A Potency of Microcellulose from Pineapple Leave Isolated by Hydrolysis-Assisted Sonication

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Abstract. Cellulose has been reported have many functions for separation and adsorption of heavy metals and organic materials waste or pollutants. This paper reported the preparation of micro size cellulose from the waste of pineapple leaves. The strategy involves delignification, bleaching, and hydrolysis process. The hydrolysis step plays an important role for cutting of the cellulose chain, and ultrasonication assisted hydrolytic procedure was applied. In addition, the isolated product was characterized by means of FTIR spectrometry to identify functional group, Scanning Electron Microscopy to inform morphology features, and Particle Size Analyzer to inform the size of cellulose particle. The yield of initial isolated cellulose was 62.07%. Then, through the ultrasonication process using 30% of sulphuric acid for 1 hour at room temperature resulted about 3.497% yield of microcellulose. The FTIR show O-H, C-O specific band for cellulose in 3402 cm\(^{-1}\), 1385 and 1200-1300 cm\(^{-1}\), respectively. This finding lead for further application of this micro/nano-cellulose for heavy-metals-and dye pollutants adsorbent.

1. Introduction
Pineapple (Ananas comosus L. Merryl) easily grow at Indonesia, a tropic country. Increasing of pineapple fruit production in Indonesia 28.64% in 2017 from the previous year 2016 [2]. Then, pineapple leaf biomass waste is easily obtained, abundant, renewable, and high in cellulose content. Pineapple leaves basically consists of substances of cellulose, hemicellulose, lignin and pectin. Cellulose can be obtained from various biomass source. The diversity of cellulose biomass sources and the extraction process will affect the type of cellulose. Pineapple fiber consist of cellulose (78-83%), hemicellulose (19%) higher than lignin, around (5-15%), pectin 1%, waxes (2-3%) and ash (1%) [1,25-27].

Cellulose compose of a linier homopolysaccharide, D-glucopyranose monomers trough (1-4) glycosidic bond. Every monomer has three hydroxyl groups. Physical properties of cellulose controlled by this important functional group. Directing the crystalline packing not only controlled by hydrogen bonds but also reignig physical properties of cellulose [8]. Fundamental structural of cellulose is micro fibrils. The chains of poly-(1,4)-D-glucosyl residues aggregate to form a fibril which is a long thread-like bundle of molecules laterally stabilized by intermolecular hydrogen bonds [10-11].

Cellulose is a semicrystalline polymer. It differs from hemicellulose and lignin, amorphous polymer. The linkage between carbohydrate groups and lignin are ester-type bond and carboxyl. First, carboxyl group of uronic acid in hemicellulose connected by ester-type bond with lignin's hydroxyl. Alkali
solution had great ability to destory this linkage. Second, hydroxyl group of lignin and carbohydrate form ether-type bond. This bond cannot be damaged by alkali solution [30].

Diameter range of single cellulose microfibrils are 2-20 nanometers. Each microfibril can be considered as a string of cellulose crystals linked along the microfibril axis by disordered amorphous domains [6,12]. Commonly microfibril term is used in high-level plants not only the length was decreased several microns, but also lose their structure. This condition depends on various diameters of microfibrils. Microfibril also used to refer nanofibrils and nanofibers. Aggregates of cellulose microfibrils will form bulky-microfiber cellulose (MFC) [13]. At the time when acid hydrolysis in progress, cellulose microfibrils lead lie athwart fission along the amorphous regions and the use of sonication yield in a rod-like material with a relatively low aspect ratio referred as cellulose whiskers [24]. Some term for cellulose whiskers puts in nano whiskers [15], nanorods [16], and rod-like cellulose crystals [17]. The individual cellulose crystals have powerful hydrogen bonding, further push re-aggregation [18] that guide to another cellulose structure called microcrystalline cellulose (MCC).

Various types of cellulose particles are obtained based on the source and method of the extraction. There is no standardization of nomenclature, so ambiguity is found in the literature [4,5]. Here we focus on extracting cellulose from pineapple leaves. The next research, we will compare between the result hydrolyzed pineapple leaves with sonication and without sonication. We predict that sonication will produce larger cellulose surface area than without sonication.

