Synthesis and Characterization of Bacterial Nanocellulose from Banana Peel for Water Filtration Membrane Application

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Abstract. This research reports the result obtained from the synthesis and characterization of Bacterial Nano Cellulose (BNC) from Nangka banana peel (Musa sp L.) media for water filtration membrane application. The BNC synthesis is successfully achieved under the condition of banana peel and water ratio of 1:3, bacterial nutrition: Glucose 10% (w/v), Ammonium Sulphate (NH₄)₂SO₄ 1% (w/v), pH: 4, and amount of starter: 15% (w/v). The duration of fermentation is 7 days. The water content obtained in BNC banana peel is 86.59%. The Scanning Electron Microscopy (SEM) analysis shows the resulting nanocellulose is nanofibril 30-50 nm in diameter. The X ray diffraction (XRD) shows the banana peel BNC crystallinity index (Ic) is 86.94% and cellulose Type I. The Fourier Transform Infra-Red (FTIR) spectra confirms the bond and functional group of nanocellulose. These results support the required properties for strong but flexible membrane filter. The potential zeta absolute value -11.39 mV from the Electrophoretic Light Scattering (ELS) shows that BNC colloidal solution has good stability that it can be further used for the manufacture of water filter catalytic membrane composites.

1. Introduction
Clean water is the primary need for humans throughout the world for their domestic, industrial, and agricultural needs. The current increase in the human population and industrialization has a negative impact on the environment quality as indicated by decreasing water catchment areas that results in decreasing ground water availability. Raw water now only covers 3% of the total water on earth whereas the remaining 97% is sea water. The solution to fulfil the need for clean water is by processing seawater into fresh water (desalination).

Recently there are many nanopore inorganic membrane materials developed for desalination processes such as zeolite, silica, alumina, zirconia, and composites from these materials [1-2]. Ceramic membrane also had developed by coating of porous geopolymer with the nanoparticles of γ-Al₂O₃, zeolite, and its combination which achieved high salt rejection rate and high flux value [3]. However, existing inorganic membranes have disadvantages such as high production costs, requiring high
operating pressure, rigid (stiff) membrane design, inflexible and easily broken. These problems can be solved by utilizing the advantages of nanocellulose material that functions as the organic network former.

Cellulose has a fiber structure (microfibril) composed of amorphous and crystalline region. These fibers are formed from nanocellulose strands with crystal structure called nanocrystalline cellulose (nanocrystal cellulose) and nanocellulose with amorphous structure. The crystalline cellulose structure is the result of interactions of non-covalent bonds, namely (inter- and intra-molecular) hydrogen bonds and Van der waals interactions [4]. Nanocrystalline cellulose has a higher tensile strength than metal or other polymers materials, the addition of nanocrystalline cellulose in paper nanocomposite can function as a reinforcing material [5]. The addition of nanocrystalline cellulose can increase the paper tensile strength up to 40% [6].

The abundant source of cellulose in Indonesia, especially from banana peel waste, is an opportunity to develop membrane with small pore size, large surface area, good mechanical, chemical, and thermal resistance, apart from solving the waste problem. The data from the Ministry of Agriculture in 2016 [7] shows that the total banana produced in 2014 was 6,862,668 tons wherein the weight of banana skin was one third the weight of unpeeled banana. Therefore the total banana peel waste in 2014 was 2,287,556 tons.

Non-wood nanocellulose like banana peel can be obtained by environmentally friendly fermentation process using *Gluconacetobacter xylinum* bacteria. The cellulose produced is usually called Bacterial Nano Cellulose (BNC) or nanocellulose bacterial. BNC has several advantages over other wood-based cellulose such as high purity without lignin, pectin, and hemicellulose, high mechanical strength and porosity [8]. Hemicellulose, lignin and pectin are undesirable contents in all types of pulp because they will interfere with the process of making sheet paper. The conventional process of removing hemicellulose, lignin and pectin requires high energy and costs. The carbohydrate content in banana peel can be used by *Gluconacetobacter xylinum* microorganisms in their growth to produce nata fermentation products as cellulose sheets. Adding inorganic or organic nitrogen would increase the activity of *Gluconacetobacter xylinum* in nata production [8]. *Gluconacetobacter xylinum* is a type of bacteria that is widely used in research and commercial production. These bacteria are non-pathogenic, in the form of short rods, gram negatives, and aerobics with high ability to produce cellulose from several sources of carbon and nitrogen [9-10]. These bacteria are ubiquitous in nature and are naturally present in sugar fermentation [10].

