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To cite this article: N. Nurfajriani *et al* 2021 *J. Phys.: Conf. Ser.* **1811** 012070

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Preparation and Characterization of Bacterial Cellulose From Culturation of Acetobacter Xylinum In Coconut Water Media

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Abstract. This study aims to analyze bacterial cellulose obtained from culturing Acetobacterxylinum in coconut water media. Coconut water media is obtained from coconut water waste in existing traditional markets. The resulting nata de coco purified with a 1% (w / v) solution of sodium hydroxide (NaOH) and 1% (v / v) acetic acid (CH3COOH). The preparation process, bacterial cellulose in the form of thin sheets, is dried under the sun or in an oven at 60 ° C; after drying, it is mashed in a blender until a solid powder is obtained. The resulting bacterial cellulose then characterized using Fourier Transform Infrared (FTIR), Thermo Gravimetric Analysis (TGA), and Scanning Electron Microscopy (SEM). The results of FTIR analysis showed that the cellulose obtained derived from microorganism activity; TGA analysis showed that the weight loss was 9.2%; this indicates the hydrophilic nature of cellulose. SEM analysis shows that bacterial cellulose's surface morphology looks very dense and dense; this shows the characteristic properties of bacterial cellulose.

1. Introduction

Bacterial cellulose gel from A. xylinum bacteria contains high quality cellulose (99.8% water and 0.2% an inert polysaccharide) compare to the plant cells-based cellulose. The cellulose does not contain lignin, hemicellulose, pectin and candle which give more benefits for its application [1]. In general, cellulose comes from plants, and some are sourced from biosynthesis by microorganisms [2].

Coconut water can be used as a media to grow A. xylinum bacteria to synthesis glucose into cellulose [3]. The data from Ministry of Agriculture reveal that North Sumatera has an abundant sources of coconut water. The coconut water production from 2015 to 2017 were 88,844 tonnes (plant area 85,808 ha), 87,682 tonnes (84,429 ha plant area) and 87,539 tonnes (plant area plant 82,743 ha), respectively. The data includes fixed figures (ATAP) for 2015, preliminary figures (ASEM) for 2016, and estimated figures (AESTI) for 2017 [4]. Therefore, based on these data, there are abundant sources of cellulose that can be extracted from coconut water which can be used as bacterial culture medium. This research will focus on the culturation of Acetobacter Xylinum in Coconut water.

The use of coconut water waste that produces nata de coco to be used as a source of cellulose can increase the economic value of coconut water waste that has been thrown away by the community, especially in traditional markets. Also, cellulose is obtained efficiently without any deforestation process [1].

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2. Materials and methods

The tools used in the manufacture of bacterial cellulose are pH universal, aluminum foil, cover paper, oven, blender, hot plate / magnetic stirrer, trays, and glassware. Tools used for the characterization of FTIR, SEM, and TGA.The materials used to make bacterial cellulose include coconut water, sucrose, sugar, ammonium sulfate, acetic acid (p.a), A. xylinum inoculum, filter paper, and 1% NaOH solution.

2.1. Starter production

A total of 30 g of sucrose and 4 g of (NH4)2SO4 were dissolved in 1 L of coconut water. The solution were heated to boiling (120°C) and then cooled. A total of 450 ml of the solution was poured into a bottle, 10 mL of concentrated CH3COOH, and 90 mL of A. xylinum bacteria were added, then covered with a cover paper. The starter solution was stored at room temperature for 1 week before being used again as a starter.

2.2. Nata de coco production

A total of 100 g of sugar and 4 g (NH4) 2SO4 were dissolved in 1 L of coconut water. The solution were heated to boiling (120°C) for 20 minutes and then cooled, after that poured into a tray, then added 10 mL CH3COOH (pH 4 - 5), add 100 mL of starter, cover with a cover paper. Then the trays were stored in a flat and stable place for 2-3 weeks at room temperature [1].

2.3. *Purification and preparation of bacterial cellulose*

The mass of newly harvested bacterial cellulose were washed with tap water then the water were removed by pressure, then immersed in 1% (w/v) NaOH solution for a week, and continued with the neutralization stage with 1% (v/v) CH3COOH for 24 hours, then rinsed with water. For the preparation of bacterial cellulose, bacterial cellulose in the form of thin sheets is dried in the sun or an oven with a temperature of 60 oC [1], after drying it is crushed with a blender until a solid powder is obtained. This product was further characterized by FTIR, SEM, and TGA.

