Effect of Different Treatment Methods on the Purification of Bacterial Cellulose Produced from OPF Juice by *Acetobacter Xylinum*

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Abstract. Bacterial cellulose (BC) is a form of cellulose produced by *Acetobacter xylinum* via fermentation process. BC contains impurities such as medium broth or the cell biomass. This study aims to determine the effect of purification method on the BC produced from oil palm frond (OPF) juice. Three purification method was presented in this study; first, BC was treated with different NaOH concentration at constant temperature of 70°C. Second, the BC was treated with different temperature while the NaOH concentration was fixed at 0.1M. Lastly, the BC was further purified in distilled water at different temperature. Fourier transform infrared spectroscopy (FTIR) and field emission scanning electron microscope (FESEM) analysis revealed that chemical composition of BC is similar to the natural cellulose without the present of biomass impurities as no peak at 1800 – 1500 cm⁻¹ can be detected. Higher percentage for loss of biomass, 22.03 % can be obtained when the BC was treated with 1.0 M NaOH at 90°C and further purified in distilled water at 90°C. As conclusion, pure BC which are free from any impurities can be obtained by using proposed method.

1. Introduction

Cellulose is the main component of plant used to maintain their structure. It is widely used in papermaking, textile, medical application and reinforcement agent because of its physical properties and lower cost [1]. The increase in demand for cellulose contributed to environmental problem due to by-product of wood pulping and deforestation. Therefore, bacterial cellulose (BC) is produced as an alternative for plant–based cellulose. Conventional method of producing BC using Hestrin-Schramm medium is expensive and requires many additional resources for cultivation [2]. Since the productivity of BC is depending on the carbon concentration, mainly glucose [3] therefore, oil palm frond (OPF) juice is used as the fermentation medium as it contains variety of sugar such as glucose, fructose and sucrose [4]. Other than that, this alternative can also reduce the environmental problem related to oil palm waste due to its limited utility.

Usually, OPF is left to decompose naturally at oil palm plantation to improve soil properties and water retention [5]. However, OPF has low rate of decomposition that will disturb the plantation process
and led to spread of disease and insects [6]. Malaysia generate approximately 80 million tonnes of dry solid biomass from oil palm industry in 2010 and the value is expected to reach up to 110 million tonnes in 2020 [7]. Therefore, an optimum used of oil palm waste as raw material is important to reduce the waste.

Consequently, the BC produced via fermentation contains other impurities such as the medium broth or the whole cell biomass [3]. Many methods have been introduced to increase the purity of the cellulose without damaging the structure of the cellulose at low cost and environmentally friendly. The most common method to purified BC was by using alkaline treatment, organic acid treatment or repeated washing with distilled water or hot tap water [8]. Alkaline treatment can be used to increase the density of the fibre. In plant cellulose, alkaline treatment was used to remove hemicellulose and lignin which are less dense thus increasing the density of the fibre. Other than that, alkaline treatment modified the crystallinity of the unit cell structure and the fibre orientation. As the crystallinity content of the fibre increase during alkaline treatment, the mechanical properties of the composite such as tensile strength will also increase [9]. It is proven that treatment method affects the morphology of BC such as porosity, mechanical properties and cell attachment even though the chemical structure and the shape of the cellulose does not change [10]. Therefore, this study is conducted to determine the effective method for purification of BC and its effect on the BC properties and morphology.

2. Materials and Methodology

2.1. Materials

The OPF was obtained from LKPP Lepar Plantation, Gambang, Pahang. The OPF was pressed by using sugar cane pressing machine at Forest Research Institute of Malaysia (FRIM) in order to get the juice. The Acetobacter xylinum strain used in this study was purchased from Malaysian Agriculture Research and Development (MARDI) in Serdang, Selangor, Malaysia. Chemicals used in this study are sodium hydroxide (NaOH), sodium hypochlorite and acetic acid with 90% purity were purchased from Sigma-Aldrich.

2.2. Fermentation medium preparation

Only pure OPF juice was used as fermentation medium in this study. The OPF juice was prepared by pressing the OPF petiole by using a press machine. Then, the OPF juice obtained was centrifuged at 4°C for 15 minutes to remove the solid material from the juice. The OPF juice was stored in -20°C freezer for further use.