Many applications are carried out for large surface area of polymer, such as an adsorbent. For further research micro cellulose produced will be tested as heavy metal adsorbents in the water system.

2. Experiment

2.1. Materials
Pineapple leaves was garnered from pineapple seller at Malang, East Java, Indonesia, distilled water, filter paper. Reagents: 6% sodium hypochlorite; 30% sulphuric acid; 6% sodium hydroxide solution.

2.2. Procedures

2.2.1. Cellulose Isolation.
a) Delignification.
Pineapple leaves was dried at 50 °C up to 48 hours, then cut in small pieces, and next delignification process was done using 6% sodium hydroxide at ratio 1:10 (w/v) at 70 °C for 4 hours. The mixture was washed until gain neutral pH.
b) Bleaching process.
The neutral residue was bleached using 6% sodium hypochlorite for 1 hour at 70 °C, using 1:42 ratio. Then, it washed until achieve neutral pH, and dried at 50 °C.
c) Acid Hydrolysis.
The neutral residue was hydrolyzed using 30% sulphuric acid at room temperature for 1 hour at ratio 1:20, then washed until achieved neutral pH.

2.2.2. Preparation of micro-cellulose.
Preparation of micro-cellulose using Ultrasonication Cleaner DC 150 DC 150 H. In the ultrasonication stage, two variations were given. First variation using sulphuric solvent, while the other with water solvent. First variation using 30% sulphuric acid (v/v) at room temperature for 1 hour at ratio 1:20.

2.3. Identification

2.3.1. FTIR-Fourier Transform Infrared spectroscopy. The FTIR spectra produced by Fourier Transform Infrared Spectrometer Shimadzu 8400S. Thin pellets were prepared with KBr powder. Then blended with the sample using wavelength range from 500-4000 cm⁻¹.
2.3.2. Scanning Electron Microscopy (SEM). The surface morphology of the pineapple leaves was measured by SEM. Sample in dry condition. Voltage of operation was 20kV.

3. Results and discussion

3.1. Composition of Pineapple Leaves Fiber (PLF) at each chemical treatment.
During the delignification process there has been a change in the color of the solution. Sodium hydroxide solvent which was originally colorless turned blackish. This indicates that lignin and hemicellulose were extracted using sodium hydroxide. The elimination of lignin and hemicellulose was calculated by percent amount in the table.

![Figure 1. Fresh PL](image1.jpg)
![Figure 2. Dried PL](image2.jpg)
![Figure 3. Dried Chip PL](image3.jpg)

![Figure 4. Powder PLF](image4.jpg)
![Figure 5. Delignification using 6% NaOH](image5.jpg)
![Figure 6. Bleaching using 6% NaOCl](image6.jpg)

![Figure 7. Hydrolysis without sonication](image7.jpg)
![Figure 8. Hydrolysis-sonication](image8.jpg)
![Figure 9. H$_2$O-sonication](image9.jpg)

NaOH solution is the best blooming solution in the pulping process. This is due to the suitability of Na$^+$ cations to infiltrate pores between fields of cellulose lattice. Infiltration of competing particles
causes expansion followed by the entry of water molecules. No specific calculation was performed to distinguish between lignin and hemicellulose. The extracted amount is shown in Table 1.

**Table 1. Chemical Composition of Pineapple Leaves (PL)**

| Treatment            | Cellulose (%) | Hemicellulose-Lignin (%) |
|----------------------|---------------|--------------------------|
| Raw material*        | 81.27         | 15.77                    |
| Delignification      | 35.33         | 8.91                     |
| Bleaching            | 64.67         | 3.22                     |
| Acid hydrolysis      | 70.11         | 2.31                     |
| Sonication:          |               |                          |
| H_2SO_4              | 72.17         | 1.77                     |
| H_2O                 | 80.13         | 0.22                     |

*Composition from literature [1, 25-27]

Hydroxyl anion can increase the pH of solution indicates that hemicellulose was dissolved. Increasing temperature can increase the rate of delignification (removal of lignin). However, using temperature above 160 °C can cause cellulose degradation.