2.4. *Characterization*

FTIR analysis

FTIR characterization of BC was carried out using an FTIR spectrophotometer. FTIR spectral analysis was performed over a wavenumber range of 500-4000 cm-1.

SEM analysis

SEM characterization was used to analyse the BC surface morphology. The acceleration stress used was 15 Kv with magnifications of 500x, 1000x, and 5000x.

TGA analysis

TGA measurement was taken to analyse the BC thermal stability at 600oC with temperature growth at 10oC/min and 50 mL/min of nitrogen gas under the atmosphere pressure.

3. Result and Discussion

The content of coconut water includes substrates such as glucose and several minerals and protein with a percentage of about 2% -5% of the wet weight of coconut water [5]. The growth of A. xylinum is influenced by several physical and chemical factors in the system and environment, including pH, temperature, nitrogen source, and carbon source [6]. Coconut water is added with sucrose or sugar as additional carbon, and urea as a source of additional nitrogen for bacteria to increase their activity. The boiling process aims to hydrolyze carbohydrates into simple sugars to facilitate the conversion of sugar by bacteria [1].

Acidification with acetic acid to reach the optimum pH, namely pH 4 so that the resulting nata has a smooth surface and soft texture [7]. Another factor is the fermentation container in the form of a large tray so that oxygen exchange can take place properly to increase yield [8]. The incubation process is carried out by covering the container with perforated aluminum foil or paper so that the container is relatively sterile and free from impurities that may come from the air. Oxygen can enter

through the pores of the paper. During the incubation time at room temperature (30 oC), A. Xylinum was responsible for the biosynthesis of sugar into cellulose. Incubated nata and purified dry bacterial cellulose are shown in Figure 1.

Figure 1. Nata incubated (a) and purified dry bacterial cellulose (b)

Harvested nata (2 weeks incubation time) with a thickness of 2 cm was washed thoroughly with tap water and pressed to separate the nata from the epidermis and most of it. Moreover, the nata is purified and separated from protein, sugar, and fat. The purification used 1% NaOH solvent at 1 week which showed a brown colour on the data surface area. The brown color represents sugars, fats, and proteins degraded due to reactions with alkaline solutions. Neutralization was carried out using 1% acetic acid to clean the remaining NaOH solution. Then the nata is pressed to remove the moisture content so that it speeds up the drying process to obtain dry cellulose sheets.

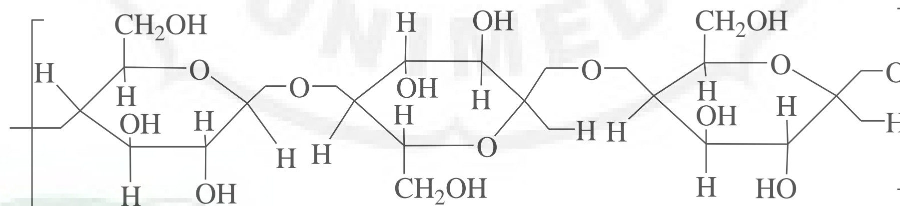

Figure 2. Cellulose structure

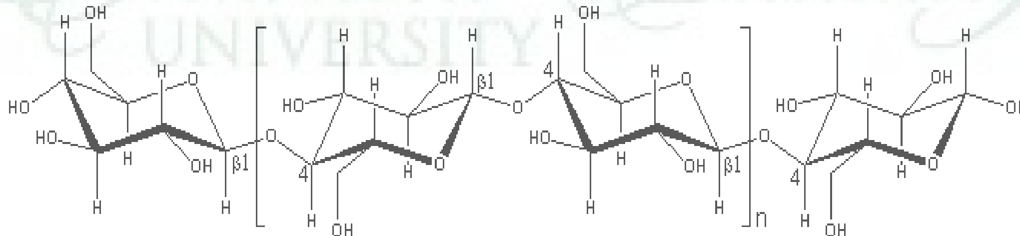

Figure 3. B (1-4) -D-glucoside linking chain on cellulose

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The purification process of bacterial cellulose uses an alkaline solution which can reduce the Iα cellulose component in a fairly large amount. Iα cellulose crystallized at the large microfibril size, whereas Iβ cellulose crystallized at the smaller microfibril size [3].

