Synthesis of nano bacterial cellulose using acid hydrolysis-ultrasonication treatment

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Abstract. Bacterial cellulose is a product from coconut water fermentation which is known as bio-cellulose. One application of bio-cellulose is by synthesising it into nano-cellulose for providing a new material. Nano bacterial cellulose will become an innovation of polymer research and application. By using nano-bacterial cellulose as reinforcement in the nano-composite manufacturer and adding it into different of polymer matrix, the product quality is improved. Provision of nano-bacterial cellulose by hydrolysis and ultrasonication methods could be done with several methods. This research aims to synthesize and study the characteristics of nano-bacterial cellulose based on the two treatments above. The results found out that strong acids can remove amorphous parts and cut off cellulose chains into nano-cellulose, whereas ultrasonic produced smaller size particles, reduce particle agglomeration and homogenous product. The best treatment in this research was HCl 5M with hydrolysis time 48 hours and 55°C process temperature. This treatment produced nano-cellulose 99.99 nm in particle size with 86.6% distribution. Characteristic of produced nano-bacterial Cellulose are β-Cellulose with grid parameter a: 8.2600Å; b: 10.3880Å; c: 7.8400Å, volume 669.27 Å³ and crystal shape is monoclinic. Index crystallinity is 76%.

1. Introduction
Bacterial cellulose is a product from coconut water fermentation which is known as bio cellulose. Bio cellulose is a type of cellulose which has privileges compare to other cellulose. Bio cellulose has unique character, such as free of lignin, higher in mechanical properties and environmentally friendly. Moreover, it also has special physical properties, for example higher in number of pure cellulose or crystalline, strength mechanical properties, high porosity, and easy to decompose. One application of bio cellulose is by synthesising it into nano-cellulose for providing a new material.

Nanotechnology could be described as everything which connected to materials, process and system that operated at 1-100 scale nanometre. It offers many kinds of innovative applications in very wide areas in industries such packaging, biomedical, electronic, optic, and agriculture. When a material transforms into nano-structure, a new phenomenon and new physical characteristics appear since the product dispersion characteristics changes.

To be specific, nano-cellulose particle is a new material of cellulose with increased in crystalline, aspect ratio, surface area, dispersion ability and biodegradable. With those new abilities, nano cellulose can be used as filler to strengthen a polymer, additive for biodegradable products,
membranes reinforce, medicine carriers and implant [1]. Nano cellulose has an incredible supra molecule structure and characteristic, high cellulose molecule, and high cellulose crystalline with moisture content up to 99% which make its application is high and important.

There are some methods to synthesis Nano cellulose such as mechanical, chemical and biological method. Previous research has been conducted which aimed to synthesis nano cellulose by hydrolyzing it with chloride acid (HCl) [2]. The result is limited only to reduce the cellulose particle till 7 μm. This extension research is aiming to produce the bacterial cellulose until its nanoform and also to develop the method to produce nano bacterial cellulose.

2. Materials and Methods

2.1. Materials

Required equipment to conduct the research were hotplate stirrer, beaker glass, beaker, vacuum pump, 41 Whatman filter papers, plastic mould, Elmasonic S 300 H ultrasonic batch and Buchi B 290 spray dryer, also analysis equipment such as Scanning Electron Microscopy (SEM), X-Ray Diffraction (XRD) Merk Xpert Pro Analytical PW30/40 and Particle Size Analyzer (PSA) Merk Malvern MAL1096681. The materials that have been used were coconut water, urea, sugar, acetic acid, *Acetobacter xylinum*, NaOH, HCl, NaOCl were purchased from a local retailer. Distilled water was produced in our own laboratory.