3.2. *Analysis Functional Group using Fourier Transform Infrared Spectroscopy (FTIR)*

Dying sample will support the sample analysis. If the amount of the sample in water was high, the hydroxyl group of the water will be read.

**Figure 10.** FTIR spectra of raw material (powder) pineapple leaves (black line), delignification using NaOH 6% (red line), bleaching using NaOCl 6% (blue line).

Based on the purpose of getting cellulose, the lignin and hemicellulose of the compounds must be removed. FTIR helps read the condition of the sample through the changes of functional groups composition from lignin and hemicellulose. Table 2 shown the specific uptake areas of lignin and hemicellulose.
Table 2. Functional Group of Pineapple Leaves (PL)

| Steps             | Stretching -OH (cm⁻¹) | Vibration C-H (cm⁻¹) | Absorbed water (cm⁻¹) | Aromatic ring vibration of Lignin (cm⁻¹) | C-C stretching (cm⁻¹) |
|-------------------|------------------------|----------------------|-----------------------|----------------------------------------|----------------------|
| Bulk materials    | 3402.96                | 2920.79              | 1632.43               | 1248.62                                | 1061.54              |
| Delignification   | 3399.10                | 2918.86              | 1645.93               | -                                      | 1059.61              |
| Bleaching         | 3364.39                | 2916.93              | 1645.93               | -                                      | 1059.61              |
| Acid hydrolysis   | 3397.17                | 2911.15              | 1647.86               | -                                      | 1061.54              |
| Sonication:       |                        |                      |                       |                                        |                      |
| H₂SO₄             | 3360.53                | 2915.00              | 1638.21               | -                                      | 1061.54              |
| H₂O               | 3449.25                | 2922.72              | 1634.36               | -                                      | 1063.47              |

Hydrolysis process did not initiate a new bond, which showed at figure 10. Based on the FTIR spectra, there are polysaccharide rings of cellulose, which showed as C-H and C-O vibration at 1382 cm⁻¹. The characteristic peak was found at 1200-1300 cm⁻¹, which is specific for aromatic ring vibration. This peak will disappeared after pulping and bleaching.

**Figure 11.** FTIR cellulose-bleaching (black line), hydrolysis-without sonication (red line), hydrolysis with sonication (blue line)

Based on figure 11, the band located at 1734.65 cm⁻¹ is related to C=O stretching of acetyl and uronic ester groups of the hemicellulose or the ester linkage of carboxylic groups of ferulic and p-coumaric acid of lignin and/or hemicellulose [24,31]. Lignin and hemicellulose are still present in small quantities. Then, the peak of lignin at 1516.71 cm⁻¹ disappeared after sonication process (blue line).

3.3. Scanning Electron Microscopy (SEM)

Figure 12 shown the dried macrofibril resembles structure like a bowl which consist of xylem and phloem tissues. The delignification process has damaged the xylem and phloem cell walls, and turned it become fibers. Lignin and hemicellulose were removed by dissolving it in 6% NaOH solution through delignification process (figure 13). Addition of 30% H₂SO₄ as hydrolysis agent cause the fibers start separated from one another indicating that the hydrogen bonds were broken (figure 14).
There are several diameters of cellulose obtained from the hydrolysis effect, such as 1.018 µm and 2.480 µm. Sonication using 30% H$_2$SO$_4$ make cellulose organize itself in a linear form (figure 15). The range of measured diameter are 1982 µm - 3715 µm. Sonication using water solvent causes irregular shape changes (figure 16). This is likely to be an interaction through hydrogen bonds between cellulose and water as solvent.

4. Conclusions
The procedure used in this research was capable for producing pure cellulose. Hydrolysis is proven to be able to cut the macro-cellulose chain become micro-sized. The FTIR provides conclusive information that the elimination of lignin and hemicellulose were occurred. Hydrolysis technique using sonication
help cellulose polymer eliminate amorphous region (which shown at SEM image). Other measurement still need to determine the size of the cellulose. Attainment of nano size is needed for further study using variations in temperature, acid type, and concentration. Increasing cellulose surface area therefore increases the chance for heavy metal adsorption.

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