**Processing of micro-nano bacterial cellulose with hydrolysis method as a reinforcing bioplastic**

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**Abstract.** Nanotechnology is the ability to create and manipulate atoms and molecules on the smallest of scales. Their size allows them to exhibit novel and significantly improved physical, chemical, biological properties, phenomena, and processes because of their size. The purpose of this research is obtaining micro-nano bacterial cellulose as reinforcing bioplastics. Bacterial cellulose (BC) was made from coconut water for two weeks. BC was dried and ground. Bacterial cellulose was given purification process with NaOH 5% for 6 hours. Making the micro-nano bacterial cellulose with hydrolysis method. Hydrolysis process with hydrochloric acid (HCl) at the conditions 3.5M, 55°C, 6 hours. Drying process used spray dryer. The hydrolysis process was obtained bacterial cellulose with ±7 µm. The addition 2% micro-nano bacterial cellulose as reinforcing in bioplastics composite can improve the physical characteristics.

**1. INTRODUCTION**

Nanotechnology is the ability to create and manipulate atoms and molecules on the smallest of scales. Their size allows them to exhibit novel and significantly improved physical, chemical, biological properties, phenomena, and processes because of their size. Nanotechnology can be defined as everything related materials, systems and processes that are operated on a scale of 1-100 nanometers (nm). Nanotechnology offers great opportunities for the development of innovative products and applicable to various fields such as packaging, biomedical, electronics, optics and agriculture. The physical characteristics and new phenomena would arise if materials on the nanoscale due to changes in the functional properties of the material, related to the changing nature of the dispersion.

Coconut water as waste has great potential as a raw material of bacterial cellulose. Bacterial cellulose as a result fermentation of *Acetobacter xylinum* bacterial in coconut water medium. Bacterial cellulose has several advantages as a source of cellulose compared to wood or non-wood resources, among others, it can be harvested in a shorter time, easily cultivated, and reduce the exploitation of forests to meet the needs of cellulose for the industry. Furthermore, not like other cellulose, bacterial cellulose has no lignin, pectin, and hemicelluloses which are impurities in cellulose which mean fewer steps in production. Bacterial cellulose has a high content of cellulose fibers and has a high mechanical characteristic. The physical characteristics of bacterial cellulose are purity, crystallinity, high mechanical strength, and low-cost polymers. The study of microbial cellulose for various areas of
application urgently needed to increase the added value the products of bacterial cellulose and is not limited to use as a food product.

Nanotechnology, every material can be lighter, more stable and increase in functionality. Nanotechnology offers many advantages and opportunities in the field of product innovation and development of bioplastic. Moreover, the strength, flexibility, and stability are considered the most important characteristic on producing bioplastic. Using filler which may increase those properties is an alternative solution for that problem [1].

There are several methods for the synthesis nano cellulose, the method of mechanical, chemical, and biological. Several previous research that use a chemical method by means of a strong acid hydrolysis. Wang et al (2008) using sulfuric acid, hydrochloric acid and distilled water in the ratio 3: 1: 6 (v / v) were accompanied by stirring 50 Hz (3000 rpm) for 10 hours, resulting nano cellulose measuring 20-90 nm [2]. Rosa et al (2010) using the base material pieces of coconut delignification in the previous stage, which then results delignification coconut pieces hydrolyzed with sulfuric acid, resulting nano cellulose measuring 5-6 x 58-515 nm[3]. Sadegifar et al (2011) using hydrogen bromide (HBr) with a concentration of 2.5 M temperature is 80°C for 3 hours, then filtered using Whatman paper. The resulting Nano cellulose measuring 100-400 x 7-8 nm [4].

Ieolovich (2012) made nano cellulose was made with variations of the reaction temperature and ratio of acid to cellulose, produced nano cellulose 1150-200 x 10-20 nm size [5]. Xiong et al (2012) with waste raw cotton which is hydrolyzed using sulfuric acid 63% by weight total of 33 mL at a temperature of 44°C with stirring and ultrasonikasi at 50 Hz for 3 hours. The adding water distillation which aims to stop the process of hydrolysis, water results distillation is added as much as 5 times the volume of the mixture. The resulting nano cellulose measuring 10-65 nm [6]. Bernando et al (2012) using a base material of bamboo fiber, bamboo fiber before hydrolysis stage soaked in NaOH 2% w / w for 4 hours and bleaching at a temperature of 80 ° C for 3 hours. Furthermore pulp already bleaching hydrolyzed using sulfuric acid 64% by weight and the resulting nano cellulose measuring 100-130 x 5-8 nm [7].

Hydrolysis of cellulose that is commonly used is strong acid. Strong acids can eliminate the amorphous part of a chain of cellulose so that the insulation on the crystalline cellulose can be done [8]. The mechanism of cellulose hydrolysis with strong acids shown in Figure 1.