3.1. FT-IR analysis

The FTIR spectra of BC show the -OH group. -CH2, and C-O-C group at wavelength of 3338 cm-1, 2893.71 cm-1, 1159 cm-1 respectively. However, there are very narrow hydroxyl group peaks and several disturbing peaks at a wavelength 1100-1050 cm-1, 1300-1150 cm-1 and 1800-1500 cm-1 that reveals the existence of nucleic acid, proteins, and lipids [5]. The FTIR spectra of BC can be seen in figure 4. The appearance of lipid, protein, and amino acid peaks in the FTIR spectrum occurred because BC still contained impurities that were missed during the BC purification stage with NaOH. However, the presence of impurities in the FTIR spectra of BC is a common occurrence in BC [9].

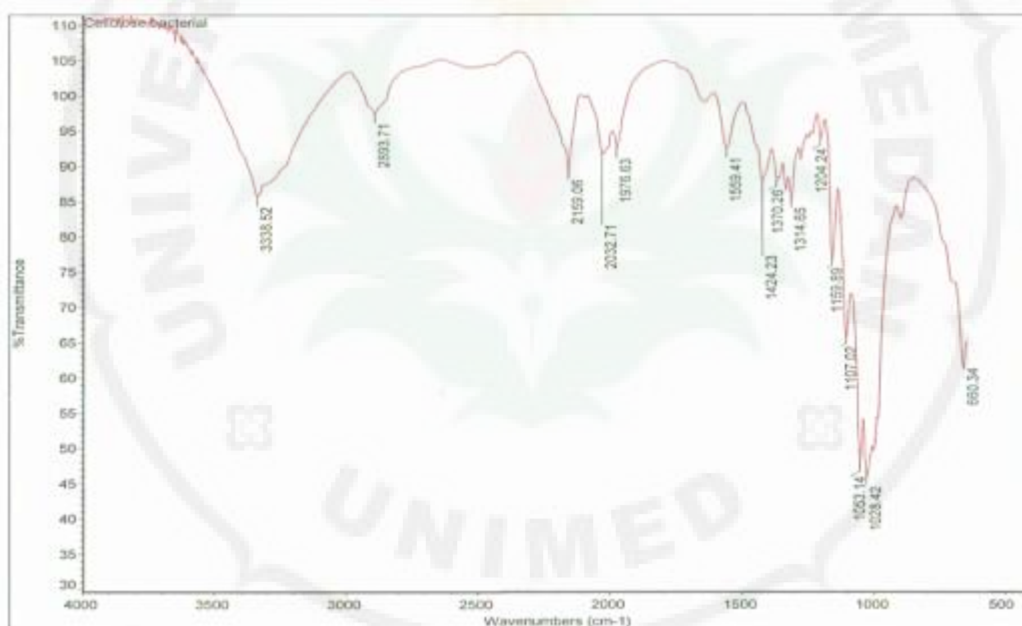

Figure 4. Bacterial cellulose FTIR spectrogram

This FTIR spectra also shows that the cellulose obtained in this study is actually derived from the activity of microorganisms, when compared to FTIR spectra from plants, the presence of this impurity substance is not obtained.

3.2. TGA analysis

Figure 3 shows the TGA graph and differential thermogravimetric analysis (DTA) for the resulting BC. The sample has 3 stages of the thermogram, namely the first stage (evaporation of bound water), the second stage (decomposition), and the third stage (carbonation). The first stage is the evaporation of water bound to the sample, usually starting at 35-150 ° C [11] and this is seen in BC, with a weight loss of 9.2%. This stage also shows the thermal stability properties of the sample before decomposition. The weight loss for BC was 9.2% indicating the hydrophilic properties of cellulose [10].

