role in the compactness of the crystalline structure and determines physical properties of cellulose. At the plant fiber, cellulose can be found in an amorphous phase which are connected with crystalline phase through intermolecular and intramolecular hydrogen bonds. This is causing cellulose not to melt the thermal degradation temperature [3,9,10]. Cellulose has a good mechanical properties, low density, and biodegradable. There are several types of cellulose (I, II, III, IV and V), cellulose I and II are commonly found in nature. Cellulose I has a best mechanical properties and a parallel chain orientation, while cellulose II has anti-parallel chain [3,11,10].
Cellulose is a natural polymer which is insoluble in water because it has a long chain and high molecular weight (more than 500,000 Da). Cellulose in nanometers or nanocellulose has a size range from 10 nm to 350 nm. Nanocellulose has a higher surface area than cellulose, so nano-cellulose can be one of the alternative solutions to make cellulose more easily to modified and dissolved in water [2,3,5].

Various methods can be used to obtained nano-cellulose, such as acid hydrolysis, ultrasonic technique, and enzymatic hydrolysis [1,4,13,14]. The method that most widely used is acid hydrolysis [2]. This method is easy and fast to produce nanocellulose that has a better properties. Some researchers have reported that crystallinity index of nano-cellulose produced by acid hydrolysis was higher than other methods. The nano-cellulose obtained from the acid hydrolysis also has a smaller size. These are the reasons that acid hydrolysis method is selected to obtained nano-cellulose.

A Strong acid such as H$_2$SO$_4$ and HCl is commonly used to break the glycoside bonds in cellulose. There are several steps in acid hydrolysis: (1) strong acid hydrolysis of cellulose under controlled conditions such as acid concentration, time, temperature, and ratio of the acid to cellulose; (2) dilution with some water to stop hydrolysis process and repeated washing with successive centrifugation; (3) dialysis which aims to fully remove free acid molecules; (4) sonication in order to form a stable suspension of nano-cellulose; (5) drying of the suspension to yield solid nano-cellulose [13,15].

Nanocellulose has unique properties such as low density, biodegradable, and good mechanical properties. In addition, nano-cellulose is also easily modified and has high surface area and typical morphology [1,2]. Nanocellulose with a small particle size and high crystallinity has a better properties and usually used as nanofiller for some polymer materials. Nanocellulose is used in the various field such as a barrier in the separation process of hazardous waste, food wrappers which replace the non-biodegradable plastics, and as nanocomposite to improve properties such as mechanical, thermal, ionic conductivity properties of the polymer [2,16].

2. Experimental

2.1. Materials
Sugarcane bagasse was collected from plantations located at Tasikmalaya, West Java, Indonesia. Other reagents used were: sodium hypochlorite; sodium hydroxide; sulfuric acid.

2.2. Methods

2.2.1. Isolation of cellulose. Sugarcane bagasse was dried in sunlight and then cut into small pieces. The cut bagasse was milled to become powder. The powder of bagasse was bleached with 250 ml of 0.735% (w/v) sodium hypochlorite for 6 hours with constant stirring at 45 °C to remove the lignin. The residue was washed with distilled water until a neutral pH. The neutral residue was refluxed with 150 ml of 17.5% sodium hydroxide for 3 hours with constant stirring at 45 °C to remove hemicellulose. The residue of this process was also washed until reach a neutral pH, and it was dried at room temperature for 2-3 days.

2.2.2. Preparation of nano-cellulose. Isolated cellulose from sugarcane bagasse was hydrolyzed with sulfuric acid with a ratio of cellulose to sulfuric acid 1:25. The hydrolysis of cellulose was carried out in several variations of sulfuric acid concentration, temperature, and time. The hydrolysis process was quenched by adding 10-fold excess distilled water (250 ml) to the reaction mixture. A colloidal suspension which produced was centrifuged at 6500 rpm for 30 minutes. Then, it was dialyzed for 5 days to neutralize and eliminate the sulfate ions. The neutral colloidal suspension was sonicated for 10 minutes to homogenize the generated nano-cellulose.
2.3. Characterization

2.3.1. Particle size analyzer. The particle size of nano-cellulose were determined by Particle Size Analyzer (PSA) with a Beckman Coulter Delsa™ Nano. The nano-cellulose was diluted with distilled water and inserted into the cuvette. The measurements were done at room temperature with the measurement range from 10 nm until 4000 nm.