2.2. Methods

This research was conducted in four steps exploratively. First step was bacterial cellulose production which made from 7 days fermentation of coconut water. Second was nano particle synthesis from bacterial cellulose which was produced in the first step. The formation of nano particle used the hydrolyzation method. This process was started with hydrolysis of bacterial cellulose using hydrochloric acid (HCl) with varied concentration and hydrolyzation time. Concentration variables named as A1 (3.5 M) and A2 (5 M), whilst three variation of time was referred to [3], named as B1 (24 hours), B2 (48 hours) and B3 (72 hours). This method was designed into six samples which are A1B1, A1B2, A1B3, A2B1, A2B2, A2B3. All treatments done in 55°C temperature, then finalizing with ultrasonication for sixty minutes. The supernatant results then filtered, bleached and neutralised. Filtration was conducted using Whatman filter papers number 41 with the help of vacuum pump. The final step was drying the filtrate using Buchi spray dryer to produce final product.

Third step was characterisation of nano bacterial cellulose. This step included surface characterisation by using Scanning Electron Microscopy (SEM), density determination, crystal characterisation by using X-Ray Diffraction (XRD) and measuring the size particle by using Particle Size Analyzer (PSA). Surface characterisation by using Scanning Electron Microscopy (SEM) was conducted in Metallurgy laboratory of mechanical engineering department of Andalas University. SEM can also cover smaller particle size than nano size. Density of particle (ρ) (g/cm³) was gained by calculating mass (m) (gram) and volume (v) (cm³) of the sample of particle with this formula:

$$\rho = \frac{m}{v}$$  \hspace{1cm} (1)

Furthermore, crystal characterisation by using X-Ray Diffraction (XRD) was done to determine the crystal system of product, such as grid parameter, type of structure, different atom structure, defect of crystal, orientation, type of crystal granule, crystallinity and size of crystal granule. The calculation results of index crystallinity value using the Segal formula [4]:

$$CrI = \left[ \frac{I_{002}-I_{AM}}{I_{002}} \right] \times 100$$  \hspace{1cm} (2)
And last analysis was PSA which aimed to have information about particle size and distribution of different size particle.

3. Results and Discussion

3.1. Bacterial Cellulose
Bacterial cellulose, which is produced with coconut water fermentation, is kind of microbial polysaccharide that formed by cellulose fibres from *Acetobacter xylinum*. As a polymer bacterial cellulose has supramolecular and high crystallinity. Physical properties of produced bacterial cellulose in this research are yellowish white in colour, transparent, and formed with slippery texture and elastic layers.

Base on analysis this cellulose has 0.9-1.0% of cellulose and 380, 56 mPa in tensile strength test. The tensile strength value (mPa) of the bacterial cellulose tends to increase as the thickness and formation of layers increase. This bacterial cellulose has modulus young approximately 18 Gpa, elongation 11.3 % and density around 1, 15 g/cm³. The produced bacterial cellulose has high porosity, hydrophilic and 700% water absorption.

3.2. Synthesizing of Nano Bacterial Cellulose
Nano cellulose can be produced by hydrolysis method with strong acid like chloride acid (HCl). Strong acid help reducing amorphous from cellulose chain that make isolation of cellulose crystalline can be done [5]. Hydrolysis mechanism by strong acid can be seen in Figure 1.

![Figure 1. Hydrolysis mechanism by acid](Figure 1. Hydrolysis mechanism by acid [6])

Hydrolyzation by strong acid is still the main process in producing nanocrystal of cellulose. Cellulose is consisted of amorphous area and crystal area. In this process small block of *amorphous* will be dislocated from cellulose chain. *Amorphous* area is low in density compare to crystal area. By giving strong acid, *amorphous* area will be unravelled and release crystalline area so then crystalline
formed. Characteristic of crystalline depend on many factors such as source of cellulose, reaction time, temperature, kind of acid that been used in hydrolysis.

After hydrolysis process was done, next step was ultrasonication by batch ultrasonic. Kinetic energy from the wave would radiate to entire water surface, and then cavitation vesicle would appear on the surface and on the wall of ultrasonic wall. This effect would increase mass transfer between material surface and the water in ultrasonic. Finally, vibration of high frequency wave would disintegrate the cellulose into smaller particle. Furthermore, mechanical effect that has been appeared, increased the penetration from the liquid into cell membrane, and released cell’s component [7]. Liu et al. stated that cavitation energy will produce power to break the cell’s wall mechanically, and it also increases mass transfer [8]. Delmifiana et al. also stated shocking wave from ultrasonication method separates agglomerated particle and produce space between particle [9].

