Sonication-Assisted Pine Cone Flower Cellulose Hydrolysis Using Formic Acid

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Abstract. Nanocellulose has many applications in industrial sectors, such as in pulp and papermaking, production of synthetic textile fibers, dye, ink, and coating materials. The cellulose itself can be isolated from the waste of pine cone flower. This paper reports formic acid in the different concentrations (10%, 30%, and 60%) for hydrolyzing of cellulose using sonication technique (48 kHz) at 45 °C for 60 mins. The hydrolyzed cellulose was analyzed using FTIR and XRD spectrometry. It was found that the hydrolyzed-cellulose isolated was 92.4%, 94.6%, and 89.6%, respectively. The FTIR spectra provided the band for O-H (3435 cm⁻¹) and C-O-C (1180-1060 cm⁻¹) functional groups. However, the spectra also showed the C=C band for lignin impurities at 1661 cm⁻¹. Furthermore, The XRD data gave similar 2-theta values for all hydrolyzed cellulose at 16°, 22°, and 34° respectively. The crystallite size was 18.34, 15.09, and 15.07 nm. Meanwhile, the crystallinity index was 50.50, 52.70, and 51.60% respectively.

1. Introduction
The waste of pine cone flower has not attracted public attention. In fact, it contains some important chemicals such as secondary metabolites, volatile chemicals, and cellulose. Some secondary metabolite were reported previously as reagent for green synthesis of nanoparticles [1-2]. The volatile components were investigated as starting materials for industrial fine chemicals [3-4]. Meanwhile, the cellulose part is undergoing investigation.

Cellulose is the linear polysaccharide constructed by β-D-glucopyranose units and linked with the β-(1,4)-glycoside bond. The length of the linkage increases the size of the polymer and also the size of the cellulose particle. Hydrolysis toward these glycoside-bonds reduces the polymer chain and also decrease the particle size of cellulose. However, cellulose chains also consist of amorphous and crystalline domains. Nanocellulose is largely used in papermaking, food, cosmetic, biomedical and biocomposite packaging material for drug delivery [5,6]. Nanocellulose composite is also used for antibacterial and antifungal.

The general methods for hydrolysis are using sulphuric acid, hydrochloric acid, and phosphoric acid [5]. However, strong acid reagent causes equipment corrosion, over-decomposition, and requires a lot of water for neutralization. Previously, Garci’a et al. [7] reported the hydrolysis of pine cone cellulose using sulfuric acid 64% at 45°C. Three different hydrolysis times (30, 45, and 90 min) were conducted and the yield reported was 32-37% and crystallinity of nanocellulose of 80.8-88.5%. In this paper, we report a greener hydrolysis under sonication condition by applying formic acid as a reagent for hydrolysis. The effect of formic acid concentration variation to the cellulose properties was studied. The

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FTIR and XRD analysis were applied for functional groups characterization, crystallinity index, and crystallite size measurement.

2. Methods

2.1. Material and Chemicals
Pine cone flowers were collected from the pine forest in Coban Rais, Batu city, Indonesia. The chemicals used for research as bought from the manufacturer include formic acid (Smart Lab), sodium hypochlorite (Smart Lab), and sodium hydroxide (Merck).

2.2. Instruments
Some instruments operated for this research includes batch sonicator (DELTA D150H 48 kHz), FTIR spectrophotometer (Shimadzu FTIR 8400s), XRD spectrometer (PanAnalytical E’xpert Pro).

2.3. Cellulose preparation
Dried pine cone flower powder (50 g) in beaker glass was added with 500 mL of sodium hydroxide solution 6%. This mixture was heated at 70°C and magnetically stirred at 300 rpm for 4 h. The result was washed with distilled water to a neutral pH, and bleached with sodium hypochlorite 6%. Then, the resulted cellulose was washed with distilled water until neutral pH and prepared as starting material.

2.4. Hydrolysis procedure
Dried cellulose powder (5.0 g) was added with 10% formic acid solution (1:20 w/v ratio) and sonicated for 60 mins. Cold water was added to stop the reaction, and left overnight. It was further separated and centrifugated. The supernatant was collected as cellulose nanoparticle (FA 10). The obtained nanocellulose were characterized by FTIR and XRD. A similar separated procedure was performed by using 30% (FA 30) and 60% (FA 60) of formic acid solution.

