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Optimization on the synthesis of bacterial nano cellulose (BNC) from banana peel waste for water filter membrane applications

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Abstract

This research reports the optimization of the synthesis of bacterial nano cellulose, gluconacetobacter xylinus bacteria, banana peel waste media of Kepok bananas (Musa paradisiaca L.) using Gluconacetobacter xylinus bacteria in a fermentation process for use in water filter membrane applications. Bacterial nanocellulose (BNC) synthesis was successfully accomplished under conditions of pH 4, 0.5% urea, and varying sucrose contents (5%, 10% and 15% (w/v)). The higher sucrose content causes the pH of the banana peel extract solution to decrease at the end of Day 8 and 10 due to the metabolic activity of bacteria, which produces acetic acid. A bacterial growth pH range of 3.93–4.26 was obtained. The Optical Density (OD) values tend to increase with respect to fermentation time due to the growth of BNC-forming bacteria. The higher the added sucrose content, the higher the total amount of the acid obtained as the G. Xylinus bacteria produces acetic acid in its metabolic processes. BNC thickness is directly proportional to the increase in sucrose level but does not increase proportionally with the increase in sucrose levels from 5%, 10% and 15% (w/v). The sucrose level at 5% (w/v) produces most optimal results. Optimal incubation time was obtained on Day 6, which had the highest rate of increase in thickness in addition to the supporting pH, OD value and total acid factors. The TEM analysis shows that the BNC surface morphology tends to be the same among all sucrose level (5%, 10% and 15% (w/v)). The difference can only be seen in the density of the nanocellulose. The nanocellular nanofiber produced from banana peels has diameter sizes between 30–50 nm which potentially be used in water filter membrane application.

1. Introduction

Currently available inorganic water filter membranes have many shortcomings such as high production costs, high pressure requirements during operation, and rigid membrane structure that are inflexible and easily broken. These issues can be addressed by utilizing the advantages of bacterial nanocellulose or Bacterial Nano Cellulose (BNC) as a binder.

BNC has a three-dimensional, nano fibrous structure with a large surface area that exhibits many unique physical and mechanical properties such as high porosity, high capacity water holding, high mechanical strength, high biocompatibility, high purity, high crystallinity up to 90% [1], high hydrophilicity and high young modulus. Nanocrystalline cellulose which is part of BNC, has superior properties such as tensile strength reaching 7.5 GPa, and unique surface chemical properties because of its many hydroxyl functional groups [2]. BNC has a reported 114 GPa with young theoretical modulus as high as 160 GPa [3]. Nanocrystalline cellulose has a higher tensile strength compared to metal or polymeric materials; the addition of nanocrystalline cellulose in paper nanocomposites can serve as a reinforcement (highly reinforcing material) [4]. The addition of nanocrystalline cellulose can increase the tensile strength of paper up to 40% [5]. Natural cellulose fibers can be synthesized by algae and several types of bacteria [6].
Nanocellulose can easily be obtained from banana peel waste through a relatively inexpensive and environmental friendly process by using the fermentation of Gluconacetobacter xylinus bacteria [7]. The banana is a popular fruit that grows in many tropical regions such as Indonesia. A number of provinces in Indonesia produce large quantity of bananas yearly. The total production of bananas in 2014 was 6,862,668 tons where the weight of the banana peels accounted for one third of banana weights [8]. Thus the total banana peel waste in 2014 was 2,287,356 tons. Banana peel has a biomass that is rich in cellulose. The increased interest in utilizing this biomass, besides aiming to reduce the environmental impacts, is to add value to cellulosic byproducts [9].

BNC has received significant attention in many fields due to its characteristics. It has been applied in reinforcing polymer composites [6]. However, there is a lack of information regarding optimal conditions for the synthesis of BNC from banana peel waste as well as the application of BNC in the composite membrane of water filter. The growth of G. xylinum bacteria in forming BNC is influenced by many factors: pH, temperature, sucrose concentration, aeration, thickness and width of growth site, banana peel concentration, type of banana and methods of banana peel extraction. However, the most important factors affecting BNC production are pH, temperature, incubation time and sucrose content.