2.3.2. Morphological analysis. The morphology measurements of the nano-cellulose were carried out on a Transmission Electron Micrograph (TEM), JEOL JEM 1400. The sample of nano-cellulose was measured in colloidal form with distilled water as a solvent.

2.3.3. Infrared spectroscopy (IR) analysis. The FTIR spectra of cellulose and nanocellulose were recorded on Fourier Transform Infrared Spectrometer Shimadzu Prestige 21. The samples were blended with KBr powder and then pressed into thin pellets. The sample was measured in the wavelength range from 4000 cm\(^{-1}\) to 500 cm\(^{-1}\).

2.3.4. X-ray diffraction (XRD) analysis. The X-ray diffraction data were collected using PW 1830 Diffractometer. This measurement was performed using Cu K\(\alpha\) with a step size 0.02, and the crystallinity index (CI) was calculated using the equation (1)[17], by measuring the peak height of the crystalline region (I\(_{002}\)) and the amorphous region (I\(_{am}\)) [18].

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CI(\%) = \frac{I_{002} - I_{am}}{I_{002}} \times 100\%
\]  

I\(_{002}\) is the intensity value for the crystalline cellulose (2\(\theta = 22.5^\circ\) for cellulose I and 2\(\theta = 20.1^\circ\) for cellulose II), and I\(_{am}\) is the intensity value for the amorphous cellulose (2\(\theta = 18^\circ\) for cellulose I and 2\(\theta = 16.3^\circ\) for cellulose II) [3,19,20].

3. Results and discussion

3.1. Infrared spectroscopy (IR) analysis

FTIR spectroscopy was used to showed that the lignin and hemicellulose have been removed during cellulose isolation process through analysis of its functional groups. The FTIR spectra of sugarcane bagasse and isolated cellulose are shown in figure 1. Based on FTIR spectrum, there are several peaks in sugarcane bagasse which is not found in the spectrum of cellulose. The peaks are around 1252 cm\(^{-1}\), 1511 cm\(^{-1}\), and 1738 cm\(^{-1}\). The absorption peak of 1252 cm\(^{-1}\) is derived from C-O stretching vibration of aryl group in lignin. The peak at 1511 cm\(^{-1}\) is attributed to the C=C stretching vibration of the aromatic ring in lignin. The C=O stretching vibration of carboxylic groups of hemicellulose and lignin is around 1738 cm\(^{-1}\) [3,21,22].
There are several factors which influence hydrolysis of cellulose such as acid concentration, temperature, and time. This work has tried some variation conditions to produce nanocellulose; the result shows that nano-cellulose was obtained in two conditions, firstly in 60% of sulfuric acid at 40 °C for 5 minutes (nano-cellulose A) and 50% of sulfuric acid at 40 °C for 10 minutes (nano-cellulose B). Both of nano-cellulose were characterized by FTIR, PSA, XRD, and TEM.

The FTIR spectra of nano-cellulose A and B are showed in Fig.2. Both of nano-cellulose FTIR spectra are not different with cellulose. It indicates that there are not new bonds formed during hydrolysis process.

The absorption peaks at 3451 cm\(^{-1}\) and around 2899 cm\(^{-1}\) were attributed to the O-H and C-H stretching vibrations, respectively. The peak absorption at 1644 cm\(^{-1}\) was reported as the O-H vibration of absorbed water. The peak for C-H and C-O vibrations contained in the polysaccharide rings of cellulose is around 1382 cm\(^{-1}\). The vibration of C-O-C in pyranose ring indicated by the absorption peak at 1060 cm\(^{-1}\) [3,5].
3.2. Particle sizes analysis
The particle size analysis shows that nano-cellulose A has an average diameter of 196.7 nm with the maximum distribution of 148.4 nm while nano-cellulose B has an average diameter of 111 nm and the maximum distribution in 95.9 nm. The results of the particle size analysis are shown in figure 3 and figure 4.