BNC has the potential to be applied on paper-making on a commercial scale. However the main problem that impedes this process is the low yield and expensive production costs for such application with low added value. Therefore, several efforts have been made to optimize the culture condition and media composition including the scale-up process. In general, BNC is produced using Hestrin-Schramm (HS) media which uses glucose as a carbon source and a combination of peptone and yeast as a source of nitrogen [11]. However using glucose as a carbon source for the production of BNC is quite expensive and causes the formation of byproducts such as gluconic acid which lowers pH of culture media and ultimately decreases BNC production [9]. In this study, the banana peel will be used as source of carbon. Another way is to apply BNC for high value-added product like membrane catalytic filter which will be explore in this research.

However the hydrophilic nature of cellulose fibers limits their use. This trait inhibits even-mixing when used as a nonpolar thermoplastic product filler. The hydrophilic nature makes it easy to absorb moisture, resulting in an open space interface which will further reduce its mechanical properties and the stability of the dimensions of its products [12]. Cellulose fibers cannot be processed at temperatures over 200° C because they will be easily degraded, so they cannot be mixed with other materials in the process of making composite materials that require high melting point. Cellulose fibers, like other biopolymers, are biodegradable or can be decomposed by microorganisms. This is a beneficial trait from the environmental aspect but biodegradable material is not durable and prone to rot in use, storage, transportation and processing [12].
2. Research Method

2.1. Materials and Chemicals
Materials and chemicals used in this study were Nangka banana peel (Musa sp L.), NaOH 98% flake produced by PT Asahimas Chemical, Ammonium Sulphate (NH₄)₂SO₄ min 98% produced by PT Petrokimia Gresik (specific for bacterial nutrition), Acetic Acid (CH₃COOH) 99% conc., Gulaku sugar brand from PT Sugar Group, Gluconacetobacter xylinus bacteria from nata de coco manufacturers from home industries in Cianjur, West Java.

2.2. Research Flow Chart
In short, the flow diagram of this research can be seen in figure 1 below:

![Research Flow Chart](image)

Figure 1. Research flow chart for the synthesis and characterization of nanocellulose from banana peel by biological method.

2.3. Synthesis of BNC
Banana peel is first cleaned and then cut into small pieces. Then weighed and soaked in water with a ratio of 1:3 (w/v). The mixture is heated to boiling and the top layer formed during the process is removed. Then the mixture is cooled for 12 hours. After that, the banana peel is squeezed, the pulp is removed and the extract is filtered. The extract obtained was cooked to a temperature of 100 °C before adding Glucose 10% (w/v) and Ammonium Sulphate 1% (w/v). Acetate acid was added to obtain pH = 4. The mixture was then put into a container, covered with newspaper to cool down for 12 hours. The starter of Gluconacetobacter xylinus bacteria 15% (v/v) was then added, and fermentation period lasted for 7 days. The fermentation results obtained are then washed and soaked in NaOH 2 M for 12 hours to obtain the banana peel BNC.

