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# Acetylation of Bacterial Cellulose from a Mixture of Palm Flour Liquid Waste and Coconut Water: The Effect of Acetylation Time on Yield and Identification of Cellulose Acetate

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**Abstract.** Cellulose acetate is a promising thermoplastic polymer to be developed since it has some characteristics, among others are easy to be formed, non-toxic, high stability, and its raw materials are renewable. The most used source of cellulose acetate raw material is bacterial cellulose because bacterial cellulose has the higher purity and the process cost is lower rather than plant cellulose. Nowadays, the production of bacterial cellulose is highly developed using coconut water media. Nevertheless, coconut water costs expensive and the supply is rare. Materials that are being potential to be developed as raw materials of bacterial cellulose through fermentation process is palm flour liquid waste since it contains high amounts of carbon and nitrogen. This study began with the synthesis of bacterial cellulose from palm flour oil liquid waste and coconut water using Acetobacter xylinum bacteria and then cellulose acetate is synthesized through an acetylation reaction. This study aims to determine the optimum acetylation time on its performance as a reinforcement filler to be applied as a packaging material. Based on the results of Scanning Electron Microscopy and Fourier Transform Infra-Red analysis on predetermined variables, it resulted particles in the form of bacterial cellulose and cellulose acetate with the highest yield of cellulose acetate at 3 hours of acetylation was 94.74%.

## Introduction

As time goes by, advancements and technological developments continue to increase. Various product innovations that contribute to human activities can be found easily. One of the innovations in the material engineering field is food packaging from natural polymers. Natural polymers as commercial packaging materials are still very limited in utilization. In general, the natural polymers used are starch and cellulose [1]. Cellulose from bacteria has a higher level of purity and lower processing costs than cellulose from plants [2]. This can facilitate the formation of cellulose acetate and result good performance in its application, especially as a packaging material.

Bacterial cellulose (BC) is produced by bacteria in a medium containing nutrients such as carbohydrates, proteins, and/or glucose through a fermentation process. Media for the formation of bacterial cellulose include sago liquid waste using *Beijerinkia fluminensis* bacteria [3], tobacco waste using *Acetobacter xylinum* bacteria [4], dan molasses using *Komagataeibacter rhaeticus* bacteria [5].

Currently, many bacterial cellulose isolations have been developed using coconut water (*nata de coco*) media, but coconut water costs expensive and the supply is rare. Palm liquid waste can be utilized as a new alternative for producing bacterial cellulose because currently palm liquid waste is only dumped into waters, polluting river water, and has not been utilized optimally. Palm liquid waste has a parameter of degree of acidity (pH) below a neutral pH that is 5 and a C/N ratio of 15 [6], so it

has the potential to meet the requirements in the production of bacterial cellulose. Bacterial cellulose resulted from palm flour liquid waste is called *nata de arenga*. *Nata de coco* and *nata de arenga* are bacterial cellulose that can be utilized as a source for the production of cellulose acetate (CA).

Cellulose acetate is an organic ester compound derived from cellulose made by the esterification process [7]. Cellulose acetate is produced by reacting cellulose with acetic anhydride $(CH_3CO)_2O$, sulfuric acid ($H_2SO_4$) catalyst, and acetic acid ($CH_3COOH$) solvent [8]. Sulfuric acid catalyst in the esterification process will accelerate the reaction between sulfuric acid and acetic anhydride, first forming acetyl sulfuric acid as an intermediate product, and then the acetyl sulfuric acid will react with cellulose to produce cellulose acetate [9].

In this study, bacterial cellulose from *nata de coco* and *nata de arenga* will be processed into cellulose acetate as a reinforcement filler. Cellulose acetate obtained through this acetylation process is then utilized as an alternative packaging material.

