Preliminary study on biosynthesis and characterization of bacteria cellulose films from coconut water

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Abstract. Bacterial cellulose produced by *Acetobacter xylinum* is a unique type of bacterial cellulose. It contains more than 90% of water. A preliminary study had shown that bacterial cellulose films has remarkable mechanical properties. The aim of this study was to investigate the optimum condition such as percentage of carbon source, time of cultivation, and pH to produce bacterial cellulose films from local coconut water, and its characterization on morphology, swelling ability and tensile strength of dried bacterial cellulose. *A. xylinum* was grown on coconut water culture medium with addition of 3%, 5%, and 7% of sugar, while the cultivation time was vary from 3 days, 5 days and 7 days. pH condition was conducted in pH 3, pH 5 and pH 7. Bacterial cellulose samples were dried using oven with temperature of 100°C until the moisture content reached 4-5%. This study showed that several parameters for optimum condition to produce bacterial cellulose films from local waste of coconut water had been obtained (5% of carbon source; pH 5; and 7 day of incubation period). The electron microscopy also showed that dried bacterial cellulose films had pores covered by fibrils on the surface. Therefore, the present work proposes the optimum formula and condition that can be used based on properties of end product needed.

1. Introduction

Cellulose is an insoluble water compound normally found in plant cell walls, especially in branches, stems, stems, other woody parts. It plays an important role in the integrity of plant cell walls [1]. Cellulose fibers from plants are known as microfibrils that are 2-20 nm in diameter and form a strong network within the cell wall of the plant. Groups of polysaccharides are arranged in parallel to form cellulose microfibrils that cluster into macrofibrils. Cellulose molecules can be arranged up to thousands of microfibrils chain units of D-glucose linked by hydrogen bonding [2].

Cellulose can be produced by a fermentation process. Bacteria that can be used to produce cellulose are Acetobacter strains, Gluconacetobacter, Agrobacter, Sarcina, among others which includes non-pathogenic bacteria and is known as cellulose bacteria [3, 4]. *Acetobacter xylinum* has been known to produce pure cellulose for more than 100 years. *A. xylinum* has been isolated from rotting sugary fruits, vegetables and fermenting coconut water [5]. Bacterial cellulose is cellulose...
produced using the help of cellulose bacteria. Actually cellulose from plants and bacteria has the same chemical structure, but the cellulose produced by the bacteria has better fiber composition. Bacterial cellulose is a polysaccharide consisting of several hundred to thousands units of D-glucose associated with β bond (1→4). The fibers of the bacterial cellulose usually have a diameter of more than 50 nm. The cellulose fiber units are interconnected to form a network structure, consisting of a three dimensional network of microfibrils and nanofibrils [6, 7]. Bacterial cellulose is pure and exhibits a higher degree of crystallinity than the cellulose obtained from plant in which the cellulose fibrils are embedded with lignin and hemicelluloses [3]. As already known, the lignin and hemicellulose that make up the cell wall components of plants is difficult to remove in the purification process. Thus, bacterial cellulose could be processes further without hazardous by-products and using low energy on the purification process.

Bacterial celluloses have transparent properties found by Klemm et al 2001 [8]. These properties can be identified during the purification and drying process of cellulose microbial on the surface of culture. After the filtering and drying process, a thin layer of bacterial cellulose can be observed transparently by placing colored paper. Bacterial cellulose has a greater water absorption capacity than plant cellulose. This is because the hydrogen bond in the bacterial cellulose is stronger and the bacterial cellulose polymer chain is also longer [9]. Applications of bacterial cellulose are extensive in many industries, such as in biomedical industries, paper production, food industries, and cosmetics. In the field of biomedical industries, bacterial cellulose can be used as biosensor, biofill, transdermal drug delivery, and wound cover [9, 10, 11]. In paper making, bacterial cellulose is used in the production of synthetic paper, insulation materials, and surface coatings. In the process of food manufacturing, bacterial cellulose is used as a stabilizer agent, making Nata de Coco, and baking [12], whereas in the cosmetic field, bacterial cellulose has been used as a health tonic drink and artificial nails [13]. In this study, the synthesis of bacterial cellulose by utilizing local waste of coconut water had been conducted. This study may be used as preliminary study in the evaluation of bacterial cellulose film as a promising candidate for application in cosmetic and biomedical industries.