2.4. Material Characterization
2.4.1. Water Content
The sample is first weighed using an electric scale to get the wet weight. Then it was put in the oven at 75 °C temperature until the weight does not change. After that the sample is weighed to get the dry weight. Water content is calculated based on the following formula:

\[
\text{Water Content} = \left( \frac{\text{Wet weigh} - \text{dry weigh}}{\text{Wet weigh}} \right) \times 100 \%
\]

(1)

2.4.2. Scanning Electron Microscopy (SEM)
SEM (Type: Hitachi SU3500, PPNN, ITB) is used to determine the microstructure of the banana peel BNC. BNC is prepared on carbon tape which is then coated with a thin layer of gold. Structure evaluation is carried out with an acceleration voltage of 5 kV.
2.4.3. X-Ray Diffraction (XRD)
XRD (Type: Bruker D8 Advanced, PPNN, ITB) is used to obtain information about the BNC crystal structure. The characterization process is carried out in the range of 2θ 10° - 55°. The anode used is Cu (1,5406 Å).

The nanocellulose crystallinity (Ic) index is calculated using the Seal method through the following equation:

\[ Ic = \left( \frac{Ia - Ib}{Ib} \right) \times 100 \% \]  

where: \( Ia \) is a peak approaching 2θ = 22° and \( Ib \) refers to the amorphous intensity of cellulose which can be searched from observing the lowest intensity between peaks approaching θ = 16° and 22°.

2.4.4. Fourier Transform Infrared Spectroscopy (FTIR)
FTIR (Shimadzu IRPrestige-21, Chemical Study Program, ITB) is used to see the effect of alkaline treatment (NaOH) and to see the content and impurities found in the BNC synthesis output. The spectrum is then analysed in the range of wave numbers 400-4,500 cm\(^{-1}\). Resolution is set at the value of 4.0 cm\(^{-1}\).

2.4.5. Electrophoretic Light Scattering (ELS)
ELS (Beckman Coulter Delsa Nano C, Pharmacy Study Program, ITB) is used to determine the potential zeta value of nanocellulose colloidal solutions. The type of solvent used is water and the electric field given is -12.4 V/cm.

3. Result and Discussion

3.1. BNC Synthesis from Banana Peel
In this study a number of trial were conducted to obtain conditions suitable for the bacteria to grow. BNC was successfully synthesized under the following conditions: ratio of banana peel and water 1:3, bacterial nutrition: Glucose 10% (w/v), Ammonium Sulphate (NH\(_4\))\(2\)SO\(_4\) 1% (w/v), pH: 4 (adjusted by adding Acetic Acid 99 % conc. and the amount of starter: 15% (w/v). In order to get good results, the banana peel is soaked and cooked for 12 hours to eliminate the peptin content that can disturb the bacterial growth. This process is simpler than the BNC synthesis process that has been carried out at this time.

3.2. Water Content
BNC obtained from the banana peel synthesis is in the hydrogel form. For this reason, it is necessary to know the water content contained in it to determine the nanocellulose dry weight. To speed up the drying, the BNC is first pressed before being put into the oven. BNC conditions before and after being put into the oven can be seen from figure 2 below:

![Figure 2. Pictures of Banana Peel BNC in Wet and Dry Conditions.](image-url)
The average water content obtained is 86.59% which indicates the largest content of banana peel BNC is water. The average cellulose content in BNC is 13.41%. The bigger the surface area and pore size of the BNC matrix, the higher the water content. The OH group of nanocellulose is very hydrophilic so it will bind to large amounts of H2O. This trait is a problem to be addressed later if the BNC is to be used in water filter membrane applications. This result is in accordance with the literature which states that Bacterial Nano Cellulose (BNC) contains high water content > 90% [10]. The water content of the BNC can be seen from table 1 below:

Table 1. Water Content of Banana Peel BNC.

| Sample Number | Wet Weight (gram) | Dry Weight (gram) | Water Content (%) |
|---------------|-------------------|-------------------|-------------------|
| 1             | 39,78             | 5,66              | 85,77             |
| 2             | 38,73             | 5,12              | 86,78             |
| 3             | 39,95             | 5,36              | 85,58             |
| 4             | 38,56             | 4,93              | 87,21             |
| Average       |                   |                   | 86,59             |
3.3. Microstructural Evaluation of SEM Figures

The banana peel BNC microstructure can be observed in figure 3 alongside both the surface (figure A and B) and cross sections (figure C and D).