## Materials and Methods

**Materials.** The materials used in this study were palm liquid waste from the palm flour industry (Lebak Peundeuy, Banten), coconut water (Cilegon, Banten), *Acetobacter xylinum* (Biotechno, Serang, Indonesia), distillate water, and materials from Merck, distributor of Darmstadt Germany, specifically ammonium phosphate $(NH_4)_3PO_4$, glacial acetic acid ($CH_3COOH$), acetic acid anhydride $(CH_3CO)_2O$, sulfuric acid ($H_2SO_4$), and sodium hydroxide (NaOH).

**Isolation of Bacterial Cellulose (BC) from Palm Liquid Waste and Coconut Water.** Bacterial cellulose isolation process starts by mixing 400 mL of palm liquid waste and 100 mL of coconut water and then heated to 100°C. The amount of coconut water composition is less than palm liquid waste because palm liquid waste is used as the newest alternative to the main raw material for producing bacterial cellulose while coconut water is used as a complementary medium for bacterial cellulose isolation. Later, added with 100 grams of glucose and 5 grams of ammonium phosphate (ZA-food grade), and the pH of medium 3-4 was adjusted by the addition of glacial acetic acid. The inoculum of *Acetobacter xylinum* bacteria was added as much as 10 %v/v to the production medium. Fermentation was done for 12-14 days in a tightly closed glass container using paper. The cellulose layer formed was separated and washed with 2 %w/v NaOH at 80°C for 1 hour. Afterwards, it is rinsed with distillate water repeatedly until the pH is neutral and dried at 70°C for 24 hours.

**Isolation of Cellulose Acetate (CA).** Ten grams of bacterial cellulose powder that has been formed was washed with glacial acetic acid to remove the water content and then filtered through a vacuum filter. The water-free cellulose acetate powder was activated with 50 mL of glacial acetic acid and 98% sulfuric acid as a catalyst at 38°C for 30 minutes. Acetic anhydride was added as a solvent in the acetylation process at a ratio of 1:3 %w/v. The acetylation process was done with variations (1; 2; 3 hours) at 38°C until a more viscous solution was formed. After the acetylation process was completed, the suspension was hydrolyzed with 12 mL of acetic acid that is diluted with a ratio of acetic acid and distillate water 1:2 and heated at 50°C for 30 minutes. Later, the solution was cooled to room temperature and then centrifuged for 15 minutes at a speed of 2,500 rpm. The resulting supernatant was added little by little into excess distillate water to form a white powder of cellulose acetate. The cellulose acetate formed was filtered through a vacuum filter and rinsed with distillate water until the acid smell vanished. Lastly, it was dried in an oven at 60°C for 6 hours.

**Morphology of Bacterial Cellulose and Cellulose Acetate Determination.** The morphology of the resulting bacterial cellulose and cellulose acetate particles can be determined using SEM (Scanning Electron Microscopy) JEOL JSM-6390 series. SEM is an electron microscope that uses a high-energy electron beam scanning process to image the surface of a sample. It is equipped with an optical microscope that is used to study the texture, topography, and surface properties of powders or solids. Due to its sharp focus, the resulting image has a three-dimensional quality.

**Functional Groups of Bacterial Cellulose and Cellulose Acetate Determination.** FTIR (Fourier Transform Infra-Red) spectrophotometry was used to determine the functional groups contained in bacterial cellulose and cellulose acetate particles. FTIR uses an optical system with a laser as a radiation source which is then interfered by infrared radiation so that the radiation signal received by

the detector has good quality and integrity. The principle of FTIR is to reach the sample through a gap in the form of infrared rays where the gap controls the energy sent to the sample. Subsequently, part of the infrared is absorbed by the sample and the other part is transmitted through the surface of the sample so that the infrared reaches the detector and later sends the measurement signal to the computer. This tool uses a Thermo Scientific type Nicolet iS5.