2. Material and Methods

2.1. Materials and General Instrumentations

Acetobacter xylinum (strain FNC-0001), was purchased from Gadjah Mada University (UGM). Coconut water was obtained from Yogyakarta Province, Indonesia. Yeast extract, sucrose, acetic acid, distilled water, urea, NaOH, universal pH paper, plate count agar were purchased from Merck. The equipments used in this study were glassware, scales, autoclave, Scanning Electron Microscopy (SEM), oven, desiccator, hot plate, a pH meter, Universal Testing Instrument and calipers.

2.2. Media culture and bacterial cellulose production

The procedure used is a modification of Almeida et al, 2014 [14] with several modifications. The culture medium used for the fermentation of A. xylinum to produce bacterial cellulose consist of coconut water (1 litre), sucrose (50 g), urea (10 g), starter of A. xylinum (10%) and the pH is set to be pH 5 with acetic acid. The media was then sterilized by autoclave at 121°C for 15 minutes. Bacterial cellulose was grown at room temperature and static conditions for 7 days in a Petri dish. After incubation, the resulting bacterial cellulose was harvested and boiled in distilled water for 2 hours, and rinsed in running water until neutral. The drying of bacterial cellulose was conducted using oven for 5 hours with temperature of 100°C.

2.3. Characterization of bacterial cellulose morphology and mechanical performance

Scanning Electron Microscopy (SEM) of the surface of dried bacterial cellulose was performed using a Hitachi SU-70 instrument operating at 4 kV. Tensile assays were performed on a Universal Testing Machine (Zwick 0.5, Lloyd’s Universal Testing Instrument), operating at a deformation rate of 10 mm/min, under ambient conditions. The films samples were cut into strip-shaped specimens 1 cm
width and 10 cm long. At least 5 specimens were tested for each composite.

2.4. Swelling Measurement
The swelling percentage was determined using method by Maneerung et al., 2008 [15] with little modifications. The samples were cut into a size of 1 cm width x 2 cm length. Then the samples were soaked in distilled water for 72 hours.

3. Results and Discussion

3.1. Physical appearances
The physical appearances of wet and dried samples of bacterial cellulose films were demonstrated in Figure 1. From the observation, physical appearances of the samples before and after drying were different.

![Figure 1. The physical appearances of wet (a) and dried (b) samples of bacterial cellulose films.](image)

At the end of drying process, the bacterial cellulose film exhibited brownish color. It probably caused by the heat applied during the drying process. The surface was also shrunk that indicating the removal of water content in drying process. In this study the water content of the obtained bacterial cellulose film is about 90-95%.

3.2. Morphology structure of bacterial cellulose film
The morphology of the surface structure of the bacterial cellulose film was analyzed using SEM (SU-3500 from Hitachi, Tokyo, Japan) with 10,000 magnification. Figure 2 shows the SEM image of the surface of the bacterial cellulose film.

![Figure 2. SEM images of bacterial cellulose films.](image)
Figure 2. SEM images of the surface morphological structure of dried bacterial cellulose film with 5-day incubation time and percentage of carbon source of 3% (a), 5% (b), and 7% (c).

The surfaces of bacterial cellulose were composed of many intertwined strings which produced an aggregated structure. Figure 2 indicates that the network of thin layers of bacterial cellulose structures is composed of very neat fibrils. As the carbon source concentration increases, it appears that the fibril density formed is also higher (from Figure 2.a to 2.c.). The width of the formed fibrils is less than 100 nm in size. This result is in agreement with the previous study [16] that reported of fine fibrils which overlapping each other as a layer of cellulose ribbons.