The results are in accordance with the literature [13] which states that nanocellulose bacteria is a 3-D random, interwoven network formed by cellulose fiber. Banana peel BNC produced has the shape of a nanofibril of 30-50 nm in diameter that it can be categorized as nanocellulose. In manufacturing membrane filters, the banana peel BNC is expected to form a binding network with other materials of a certain porosity.

3.4. Analysis of XRD Characterization Test

XRD results from BNC produced from the banana peel medium are shown in figure 4. The crystallinity index (Ic) is measured by using the Seal method described in Chapter 2.4.3. From the calculation, the obtained BNC Crystallinity Index (Ic) is 86.94%. The result is in accordance with the literature which shows that the Ic of Bacterial Nano Cellulosa (BNC) is generally high. This is one of the advantages of making nanocellulose from biological processes compared to other chemical and mechanical processes. This trait is later useful when making catalytic filter membranes with the addition of BNC banana peels. Nanocrystalline cellulose has a higher tensile strength than metal or other polymers. The addition of nanocrystalline cellulose in nanocomposite can serve as a reinforcing material [4].

![Figure 3. SEM Result of Banana Peel BNC on Surface view (A,B) and Cross Section View (C,D).](image1)

![Figure 4. XRD Result of Banana Peel BNC](image2)
The crystallinity index (Ic) of banana peel BNC can still be optimized in future research by varying the amount of bacterial nutrition, type of sugar, duration of fermentation, and pH. The $2\theta$ peaks are visibly seen in the range 16°-22° and can be observed from the BNC sample. The peaks indicate that the nanocellulose that was isolated has a type I cellulose crystal structure [14] which is commonly found in cellulose derived from natural sources like bacteria.

3.5. **FTIR Spectra Analysis**

The FTIR spectra analysis provides an overview of the banana peel BNC composition as can be seen in figure 5 and table 2 below.

![Figure 5. FTIR Spectra of Banana Peel BNC.](image)

**Table 2.** Banana peel BNC peaks FTIR analysis after being soaked with NaOH

| Peak (cm$^{-1}$) | Bond and Type of Functional Group |
|-----------------|----------------------------------|
| 3448            | The stretching OH hydrogen bond  |
| 2922            | CH stretching                    |
| 1629            | OH bending from absorbable water |
| 1436            | HCH and OCH vibration bending   |
| 1163            | The C-O-C bond                  |

Peak of banana peel BNC above corresponds to the characteristics of BNC absorption bands like 3,348 cm$^{-1}$ as hydroxyl group, 2,942 cm$^{-1}$ as CH stretching bond, 1,161 cm$^{-1}$ as C1 O C4 streching bond in β-glycosidic as found in plant-based cellulose [15].
3.6. **Analysis of Zeta Potential Value**

ELS testing was carried out to obtain information about potential zeta of BNC in colloidal solutions. The potential zeta value indicates the degree of repulsion between particles with the same surface charge in a disperse [16]. The particle’s high dispersion trait is important if it is to be considered as reinforcing agents in composite manufacturing [17].

Based on the test results, the obtained potential zeta value for banana peel BNC was ranging from -11.12 and -11.66 mV with the average is -11.39 mV. The potential zeta absolute value indicates that the BNC colloidal solution has good stability with water solvents and is able to prevent rapid particle aggregation. These results support the further use of BNC as a mixture material in manufacturing filter membrane composites with ceramic materials and other chemicals.

4. Conclusion

The conclusions drawn from this study are nanocellulose synthesis was successfully done with a ratio of banana peel and water 1:3, bacterial nutrition: Glucose 10% (w/v), Ammonium Sulphate (NH4) 2SO4 1% (w/v), pH: 4 and starter number: 15 % (w/v) with duration of fermentation is 7 days. The obtained water content in banana peel BNC is 86.59%. The banana peel BNC has a nanofibril shape the size of 30-50 nm in diameter, crystallinity index (Ic) 86.94% and nanocellulose that was isolated has a type I cellulose crystal structure. From FTIR spectra confirms the nanocellulose bonds and functional groups. The potential zeta absolute value of -11.39 mV indicates that the BNC colloidal solution already has good stability.