Gluconacetobacter xylinus is a type of bacteria that is widely used in research and commercial production. This bacterium has a short stem, is non-pathogenic, gram-negative, and aerobic and has a high ability to produce cellulose from several carbon and nitrogen sources [10]. This bacterium is ubiquitous in further producing cellulose [11]. Cellulose is a secondary metabolite of G. xylinus bacteria. Its primary metabolites are: acetic acid, water, and the energy that is reused in the metabolic cycle. Acetic acid is used by G. xylinus bacteria as a substrate to create optimum conditions for bacterial growth and to form CO$_2$ and H$_2$O. The G. xylinus bacteria is an ‘over oxidiser’ which means it can change the acetic acid in the fermentation medium into CO$_2$ and H$_2$O, if the sucrose in the fermentation medium has been completely metabolized [12].

The carbohydrates content in banana peels can be used by G. xylinum microorganisms in the growth period to produce nata fermentation products in the form of cellulose sheets. The addition of inorganic or organic nitrogen sources will increase the Gluconacetobacter xylinus activity in nata production [13].

The metabolism of G. xyline’s begins with the breakdown of carbohydrates into glucose in banana peel media. The glucose will then bind with fatty acids (Guanosin triphosphate) to form precursors that characterize cellulose by enzyme cellulose synthases, which will be released into the environment to form interwoven cellulose on the media surface. Glycolysis also occurs during the carbohydrate metabolism by the G. Xylinus bacteria, which starts with the glucose changing into glucose 6-phosphate and ends with the formation of pyruvic acid. Glucose 6-phosphate is used by G. xylinus to further produce cellulose. Cellulose is a secondary metabolite of the G. xylinus bacteria. Its primary metabolite are acetic acid, water, and the energy that is reused in the metabolic cycle. This acetic acid is used by G. xylinus bacteria as a substrate to create optimum conditions for bacterial growth and to form CO$_2$ and H$_2$O. The G. xylinus bacteria is an ’over oxidiser’ which means it can change the acetic acid in the fermentation medium into CO$_2$ and H$_2$O, if the sugar in the fermentation medium has been completely metabolized [12].

In previous research, nanocellulose synthesis from banana peels was successfully carried out at a 1:3 ratio of banana peels to water; bacterial nutrition: Sucrose 10% (w/v), Ammonium Sulfate (NH$_4$)$_2$SO$_4$ 1% (w/v), pH: 4; number of starters: 15% (w/v). The fermentation time is seven days [14]. For further optimization, the sugar levels (sucrose) will be varied; at 10% (w/v) lower and higher, the Nitrogen source will be changed replacing Ammonium Sulfate (NH$_4$)$_2$SO$_4$ with Urea which is more economical. Likewise, the length of fermentation time will be varied, either seven days faster and longer.

2. Research method

2.1. Materials

The materials and chemicals used in this study were: Kepok Banana peels (Musa paradisiaca L.) from Kalimantan Province after 2 days peeling; NaOH 98% flakes produced by PT Asahimas Chemical; Urea (NH$_2$)$_2$CO; Acetic Acid (CH$_3$COOH) 99%; ‘Gulaku’ brand of sugar from P.T. Sugar Group; Gluconacetobacter xylinus bacteria from ‘Nata de Coco’ manufactured in home industries in Cianjur, West Java.

2.2. Experimental procedure

The banana peels were cleaned, cut into small pieces and weighed. They were then heated to the boiling point and cooled. The were the banana peel was soaked in water at a ratio of 1: 3 (w/v), after which it was reheated to boiling point, then cooled for 12 h. Then the banana peels were then pressed and filtered to take out the filtrate. The resulting filtrate was cooked at a temperature of 100 °C; sucrose was added in the percentage of 5%, 10%, and 15% (w/v), and 0.5% urea (w/v), and acetic acid until the solution reached pH value = 4. Afterwards, the solutions was put inside beaker glass, covered with newspaper and cooled. Bacterial starter 10% (v/v) OD 0.5
was added on fermentation and observe on day 2, 4, 6, 8, and 10. The fermentation result obtained was analyzed to measure the thickness, pH, optical density (OD), total acid, and characterization.

2.3. Characterisation of materials

2.3.1. Thickness
The thickness of BNC produced in the beaker glass was measured at several points and the averages were taken.