3.3. Nano Bacterial Cellulose Characteristic
Base on Particle Size Analyzer (PSA) result as can be seen in Figure 2a to Figure 2f, showed that A1B1 treatment (Figure 2a) contains 31.3 % 74.92 nm particle size; 68.3 % 481.2 nm particle; and 0.4 % 3873 nm particle. Furthermore, A1B2 treatment (Figure 2b) produced nano bacterial cellulose which has 100% 531 nm particle which means relatively similar size. On the other hand, A1B3 treatment (Figure 2c) produced nano bacterial cellulose with varied particle size with distribution 24.5% 117.8 nm; and 75.5% 665.5 nm.

A2B1 treatment as can be seen in Figure 2d also contains relatively similar particle size which is 100% 427.8 nm. A2B2 treatment as presented in Figure 2e, produced 86.6% 99.9 nm particle and 13.4% 568.9 nm. Last treatment, A2B3 as seen in Figure 2f contains 6.1% 146.9% nm particle and 93.9% 491,9 nm particle.

To be conclude all the treatments produce nano particle bacterial cellulose. It can be seen by the size of the particle, which has been produced, are between 1-100 nm. Hosokawa et al. (2007) stated that nano particle is particle that has size between 1-100 nm [10]. If we refer to that definition, we can conclude that the treatment that produced nano particle and had the best distribution was A2B2 with 86.6% 99.9 nm particle size. Based on all treatments, the trend shows that increasing concentration of HCl tend to influence the particle size of nanto cellulose. The particle is reported to be smaller for 5M HCl than nano cellulose with 2.5 M HCl hydrolyzation. As for the time of hydrolyzation the interaction between the factors should be further examined.
Figure 2a. A1B1 Treatment

Figure 2b. A1B2 Treatment
Figure 2c. A1B3 Treatment

Figure 2d. A2B1 Treatment
In this research, ultrasonication has proven to have positive impact to particle size and distribution. It makes particle size homogeneity and stable without any agglomeration in packaging during storage time, which can be seen in SEM result. Results also give information that particle was distributed in varied size, where A2B2 treatment seems to have homogenous particle size than others. SEM results with 5000 x zoom can be seen in Figure 3.
In addition, XRD test has also been conducted. The results give us information that material that has been produced by hydrolysis process was β-cellulose \((C_6H_{10}O_5)_n\) and NaCl as by product. β-cellulose is cellulose classification based on its polymerizing degree and its solubility in Sodium hydroxide 17.5% solutions. The classification is divided on to 3 type [11], which are α-Cellulose as cellulose that has long chain, insoluble in NaOH 17.5% or other strong alkali and has polymerizing degree 600-1500; β-Cellulose, refers to Cellulose with short chain, dissolve in NaOH 17.5 % or other strong alkali, has polymerizing degree 15-90 and sediment when neutralise; and γ-Cellulose is the same cellulose with β-Cellulose but different in polymerizing degree less than 15.The by product, NaCl was formed due to the reaction between HCl and NaOH 10% and imperfect neutralization through the washing process. The XRD test also classified that produced cellulose has dimension which was equal in the term of grid parameter \(a : 8.2600\AA; b : 10.3880\AA; c : 7.8400\AA\), volume 669.27 Å³ dan crystal shape is monoclinic. The index crystallinity at the best treatment is 76%. XRD result can be seen in Figure 4.
4. Conclusion
Taking everything into consideration, it can be concluded that bacterial cellulose that have been produced in this research has nano particle size. The best treatment was HCl 5M with hydrolysis time 48 hours and 55°C process temperature. This treatment produced nano-cellulose 99.99 nm in particle size with 86.6% distribution. The nano bacterial cellulose has certain characteristic, which is β-Cellulose with grid parameter a : 8.2600Å; b : 10.3880Å; c : 7.8400Å, volume 669.27 Å³ dan crystal shape is monoclinic. The index crystallinity is 76%.

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