Cellulose nanocrystal (CNC) produced from the sulphuric acid hydrolysis of the pine cone flower waste (*Pinus merkusii* Jungh Et De Vriese)

S E Lusiana¹, A Srihardyastutie¹ and M Masruri¹*

¹Chemistry Department, Faculty of Mathematics and Natural Sciences, Brawijaya University, Jl. Veteran 65145 Malang Indonesia

*Corresponding author’s email: masruri@ub.ac.id

Abstract. Cellulose nanocrystal (CNC) has been produced up on the hydrolysis of the cellulose isolated from the waste of pine flower (*Pinus merkusii* Jungh Et De Vriese). The hydrolysis process is undergone using 10%, 30% and 60% of sulfuric acid solution. The hydrolysis process is also performed using normal heating at 70 °C under stirring with magnetic stirrer for 4 hours. The CNC afforded is characterized by means of infra red spectrophotometry (FTIR), x-ray diffraction spectrometry (XRD) and transmission electron microscope (TEM) analysis. The TEM analysis afford the dimension of CNC in about 15 nm x 185 nm. This finding open the way for further prospective application.

1. Introduction

Nanocellulose is a cellulose in a nanometer dimension, which can be isolated from biological sources, such as plants, animal and microorganism. Nano size (1-100 nm) of cellulose has many industrial applications, such as gel antibacterial, functional biodegradable plastic for food packaging, material for medical tool and device, and drug delivery materials [1, 2]. Previously, it has been reported that the waste of Indonesian pine industry can come from the cone flower dropped around the tree [3, 4]. It is about 40-60% cellulose contained in the cone flower. The direct cellulose isolation and purification is already reported elsewhere recently, and the strategy to convert cellulose into nanometer in size is reported in this paper. Previous research reported the methods for producing nanocellulose, such as hydrolysis in some acid media [5, 6], hydrolysis-assisted sonication [7] and microwave [8, 9], oxidative-hydrolysis using some oxidation reagents [5]. This strategy applies sulfuric acid in different concentration by conventional stirring to afford cellulose nanocrystal (CNC) from cone flower waste of *Pinus merkusii* Jungh Et De Vriese.

2. Experiment section

2.1. Sample and chemicals

A pine cone flower’s sample was collected from local forest in Malang Region, East Java. Then, it was washed by immersion in hot water for 2 h, and further dried in oven at 100 °C for 24 h. The dried sample was undergone milling to afford a dried powder.
2.2. Cellulose isolation
Cellulose preparation is conducted following previous reference [10] with modification. A 50 g of pine cone powder was mixed with 500 mL sodium hydroxide 6%. This mixture was heated at 70 °C under magnetic stirring for 4 h. The solid part was separated by filtration and further washed with distillated water until neutral pH. From this product, was undergone bleaching process by mixing with 300 mL of 6% sodium hypochlorite solution. The mixture was stirred at 70 °C for 2 h and filtered off. This process was repeated for 4 times.

2.3. Cellulose nanocrystal preparation
The procedure follows the previous reference [5] with modification. A 1.0 g of dried cellulose in Erlenmeyer, which has been afforded in the previous step is added with 20 mL of 10% sulfuric acid solution. The mixture is warmed at 45 °C and stirred for 60 minutes. A cold distillated water is added to stop the reaction. The mixture is washed and undergone centrifugation until neutral pH. The solid product precipitate in bottom is collected as nanocellulose and dried before further characterization. Similar procedure is undertaken using different sulfuric acid concentration, i.e. 30% and 60%. The nanocellulose afforded is also characterized using similar methods.

2.4. FTIR analysis
The FTIR analysis of cellulose nanocrystal is undertaken using FTIR spectrophotometer Shimadzu. The sample is homogeneously mixed with potassium bromide and pressed to form a clear and transparent disk with 1.5 cm in diameter. The spectra analysis is scanned in between 4000 and 400 cm⁻¹ of wavelength.

2.5. XRD analysis
Crystallinity analysis is performed in x-ray diffraction spectrometer (PanAnalytical, Type E’xpert Pro). CuKα radiation 10-90 (2-theta), scanning rate 5 °/min, the operation voltage 40 kV and current 25 mA.