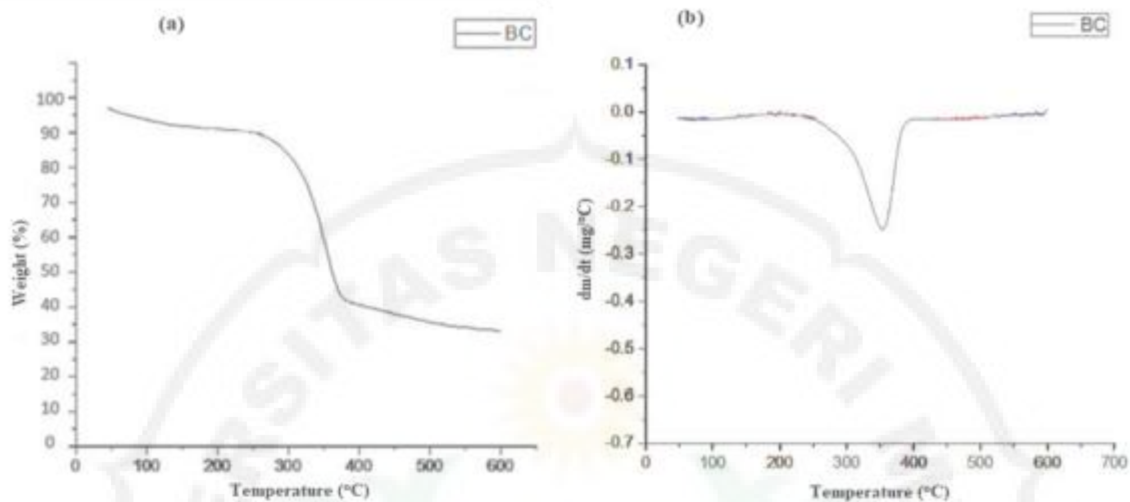

Figure 5. TGA graph (a) and DTA graph (b) from BC

The decomposition stage is the step of removing a very large sample weight due to the breaking of hydrogen and hydroxyl bonds in the sample. The decomposition of BC at a temperature of 272.78-381.16°C (250-350°C), BC decomposition is caused because BC has a hydroxyl group that is easily dehydrated both intra- and inter-molecularly when energized [12-14]. peaks that can be seen on the DTA graph. These peaks indicate the properties of thermal stability or more precisely the nature of crystallinity (glass transitions and melting points). The third stage is carbonation, which usually occurs at temperatures over 400 oC [2]. This temperature represents the carbon content in the sample. The residual weight on BC was 41%.

3.3. SEM analysis

Figure 6. show the SEM imaging of BC that reveals a strong dense like a cellulose plant with a space and holes characteristics [2]. The surface morphology image of BC is microfibrils which are interwoven, closely attached, and very strong as in Figure 4a. The hydrophilic properties and surface tension of water are strong in dry BC [15].

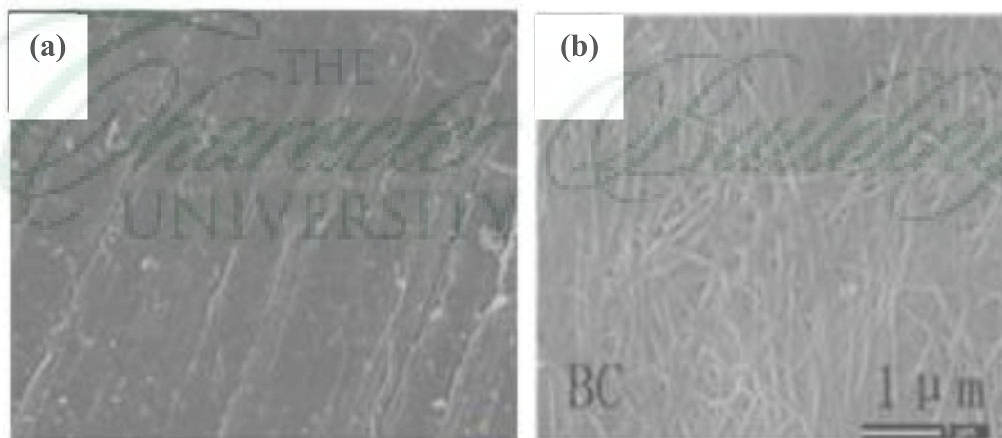

Figure 6. The surface morphology of BC (a) and the morphology of BC microfibrils by Lin et al., 2014

4. Conclussion

Nata fiber produced from the cultivation of A. Xylinum with coconut water as the medium can be done simply and can be used as an alternative, environmentally friendly source of cellulose. BC can be a good alternative as a source of cellulose for further research on cellulose modification.

Acknowledgment

The author would like to thank the DRPM-Kemendikbud and LPPM Universitas Negeri Medan for research funding provided via the Applied Research scheme-2020 with grant number of 024/UN-33.8/PL-DRPM/2020.

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