![Figure 1. Mechanism of Acid Hydrolysis](image-url)

Acid hydrolysis is main process used in producing cellulose nanocrystal, where the arrangement of small blocks separated from the cellulose fibers. Cellulose is composed of amorphous regions and crystalline regions. The amorphous area has a lower density than crystalline regions, so that when the cellulose is given treatment using strong acids, the amorphous regions will break and release the crystalline regions. The nature of cellulose nanocrystal depends on various factors, such as a source of cellulose, reaction time, temperature, and type of acid used for the hydrolysis process. Sulfuric acid
and hydrochloric acid is often used in the production of nanocrystals of cellulose. Dispersabilitas of nanocrystal cellulose derived from both acid type is different, because of the abundance of sulfate groups on the surface. The nanocrystal cellulose derived from hydrolysis using sulfuric acid can be dispersed easily in water while nanocrystal cellulose derived from hydrolysis using hydrochloric acid does not disperse and the suspension solution tends to flocculate [10]. The purpose of this research is obtaining micro-nano bacterial cellulose as reinforcing bioplastics and knowing the characteristics of bioplastics.

2. MATERIAL AND METHOD

2.1. Materials and tools
Materials were used coconut water, urea, sugar, acetic acid, hydrochloric acid, sodium hydroxide, sodium hypochlorite, sago starch, and glycerin, and distilled water. Tools were used hotplate stirrer, beaker glass, 41 Whatman filter papers, plastic mold, and Buchi B 290 spray dryer.

2.2. Methodology
This research was conducted in three stages: the first is a production of bacterial cellulose, the second is making of micro-nano from bacterial cellulose that is produced in the first stage, and final stage is manufacturing bioplastics. The method of making micro-nano cellulose bacteria is acid hydrolysis process. Firstly, bacterial cellulose powder was heated in NaOH 5%, 100 °C for 4 hours. After that washed and filtered and then blended. Hydrolysis process with hydrochloric acid (HCl) at the conditions 3.5M, 55 °C, 12 hours. The results, micro-nano cellulose bacteria from this step, was bleached, neutralized, and then dried with a spray dryer. Casting method was used to manufacture the bioplastics. Treatment of adding nano cellulose (0%, 2%, 4%) into 100 ml of distilled water plus 3 ml of glycerol and 10 g of sago starch and then stirred for 1 hour at 80-85 °C. After 1 hour, the solution then printed in the mold.

3. RESULT AND DISCUSSION

3.1. Bacterial Cellulose
Bacterial cellulose (BC) was made from coconut water for two weeks (Figure 2a). Base on analysis this cellulose has 0.9-1.0% of cellulose and 380, 56 Mpa in the tensile strength test. The more layers formed, the higher tensile strength number. Modulus young approximately 18 Gpa, elongation 11.3 % and density around 1, 15 g/cm³. Bacterial cellulose has high porosity, hydrophilic and 700% water absorption. Bacterial Cellulose was dried and grinded as the Figure 2b and 2c. SEM testing results of bacterial cellulose can be seen in figure 3. Bacterial cellulose was given purification process with NaOH 5% for 6 hours.

Figure 2. Bacterial Cellulose
3.2. Nano bacterial cellulose

Hydrolysis process using hydrochloric acid (HCl) with 3.5M concentration conditions, 55°C temperature for 6 hours. This process has been work out in reducing the size of the bacterial cellulose fibers to ± 7 μm. SEM testing results against bacterial cellulose can be seen in Figure 4. To the extent, this has not been obtained yet cellulose particle size with nanometer size.

Figure 3. SEM test of bacterial cellulose

Figure 4. SEM test of micro-nano bacterial cellulose

Preparations of micro-nano bacterial cellulose can be stored in a wet form can be seen in Figure 5a. Drying is done by spray drying to obtain micro-nano bacterial cellulose in a powder, as in Figure 5b, 5c.
3.3. Physical characteristic of bioplastic

Micro-nano bacterial cellulose produced from hydrolysis at 3.5M concentration 55°C temperature for 6 hours selected as a filler in the manufacture of bioplastics. Distribution of micro-nano bacterial cellulose in bioplastics can be seen from the test results of SEM in Figure 6. Micro-nano bacterial cellulose distributed within the matrix of starch, this means that the process of adding nanofiller bacterial cellulose to the matrix goes well. Bioplastics surface that looks less flat due to the molding process of bioplastics with the casting method manually.

Test FTIR (Fourier Transform Infrared Spectroscopy) as an analysis conducted to determine the functional groups on the polymer. FTIR spectra generated from testing bioplastics are presented in Figure 7. Based on the image seen on the identification of functional groups contained in each of the constituent components of bioplastics. So it can be seen clearly that the bioplastics obtained a bioplastic produced through a process of mixing (blending), it can be seen because no new functional groups. Also be concluded that the plastic is formed while still having hydrophilic properties such as its constituent components. The existence of functional groups such as CH (frequency 2900-3010 cm⁻¹), C-NO₂ (frequency 1300-1500 cm⁻¹), -N = N (frequency from 1575 to 1650 cm⁻¹) and - (CH₂) n (frequency 750 cm⁻¹), shows the plastic film can be degraded properly.