2.6. TEM analysis
The morphology analysis using transmission electron microscope is recorded using JEOL JEM-1400 operated in 120 V. The sample is coated in carbon prior to analysis.

3. Result and discussion
Cellulose in the plant mixes with other cell matrix. Protein, amino acid, organic acid, lignin, and other chemical components. Isolation from its mixture requires separation by destructing of the other chemicals. A basic hydrolysis generally undergone to dispose protein, lignin, organic acid, and other chemicals. Table 1 is displayed cellulose isolated after delignification and bleaching process [1]. In average 22.22 ± 1.07 g of cellulose is able to isolated from 50.0 g of a dried powder of cone flower waste.

| Entry | Cellulose code | Yield (g) |
|-------|----------------|-----------|
| 1     | C1             | 21.05     |
| 2     | C2             | 21.37     |
| 3     | C3             | 22.03     |
| 4     | C4             | 23.32     |
| 5     | C5             | 23.35     |
|       | **average yield** | **22.22 ± 1.07** |

Hydrolysis process of the cellulose breaks the poly-glucose into smaller chain to afford nanosize of cellulose. The sulfuric acid hydrolysis, using 10%, 30% and 60%, respectively increase glycoside
bonds. Moreover, applying 60% of sulfuric acid intensify the carbonyl band absorption in FTIR (Figure 1). The increasing of carbonyl band means the opening of glucofuranose chain. Sulfuric acid 10% and 30% give in about 35% absorption unit, however sulfuric acid 60% give the carbonyl peak in 50% of absorption unit. Furthermore, the rest of functional groups bands remain similar; such as the hydroxyl (O–H) band in 3485 cm⁻¹, C–H alkyl groups in between 2950 cm⁻¹ and 2800 cm⁻¹, the C–O–C band absorption in 1300-950 cm⁻¹.

The opening of glucofuranose chain also affects the crystallinity of the cellulose nanocrystal (Figure 2). An amorphous nanocellulose is produced from hydrolysis using 60% of sulfuric acid. Conversely, 10% and 30% sulfuric acid provide a similar crystallinity of nanocellulose. The peak at 2-theta 15° (101) and 18° (101) is detected for nanocellulose from 10% and 30% sulfuric acid hydrolysis. The sharp peak 22° (002) is recorded, includes the peak at shoulder in 21° (021). Similar reference is reported for nanocellulose [11]. However, for nanocellulose afforded by 60% sulfuric hydrolysis provide a broad peak 22° (002).

**Figure 1.** The FTIR spectra of cellulose nanocrystal from pine cone flower waste. The nanocellulose produced by hydrolysis using 10% and 30% of sulfuric acid (a), and 60% sulfuric acid (b).

**Figure 2.** The XRD diffractogram of cellulose nanocrystal from pine cone flower waste.
The nanocrystal size of cellulose is determined based on the direct measurement from TEM image (Figure 3). Cellulose nanocrystal produced from sulfuric acid hydrolysis using 10% and 30% give similar dimension in size. It is about 15 nm in wide and 185 nm of length (Figure 3a). However, nanocellulose afforded from 60% sulfuric acid hydrolysis give a variation in dimension. A shorter nanosize than that in 10% and 30% hydrolysis products. It is about 110-125 nm in length and 15-35 nm in wide.

![Figure 3](image_url)

**Figure 3.** The transmission electron microscope (TEM) image of bionanocellulose from pine cone flower waste. Bionanocellulose produced by hydrolysis using 10% and 30% of sulfuric acid (a), and 60% sulfuric acid (b).

4. **Conclusion**

Cellulose nanocrystal is able to be isolated from the hydrolysis of pine cone flower waste-rich of cellulose. The sulfuric acid concentration affects the nanocrystalinity and nano size of the cellulose. A 10% and 30% sulfuric acid give a high crystallinity with nano dimension 15 nm (wide) and 185 nm length. However, amorphous nanocellulose is afforded from 60% hydrolysis of sulfuric acid.

5. **Acknowledgement**

Authors thank to Directorate General for Research and Community development (DRPM) of Ministry of Research, Technology and Higher Education for Penelitian Unggulan Dasar Perguruan Tinggi (2019) under contract number 167/SP2H/LT/DRPM/2019.

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