2.5. Crystallite dimension measurement
Crystallite size \((D_{hkl})\) was calculated using the modified Debye Scherrer equation (1) [8]

\[
D_{hkl} = \frac{0.9\lambda}{\beta_{1/2}\cos\theta}
\]

Where \(D_{hkl}\) is the crystal dimension upright to the diffracting planes, \(\lambda = 1.5406 \, \text{Å}\). \(\beta_{1/2}\) is the full width at half-maximum of diffraction peak. \(\theta\) is the correlation Bragg angle.

3. Result and discussion

3.1. Cellulose preparation
According to Rambabu et al. [9], the composition of the pine cone flower was cellulose (44 wt%), hemicellulose (27 wt%), and lignin (27 wt%). Besides, other secondary components such as waxes, fats, tannins, gums and other phenolic and terpenoid compounds were also reported [9]. To obtain a pure and high strength of cellulose, several treatments have to be undertaken. Hot water treatment generally removes the waxes, fats, tannins, and gums from cellulose. The alkaline treatment using sodium hydroxide solution displaces the hemicellulose and pectin. Moreover, the mixture of cellulose with sodium hypochlorite can reduce lignin attached in cellulose. Cellulose produced from the waste of pine cone flower is depicted in Figure 1. Alkali treatment of cellulose using sodium hydroxide provides slightly brown cellulose (b), and after the bleaching process, it gives a light yellow cellulose (c).
3.2. Hydrolysis using formic acid

Cellulose hydrolysis is more affected by acidity of the reagent, due to acidic provided by proton to hydrolyze the glycoside bond in cellulose. Sonication can accelerate the process \[10\]. Table 1 summarized the data obtained from the hydrolysis process of cellulose in three different concentrations of formic acid. The recorded data correlated the yield of cellulose during hydrolysis, crystallinity index, and crystallite size achieved from XRD analysis.

| Sample | Yield (%) | Crystallinity index (%) | Crystallite size (nm) |
|--------|-----------|------------------------|----------------------|
| FA 10  | 92.4      | 50.50                  | 18.34                |
| FA 30  | 94.6      | 52.70                  | 15.09                |
| FA 60  | 89.6      | 51.60                  | 15.07                |

**Figure 1.** Preparation cellulose from the waste of pine cone flower: waste powder (a), after alkali treatment (b), and after bleaching (c).

**Figure 2.** XRD pattern of three samples hydrolysis result.

The yield of nanocellulose obtained between 89.6% and 94.6%. The highest yield of nanocellulose is produced from 30% of formic acid hydrolysis (FA 30), and the lowest yield is provided using 60% of formic acid. In addition, the XRD analysis provided diffractogram (Figure 2). Three peaks diffractogram
showed similar region for all nanocelluloses (FA10, FA30, and FA60) at peak (2θ) = 16°, 22°, and 34°. These values correlated to the crystalline plane of 110, 200, and 004 indicating cellulose [11]. The crystallinity index was calculated using Origin Pro software and crystallite size was calculated using the Scherrer equation as tabulated in Table 1. The crystallinity index of the three samples was obtained between the range of 50.50% and 52.70%. The highest crystallinity index was shown at 10% formic acid treatment. On the other hand, the enhancement of formic acid concentration showed a little decrease in crystallite size of hydrolysis products. The value of crystallite size FA 30 was similar to FA 60.

Figure 3 shows the FT-IR spectra of three nanocellulose samples.

Figure 3 shows the FT-IR spectra of nanocellulose achieved from hydrolysis using three concentrations of formic acid (FA10, FA30, and FA60). The nanocellulose samples provide identical absorption band spectra. The hydroxyl group stretching of nanocellulose is observed at 3435 cm⁻¹. The C-H symmetric and asymmetric stretching bands are shown at 2914 cm⁻¹. Moreover, the absorption band at 1180 and 1060 cm⁻¹ correlated to C-O-C pyranose ring bending vibration, and also peak at 905 cm⁻¹ is a band for glycosidic-bond bending vibration. However, remanence of phenolic or tannin compounds are still presence in the nanocellulose produced. It is indicated by the observed band at 1661 cm⁻¹. This finding is in agreement with that reported by Kian et al. [12].

4. Conclusion
Nanocellulose can be prepared with sonication-assisted formic acid hydrolysis. The optimum result is obtained using formic acid of 30%. It gives a higher yield of nanocellulose with the highest crystallinity index and a lower of the crystallite size. This finding lead in further application of nanocellulose for nanohydrogel-based nanocellulose.

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