3.3. Yield
Yield is the result of the percentage of the division between the weight of bacterial cellulose produced and the weight of the starting material. The more sugar concentrations added to the medium, the yield of the resulting bacterial cellulose also increases to a certain concentration. pH is also an important factor for the growth and formation of bacterial cellulose film.
Sugar as a carbon source was used in the bacterial cellulose fermentation process. The relationship between culture time and sugar concentration to bacterial cellulose yield can be seen in Figure 1. Figure 1 shows that the addition of 5% of sugar give the maximum yield of bacterial cellulose. The yield of 5% sugar on the 3rd, 5th or 7th day were 15.4%; 30.7%; and 53.4%, respectively. The yield were decreased at 7% of sugar i.e. 12.5%; 24.5%; 40.3%; on the 3rd, 5th or 7th day respectively, while the addition of 3% sugar resulting in low yield. This indicates that the addition of 5% sugar concentration is the optimum condition for bacteria Acetobacter xylinum growth. The addition of more than 5% of sugar cause the plasmolysis in these bacterial cells, thus decreasing the formation of cellulose and also resulting in low yield.

3.4. Thickness
Table 1 indicates that the optimum pH in this study was pH 5, in accordance with the literature that the optimum pH for A. xylinum growth was pH 5.5 [17].

| pH | Thickness, cm |
|----|---------------|
| 3  | 0.031± 0.001  |
| 5  | 0.072± 0.003  |
| 7  | 0.058± 0.002  |

The thickness of the bacterial cellulose film obtained at pH 5 is 0.072 cm (Table 1). The thickness of the bacterial cellulose layer at pH 3 was only 0.031 cm and increased up to pH 5. The thickness of the bacterial cellulose layer decreased at pH 7 of 0.058 cm. This means that pH 5 is the optimum pH of the formation of bacterial cellulose film.

3.5. Tensile Strength
The characteristic of the thin layer of bacterial cellulose is highly dependent on the tensile strength and elongation of the thin film. Tensile strength is one of the mechanical properties to measure the strength of the thin film. Tensile strength is the maximum pull force that can be retained by a thin layer during the measurement takes place until the thin layer is disconnected.
3.5. Tensile strength

Figure 4 shows the tensile strength of the bacterial cellulose film. It can be seen that the incubation time for 10 days gives the highest tensile strength of 116.3 MPa, followed by incubation time for 7 days and 5 days respectively of 73.9 MPa and 56.4 MPa. The high and low of tensile strength of bacterial cellulose films can be linked to its morphology structure. A few pores that covered by fibrils string at the bacterial cellulose surface increased the surface area where larger surface area may lead to lower strength, this result is also in agreement with the literature [18].

3.6. Elongation

The elongation at break of the bacterial cellulose films was shown in Fig. 5.

Figure 5 shows the elongation of the bacterial cellulose film. It can be seen that 5 days incubation time gives the highest elongation of 3.65%, followed by incubation time for 7 days and 10 days respectively 3.5% and 2.7%. The elongation at break of bacterial cellulose films show the inverted pattern of tensile strength, this result is also in agreement with the literature [18, 19].

3.7. Swelling ability

Swelling is the ability of water to penetrate into the structure of the thin layer of bacterial cellulose. In general, amorphous structures have greater hydrophilic properties than materials with high crystallinity.
Figure 6. Percentage of swelling from bacterial cellulose film with 7 days incubation time and sugar variation of 3%, 5% and 7% (G3H7, G5H7 and G7H7).

Percentage of swelling ability was determined by Maneerung et al., 2008 method [15] with slight modification, i.e. dried sample with size of 1 x 2 cm was soaked in aquades for 72 hours. Figure 6 shows that the highest percentage of swelling is owned by a thin layer of bacterial cellulose with 5% sugar percentage and 7 days incubation time. The swelling of G3H7 (3% of sugar) and G7H7 (7% of sugar) were lower than G5H7 sample (5% of sugar). This might due to the lower structure of rigidity that samples could not retain more water. This result also in agreement with the yield that the optimum of carbon source is around 5%. As fibrous films, bacterial cellulose can hold water by physically entrapping it in fine capillaries and internal pores [18].

4. Conclusion
This study showed that several parameters for optimum condition to produce bacterial cellulose films from local waste of coconut water had been obtained (i.e. 5% of carbon source; pH 5; 7 day of incubation period). The electron microscopy also showed that dried bacterial cellulose films had pores covered by fibrils on the surface. This result on preliminary study of bacterial cellulose film (surface morphology, mechanical and swelling properties) may be used as a consideration promising candidate for application of bacterial cellulose film in cosmetic and biomedical industries.

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