**Yield Percentage Determination.** The ratio between the mass of the cellulose acetate product obtained from the acetylation reaction and the mass of the cellulose raw material yields the percent yield. The formula for calculating yield [10] is written as follows:

$$\text{Yield}(\%) = \frac{\text{Product mass}}{\text{Raw material mass}} x100\% \quad (1)$$

## Results and Discussion

**Physical Appearance of Bacterial Cellulose and Cellulose Acetate.** The physical appearance of bacterial cellulose and cellulose acetate on each variable of acetylation time can be seen in Fig. 1.

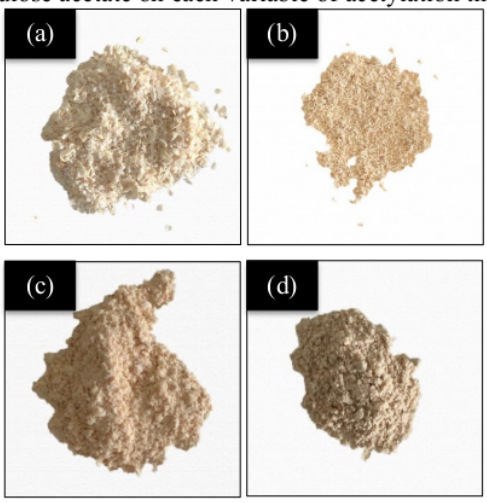


**Figure 1.** Physical appearance of (a) bacterial cellulose, (b) cellulose acetate at 1 hour, (c) 2 hours and (d) 3 hours acetylation time.

In this study, bacterial cellulose was resulted in the form of a brownish white fine powder. According to Cazón et al. (2020), some of the characteristics of bacterial cellulose are that it is non-toxic, can be degraded, has high mechanical strength, and has a distinctive white color. Other than that, the bacterial cellulose resulted is a biopolymer produced by the *Acetobacter xylinum* bacteria and has a higher purity level than other cellulose sources. This indicates that the particles obtained are bacterial cellulose.

Fig. 1 shows that the color of cellulose acetate powder looks darker as the acetylation time increases. When it was taken out from the oven, cellulose acetate at the acetylation time of 1 and 2 hours still looked wet and produced a small amount of cellulose acetate powder. This was because the acetylation time of 1 and 2 hours is not the optimal time for bacterial cellulose degradation to become cellulose acetate. Cellulose acetate at the acetylation time of 1 and 2 hours still contains impurities in the form of moisture, volatile content, and pollutant. The presence of acetic acid also causes the sample to look wet because it is hygroscopic. These impurities are then removed by washing with a centrifuge which utilizes the principle of differences in the specific gravity of the material.

**Functional Group Analysis of Bacterial Cellulose and Cellulose Acetate.** FTIR (Fourier Transform Infra-Red) analysis used to know the resulted of particle functional group. The results are shown in Fig. 2.