2.3.2. Level of acidity (pH)
Level of acidity (pH) was measured by using a digital pH indicator meter 0–14.

2.3.3. Optical density (OD)
The optical density (OD) value was measured using the Optima Sp-300 Spectrophotometer. The measurement procedure was as follows: 1 ml of sample solution put in the cuvet and measured using the spectrophotometer with a wavelength $\lambda = 600 \, \mu m$, OD result was obtained.

2.3.4. Total acid
Total acid determines the total amount of acid in solution by measuring the acetic acid level. The total acid was tested by using titration to measure the acetic acid level following the method guidelines [15]. As much as 10 ml BNC was titrated, from which the total acid was measured. Before being titrated, two drops of 2% phenolphthalein (PP) indicator were added to the sample; afterwards, the sample was titrated with 0.1 N NaOH until a consistently pink color appeared. Acid levels were calculated using equation (1).
3. Results and discussion

3.1. Synthesis optimization

This research was conducted by varying bacterial nutrition; namely, the added sucrose content of 5%, 10% and 15% (w/v) as sources of Carbon (C) while the source of Nitrogen (N) from Urea was maintained at 0.5%.

All samples were incubated for 10 days at room temperature. Figures 1–3 show the banana peels bacterial nanocellulose (BNC) produced during the experiment.

From the overall results, it can be seen that the BNC surface is smooth despite some lumpy and uneven parts. However, there is a visible growth of fungi or other contaminants which can affect the growth of bacteria that produce BNC.
The thickness of the BNC samples produced was measured every two days. The banana peel extracts with the added bacteria were then observed for pH, OD and total acid to determine the optimal conditions for *G. xylinus* bacteria growth.

### 3.2. Acid level (pH)

The results obtained are as follows:

Figure 4 indicates that increased sucrose levels influence the pH level of the sample. The samples showed an increase in pH until Day 6 due to the lysis process. On Day 8, however, there was a decrease in the pH due to changes in the number of BNC-forming bacteria; the longer the fermentation process, the more bacteria were...
During the fermentation process, *G. xylinus* bacteria will grow and produce acetic acid in the metabolic process so that the longer the fermentation, the more acetic acid results, which causes the liquid media to become more acidic, thereby lowering the pH. The bacterial growth pH range was obtained was 3.93–4.26.

### 3.3. Optical density (OD)

Below are the results obtained for OD measurements:

Figure 5 shows the decrease in OD due to the presence of dead *G. xylinus* bacteria when incubation has begun, from Day 2 until Day 4. This is due to the adjustment of bacterial conditions, changing from *Nata de Coco* to the banana peel medium. The longer the incubation time, however, the more the bacteria begin to adapt and grow as indicated by decreasing pH rate. The OD values tend to increase at the end of each treatment. The higher OD values indicate greater turbidity in the media liquid, which is due to the increase in BNC-forming bacteria. The OD value of 5% and 15% sucrose levels start to increase on Day 4, while the 10% sucrose level starts increase on Day 6.

### 3.4. Total acid

Figure 6 represents the total acid obtained during synthesis.
Figure 6 shows that from the beginning of the incubation period until Day 6 there was a decrease in total acid. This occurred because of the adaptation process of G. xylinus bacteria, in which there are a number of dead bacterial cells. Total acid decrease from Day 2 to Day 6 before it increases for all treatments of 5%, 10% and 15% (w/v) sucrose content. This is due to the fact that the G. xylinus bacteria has adapted and can grow optimally to produce acetic acid metabolically so that more acetic acid is formed. This causes the liquid media environment to be more acidic, thereby lowering the pH. The highest increase in total acid of 0.20 (60.6%) was obtained on Day 6 at 5% sucrose content. From these results it can be concluded that the best sucrose content is 5%.

3.5. Thickness

The thickness of the BNC formed can be seen in figure 7.

Figure 7 shows, that the higher the percentage of sucrose content added, the thicker the resulting BNC produced. The BNC thickness increases due to the growth of the BNC—forming bacteria. There is an increase in BNC yield because there more sucrose is available as a source of calories and as an ingredient to be synthesized into cellulose or BNC [17].