References

[1] Lee K P, Arnot T C and Mattia D 2011 A review of reverse osmosis membrane materials for desalination—development to date and future potential, *Journal of Membrane Science* 370 (1) 1-22

[2] Zhu B, Kim J H, Na Y H, Moon J S, Connor G, Maeda S, Morris G, Gray S and Duke M 2013 Temperature and pressure effects of desalination using a MFI-type zeolite membrane *Membranes* 3 (3) 155-168

[3] Heri Setiawan, Raisa Khairani, Mukhlis A. Rahman, Rifki Septawendar, Rino Rakhmata Mukti, Hermawan K. Dipojono and Bambang Sunendar 2017 Synthesis of zeolite and γ-alumina nanoparticles as ceramic membranes for desalination applications *Journal of the Australian Ceramic Society* 53 (2) 531-538

[4] Kang Y J, Chun S J, Lee S S, Kim YY, Kim J H, Chung H, Lee and Kim W 2012 All-solid-state flexible supercapacitors fabricated with bacterial nanocellulose papers, carbon nanotubes, and triblock-copolymer ion gels. *ACS nano* 6 (7) 6400-6406

[5] Habibi Y, Lucia L A and Rojas O J 2010 Cellulose Nanocrystals Chemistry Self-Assembly and Applications *Chemical Reviews* 110 (6) 3479-3500

[6] Salam A, Lucia L A and Jameel H 2013 A novel cellulose nanocrystals-based approach to improve the mechanical properties of recycled paper *ACS Sustainable Chemistry & Engineering* 1 (12) 1584-1592

[7] Kementerian Pertanian (2016) Outlook Komoditi Pisang 2016 Pusat Data dan Sistem Informasi Pertanian

[8] Lapuz M M, Gallerdo E G and Palo MA 1967 The Nata Organism-Cultural Requirements Characteristic and Identify *Philippines J Sci* 96 91-102

[9] Chawla P R, Bajaj I B, Survase S A and Singhal R S 2009 *Food Technol. Biotechnology* 47 107

[10] Klemm D, Schumann D, Udhart U and Marsch S 2001 *Prog. Polym. Sci.* 26 1561

[11] Hestrin S and Schramm M 1954, Synthesis of Cellulose by Acetobacter xylinum *Biochem J* 58 345

[12] John M and Thomas S 2008 Bifibres and biocomposites *Carbohydr Polym* 71, 343-365
[13] Dankovich T A and Hsieh Y L 2007 Cellulose 14 469
[14] Rosa M F, Medeiros E S, Malmonge J A, Gregorski A S, Wood D F, Mattoso L H C, Glenn G, Orts W J, and Imam S H 2010 Cellulose Nanowisker from Coconut Husk Fibers Effect of Preparation Conditions on Their Thermal and Morphological Behavior Journal of Carbohydrate Polymers 81 83-92
[15] Shurma-Slusarka B, Presler S and Danielewicz 2008 Characteristic of Bacterial Cellulose obtain from Acetobacter xylinum culture for application in papermaking, Fibres Text Eastern Eur 16 108-111
[16] Pellisari F M, Sobral P J A and Menegalli F C 2014 Isolation and Characterization of Cellulose Nanofibers from Banana Peels Journal of Cellulose 21 417 – 432
[17] Lam E, Leung A C W, Liu Y, Majid E, Hrapovic S, Male K B and Luong J H T 2012 Green Strategy Guided by Raman Spectroscopy for the Synthesis of Ammonium Carboxylated Nanocrystalline Cellulose and the Recovery of Byproducts Journals of ACS Sustainable Chemistry and Engineering 1 278 – 283

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