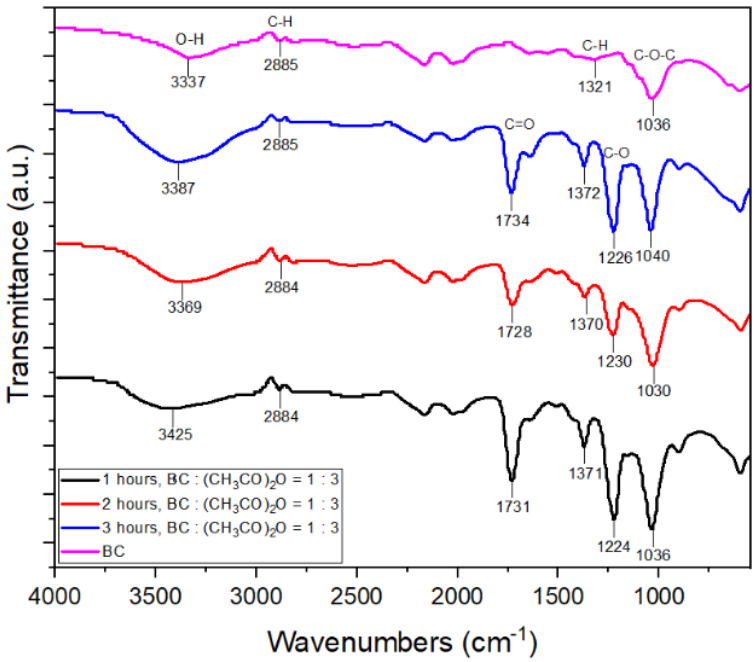


**Figure 2.** FTIR pattern of bacterial cellulose and cellulose acetate at 1, 2 and 3 hours acetylation time.

Fig. 2 shows that all samples of cellulose acetate and bacterial cellulose have four main peaks located in the range of wave numbers 1,030-1,040; 1,321-1,372; 2,884-2,885; and 3,337-3,425 $cm^{-1}$. Wave numbers in the range 1,030-1,040 $cm^{-1}$ are C−O−C functional groups [12], wave numbers in the range 3,337-3,425 $cm^{-1}$ are −OH functional groups [13], while the range in wave numbers 1,321-1,372 and 2,884-2,885 $cm^{-1}$ indicate the presence of C−H functional group which is the main bond in cellulose [12] [14]. The two said bonds are bonded to each other and cause the formation of a hexagonal structure of carbon atoms.

It is seen that the −OH and C−H functional groups in the three samples of cellulose acetate have a larger wave number within the increasing acetylation time from 1 hour to 3 hours. The −OH group resulted is a hydroxyl group from bacterial cellulose which is substituted by an acetyl group [15]. This shows that the acetylation time affects the cellulose acetate functional groups' characteristics.

The wave numbers in the range of 1,224-1,230 $cm^{-1}$ and 1,728-1,734 $cm^{-1}$ are the C−O and C=O functional groups which indicate that there has been a functional group bond between bacterial cellulose and acetic anhydride in the acetylation process [16]. This confirms the statement that cellulose acetate has been successfully synthesized.

**Morphological Analysis of Bacterial Cellulose and Cellulose Acetate.** To determine the particle morphology of bacterial cellulose and cellulose acetate, analysis was done using SEM as shown in Fig. 3 and Fig. 4.

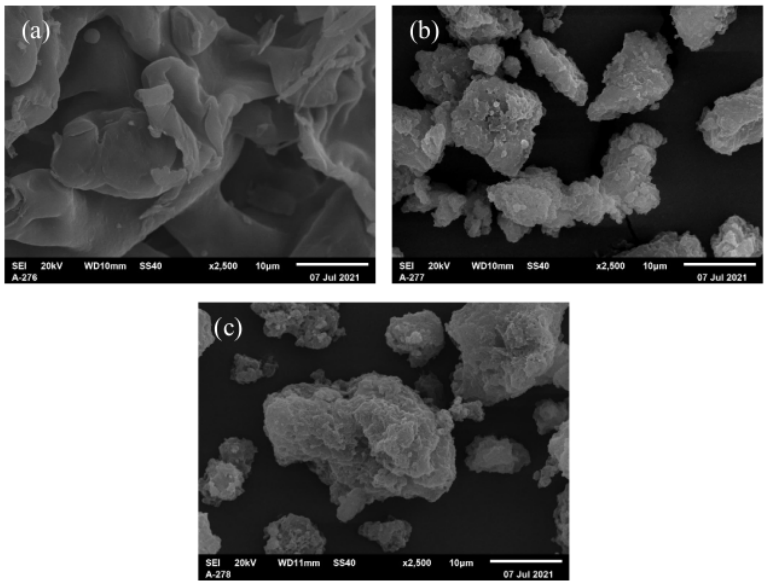


**Figure 3.** Morphology at 2500x magnification (a) bacterial cellulose, (b) cellulose acetate 1 hour and (c) 3 hours acetylation time.