Carbohydrates are the source of carbon used in BNC fermentation; these are classified as monosaccharides and disaccharides. Media containing glucose, sucrose and lactose can help in the formation of BNC. The most widely used sucrose media are sucrose or granulated sugar. Granulated sucrose can also function as an inducer that plays a role in the formation of extracellular polymerase enzymes that work to compose BNC threads so that the formation of BNC is maximized [18].

From these results it can be observed that BNC thickness is linearly proportional to the increase in sucrose content. The best thickness is obtained at 15% (w/v) sucrose content. The highest rate of increase of 15% sucrose content occurred on Day 6 and was equal to 0.30 cm (85.71%). However, the increase in thickness does not correlate with the multiplied increase of the sucrose content from lower sucrose levels 5%, 10% (2 × higher) and 15% (w/v) (3 × higher). This can be seen from the final thickness for 5% sucrose content, which is 0.50 cm while the 10% (w/v) sucrose content is 0.75 cm instead of being 2 × thicker at 1.00 cm and a sucrose content of 15% (w/v) at 0.95 cm which should be 1.50 cm. This is because the process of bacterial metabolism takes time to break down glucose into new energy and cellulose. From these results the thickness achieved at 5% sucrose content is considered more optimal either 10% or 15% (w/v). To obtain more optimal results at 10% and 15% (w/v) sucrose levels, the system can be modified by adding sucrose in stages (fed batch) to provide enough time for bacteria to break down the carbohydrates in the banana peels to glucose and then into cellulose. The most optimal incubation time was obtained on Day 6, as indicated by the highest rate of increase in BNC thickness for all sucrose content levels: 5% (15 cm), 10% (20 cm), and 15% (30 cm) in addition to other supporting factors, such as pH, OD, and total acid. After Day 6, BNC thickness tends to decrease.

3.6. TEM analysis results

The TEM test was conducted on the synthesis results to determine the differences in BNC shape and size. The sample was taken on the last day of observation (Day 10). The BNC for 10% and 15% was then visibly thicker and
the nano-fibers appeared to be more piled up and concentrated than 5%, but the cellulose structure remained same (as shown in figure 8). The nanofiber produced has a diameter size of 30–50 nm (figures 8 and 9).

These results are in accordance with the findings of Feng et al 2015 [19] and Lai et al 2014 [20] who explain that BNC fiber with a diameter of approximately 30 nm, naturally categorized as nano-scale cellulose.

From figures 8 and 9 can be seen that the BNC from banana peel has a three-dimensional nano fibrous structure with a large surface area. This properties is essential in the application in water filter membrane application. BNC exhibits many unique physical and mechanical properties such as high porosity, high water holding capacity, high mechanical strength, high biocompatibility, high purity, high crystallinity up to 90% [1].

Figure 8. TEM Results at 20,000 × magnification of sucrose levels of 5%, 10%, and TEM Results at 10,000 × magnification at Sucrose Levels of 15% (w/v).
4. Conclusions

The BNC synthesis from Kepok Banana peels was successfully performed under conditions of pH 4, 0.5% urea, and varying percentages of sucrose content (5%, 10% and 15% (w/v)). The thickness of the BNC is directly proportional to the increase in sucrose content, but does not proportionally when the sucrose levels is increased from 5%, 10% (2 times) or 15% (w/v) (3 times). The 5% sucrose content gives the most optimal. A higher sucrose level will cause the pH of the banana peel extract solution to increase at the end of Day 8 and 10 due to bacterial complications in the metabolic process that produce acetic acid. The bacterial growth pH range obtained was 3.93–4.26. The OD values decreased on Day 2 and 4 and decreased even more on Day 8 because of the growing numbers of BNC-forming bacteria. The higher the percentage of added sucrose content, the higher the total amount of acid because *G. xylinus* bacteria produce acetic acid in the metabolic process. The 5% sucrose content has a more optimal value after the adaptive process because is has the highest increase in total acid. Optimal incubation time was obtained on Day 6 as the highest rate of increase in BNC thickness for all 5%, 10%, and 15% (w/v) sucrose levels was obtain on that day, in addition to the supporting pH, OD value and total acid factors. The difference can only be detected from the nanocellulose density. The type of nanocellulose obtained from banana peel is nano fiber with a size of 30–50 nm.

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