Nanocellulose A has the bigger size than nano-cellulose B, due to the hydrolysis time to produce nano-cellulose B was longer than nano-cellulose A so that the chain of cellulose B was more hydrolyzed than cellulose A and generates a smaller particles size for nano-cellulose B. The results of PSA indicate that acid concentration does not significantly influence in particle size of nano-cellulose. This is evidenced by the particle size of nanocellulose A is bigger than nano-cellulose B, whereas the acid concentration to produce nano-cellulose A was higher than nano-cellulose B (60% of sulfuric acid for nano-cellulose A and 50% for nano-cellulose B). Some studies have reported that acid concentration influenced on the value of index crystallinity of nano-cellulose, the higher of acid concentration used during hydrolysis process produces the nano-cellulose with the lower crystallinity index [13].

3.3. X-ray diffraction analysis
The crystallinity index of isolated cellulose, nanocellulose A, and B was analyzed by X-Ray Diffraction (XRD). The diffractograms are shown in Fig. 5. Cellulose and both of nano-cellulose have three diffraction peaks; they are 2θ = 12.2 °; 20.1 °; 22°. The peaks are typical for cellulose II, which has antiparallel structure. The crystallinity index for cellulose and nanocellulose was calculated by using equation (1) with I_{200} at 2θ = 20.1° and I_{am} at 2θ = 16.3°, the results are shown in Table. 1.

The crystallinity index for isolated cellulose from sugarcane bagasse is about 70.62%. Nanocellulose A has the lower index crystallinity in the amount of 67.83% while the crystallinity index for nano-cellulose B is 76.01%. This difference is due to the different of acid concentrations used in hydrolysis process, nano-cellulose A by using 60% of acid concentration while nano-cellulose B by using acid concentration of 50%. The higher acid concentration produces nano-cellulose with lower crystallinity, and it indicates that the addition of acid with higher concentration is not only to break the amorphous region of the cellulose but also to damage parts of the crystalline structure.
Cellulose has amorphous and crystalline parts; the amorphous will be more vulnerable to hydrolysis process. The hydrolysis by using 50% of acid concentration will break the amorphous region of cellulose to produce nano-cellulose B with higher crystallinity index, however when the acid concentration that used is higher such as 60%, the crystalline part can be damaged during hydrolysis process, and the crystallinity index is decreased.

|                      | Crystallinity Index (%) |
|----------------------|-------------------------|
| Cellulose            | 70.62                   |
| Nanocellulose A      | 67.83                   |
| Nanocellulose B      | 76.01                   |

3.4. **Morphological analysis**

The morphology of nano-cellulose A and B was characterized by Transmission Electron Microscope (TEM). The results can be seen in figure 6 and figure 7.

**Table 1.** Crystallinity index of cellulose and nanocellulose
Based on the results of TEM, both of produced nano-cellulose have a spherical morphology, but nano-
cellulose A has less clear morphologies than nano-cellulose B. This shows that nano-cellulose A has
more amorphous region than nano-cellulose B. It is correlated with the crystallinity index of nano-
cellulose that has discussed before.

4. Conclusion
Nanocellulose can be obtained from isolated cellulose from sugarcane bagasse and followed by
sulfuric acid hydrolysis. There are two conditions used in this report to produce nano-cellulose, that
are sulfuric acid concentration of 60% of at 40 °C for 5 minutes for nano-cellulose A and 50% at 40
°C for 10 minutes for nano-cellulose B. Nanocellulose B has better properties than nano-cellulose A.
Nanocellulose B has the average diameter of 111 nm with the maximum distribution of 95.9 nm. The
crystallinity index of nano-cellulose B is higher than the isolated cellulose in the amount of 76.01%.
The result of TEM analysis shows that nano-cellulose B has a spherical morphology.

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