Based on the results of morphological analysis, at 1 hour acetylation time, particles obtained are still in the form of lumps with pores that tend to be small and in small numbers. This is due to the presence of acetic acid that causes the previously sifted particles to rejoin to form aggregates. The increase in acetylation time from 1 hour to the next 3 hours resulted in cellulose acetate starting to form a layer. Other than that, the increase in acetylation time also causes the pores to enlarge and increase the amount which can be seen in Fig. 4. The morphology of bacterial cellulose has a morphology similar to the bacterial cellulose in the study [17] that is like a stacked random sheet structure.

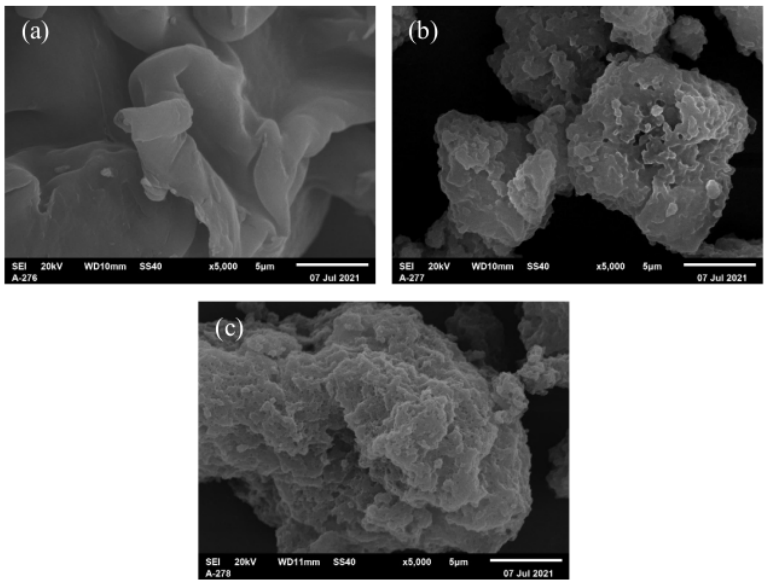


**Figure 4.** Morphology at 5000x magnification (a) bacterial cellulose, (b) cellulose acetate 1 hour and (c) 3 hours acetylation time.

Fig. 4 shows a higher magnification that can be seen where the increasing acetylation time from 1 hour to 3 hours results particles with a hexagonal pore arrangement. The amount of pores obtained is increasing and there are pores in the pores so the surface area is higher. This shows that the acetylation time of 3 hours is the optimum time for acetylation. The resulting cellulose acetate has pores that are almost evenly distributed on the surface of particles in a uniform size. The morphology has similarities to the study [18].

**Yield Cellulose Acetate.** Yield of cellulose acetate at each of acetylation time variable is shown in Table 1.

**Table 1.** Yield calculation results

| Acetylation time [hour] | Raw material mass [gram] | Product mass [gram] | Yield cellulose acetate [%] |
|---|---|---|---|
| 1 | 10 | 7.349 | 73.49 |
| 2 | 10 | 7.664 | 76.64 |
| 3 | 10 | 9.474 | 94.74 |

The results of the analysis show that the yield is affected by the acetylation process time. A significant increase in yield occurred in the acetylation time range of 2 to 3 hours which the difference obtained was 18.1%. The maximum yield was obtained at 3 hours of acetylation which was 94.74%. This is in line with the greater the acetylation time, the greater the yield produced. Acetylation time is one of the factors that affect the rate of reaction. The reaction occurs when the particles crash with each other and cause collision thus affecting the degradation process of bacterial cellulose into cellulose acetate.

## Summary

Based on the study and discussion that has been done, it can be concluded that cellulose acetate based on bacterial cellulose material from palm flour liquid waste and coconut water was successfully synthesized through the acetylation process. The optimum acetylation time was obtained at 3 hours with a yield reaching 49.74%. Morphological testing of cellulose acetate with SEM, there was an increase in pore size from the acetylation time of 1 to 3 hours. These results are consistent with the FTIR analysis that shows that the −OH and C−H functional groups have a higher wave number along with the increasing of acetylation time.

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