Figure 5. Micro-nano bacterial cellulose

Figure 6. SEM test of bioplastic (treatment 2%)
The addition of micro-nano bacterial cellulose as filler bioplastics significant effect increases the value of tensile strength and tensile modulus. The highest tensile strength at treatment addition of micro-nano cellulose 2% of 25.62 Mpa. This value is higher than the tensile strength in bioplastics breadfruit starch-chitosan-sorbitol with a value of 16.34 Mpa [11]. The resulting rigid bioplastic indicated by the tensile modulus values high despite already plus plasticizer glycerol. The tensile modulus value was higher when compared to the starch bioplastics breadfruit-chitosan-sorbitol with a value of 2.72 Mpa [11]. According to Bourtoom (2006) To increase the elasticity of the bioplastic can be done by the addition of plasticizers [12]. The tensile strength value meets into standard tensile strength values, with the value of biodegradable tensile strength of 10-100 Mpa and tensile strength values seen from polypropylene (conventional plastic) value of 24.7 which was close enough [13]. The mechanical properties of bioplastics with sago starch matrix and filler micro-nano bacterial cellulose when compared to conventional plastics and biodegradable plastics produces mechanical properties are not much different. Values for tensile strength bioplastic is now approaching the standard, but the young's modulus and elongation still not meet the standards.

4. CONCLUSION
Micro-nano-sized bacterial cellulose which was produced is ± 7 μm. The addition of micro-nano bacterial cellulose as filler bioplastics significant effect increases the value of tensile strength and tensile modulus. The physical characteristics of bioplastics by adding 2% of micro-nano bacterial cellulose which are tensile strength of 25.65 Mpa, Young modulus of 3.73 Mpa, elongation of 4.98% and density of 0.32 g / cm³. The tensile strength value has fulfilled the standard of bioplastics.

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REFERENCES
[1] Wicaksono R, Syamsu K, Yuliasih I, Nasir M, 2013. Karakteristik Nanoserat Selulosa dari Ampas Tapioka dan Aplikasinya Sebagai Penguat Film Tapioka. J Tek Ind Pertanian. 23 (1) : 38-45.
[2] Wang, N.; Enyong, D.; Rongshi, C., 2008. Preparation and Liquid Crystalline Properties of Spherical Cellulose Nanocrystals. Langmuir, Vol. 24, 5-8
[3] Rosa, M. F.; Medeiros, E. S.; Malmonge, J. A.; Gregorski K. S.; Wood, D. F.; Mattoso, L. H. C.; Glenn, G.; Orts, W. J.; Imam, S. H., 2010. Cellulose nanowhiskers from coconut husk fibers: Effect of preparation conditions on their thermal and morphological behavior. Carbohydrate Polymers, 81, 83-92
[4] Sadeghfar, H.; Ilari, F.; Sarah, P. C.; Dermot F. B.; Dimitris S. A., 2011. Production of
cellulose nanocrystals using hydrobromic acid and click reactions on their surface. Springer. *Journal Material Science*.

[5] Ioelovich Michael. 2012. Optimal Conditions for Isolation of Nanocrystalline Cellulose Particles. *Journal of Nanoscience and Nanotechnology*, 2(2): 9-13

[6] Xiong, R.; Xinxing, Z.; Dong, T.; Zehang, Z.; Canhui, L., 2012. Comparing microcrystalline with spherical nanocrystalline cellulose from waste cotton fabrics. *Cellulose*, 19, 1189–1198.

[7] Bernardo, S. L. B.; Fabiano V. P.; Jean, L. P.; Bruno J., 2012. Preparation morphology and structure of cellulose nanocrystals from bamboo fibers. *Cellulose*, 19, 1527–1536.

[8] Isdin O. *Nanoscience in nature: cellulose nanocrystals*. Surg, 2010, 3(2)

[9] Yue, Y., A. 2007. Comparative Study of Cellulose I and II Fibers and Nanocrystals. *Thesis*. Heilongjiang Institute of Science and Technology.

[10] Peng, B.C., Dhar, N., Liu, H.L., dan Tam, K.C. 2011. Chemistry and Applications of Nanocrystalline Cellulose and Its Derivatives: A Nanotechnology Perspective. *The Canadian Journal of Chemical Engineering*. 89: 1191-1205.

[11] Setiani, W., T. Sudiarti dan L. Rahmindar. 2013. Preparasi dan Karakterisasi Edible Film dari Poliblend Pati Sukun-Kitosan. *Jurnal Kimia Valensi* 3(2) : 100-109 . Jurusan Kimia Fakultas Sains dan Teknologi UIIN Sunan Gunung Djati. Bandung

[12] Bourtoom, T. 2006. Plasticizer Effect on the Propertes of Biodegradable Blend Film From Rice Starch-Chitosan. *Songklanakarin Journal of Science and Technology*. 30 (Suppl.1), 149-155.

[13] Krochta, J.M and M. Johnston.1997. Edible and Biodegradable Polymer Film. Challenges and Opportunities. *Food Tech*. 51(2):61-74