2.3 Production of BC pellicle

The fermentation process was carried out in an incubator at 30°C for 7 days [11]. The initial pH of the medium was maintained at 4.5 by adding sodium hydroxide or acetic acid solution. The medium was sterilized by using autoclave machine at 121°C for 15 minutes. After sterilization, the medium was left to cool down to room temperature before 10 mL of Acetobacter xylinum inoculum was added into the 100 mL of fermentation medium (10 % v/v).

2.4 Purification of BC pellicle

2.4.1 Purification of BC at different NaOH concentration. The BC pellicle was immersed in 0.1 M, 0.5 M and 1.0 M of NaOH solution. Then, the solution was heated up to constant temperature of 70°C and stirred for two hours. The treated BC pellicle was further purified by immersing in distilled water and left for one day until the yellowish colour change to white.

2.4.2 Purification of BC at different temperature. The BC was immersed in 0.1M NaOH solution and heated to three different temperatures, 70°C, 80°C and 90°C. Then, the BC was stirred for two hours. The treated BC was further purified by immersing the in distilled water and was left for one day until the yellowish colour change to white.
2.4.3 Subsequent treatment of BC using distilled water at different temperature. The BC was immersed in 0.1M NaOH solution and heated at temperature, 90°C. Then, the BC was stirred for two hours. The treated BC was further purified by immersing it in a distilled water and heated at three different temperatures, 70°C, 80°C and 90°C. The BC was heated until the yellowish colour change to white.

2.5 Analysis of the BC
The purified BC was dried in oven at 80°C for a day and the dry weight of the BC was recorded before further analysis. The functional group of BC was analysed by using FTIR and the morphology was characterized by using FESEM analysis. The loss of biomass in BC was calculated from the following equation.

\[
\% \text{loss of biomass} = \frac{W_{\text{before}} - W_{\text{after}}}{W_{\text{before}}} \times 100
\]

Where, \(W_{\text{before}}\) is the mass of the BC before purification treatment and \(W_{\text{after}}\) is mass of the BC after purification treatment.

3. Result & Discussion

3.1 Physical appearance of the purified BC
The colour different of the BC before and after purification treatment was shown in Figure 1a and Figure 1b. From the figures, the difference in the colour can be observed where purified BC is changed from yellowish to white colour indicating that the medium broth components has been removed from the BC matrix.

![Figure 1a. Wet BC before treatment](image)

![Figure 1b. Wet BC after treatment](image)

3.2 Morphology characterization by using FESEM
The morphology of BC from FESEM is shown in Figure 2. From the figure, it can be observed that the BC is made of ultrafine layer of cellulose ribbons that are crossed, superimposed and randomly oriented. These layers also called as micro fibrils are irregular in size where the size varies from 50 nm to 60 nm. Other than that, there is no presence of the cell biomass embed in the BC matrix as the NaOH solution are able to hydrolyse and remove the cell impurities even at low concentration [12].
3.3 **Analysis of functional group by using FTIR analysis**

The summary for the wave number correspond to the functional group of the BC interpreted from the FTIR spectroscopy is shown in Table 1 and the clear peak is shown in Figure 3.

**Table 1. Summary of the BC wave number and it’s corresponded functional group from FTIR analysis**

| Wave number | Functional group                                      | References |
|-------------|------------------------------------------------------|------------|
| 3340 cm$^{-1}$ | O–H stretching of cellulose I                        | [12]       |
| 2896 cm$^{-1}$ | C–H stretching                                      | [3]        |
| 1200 – 1000 cm$^{-1}$ | H–C–H and C–OH bending                             | [3]        |
| 1800 – 1500 cm$^{-1}$ | amine and carbonyl                                  | [14]       |
| 850 – 1150 cm$^{-1}$ | the C – O stretching of the primary alcohol          | [13]       |

From Figure 3, it can be observed that the functional group of BC obtained from the three treatment method is similar with the natural cellulose obtained from plant. The crystalline structure of cellulose can be observed at 3340 cm$^{-1}$ absorbance peak that indicate the O–H stretching of cellulose [13]. C–H stretching can be observed at 2896 cm$^{-1}$ where both regions indicate the chemical composition of the main chain of bacterial cellulose. The intensity of the peak at 850 – 1150 cm$^{-1}$ indicated the crystallinity of the BC where treatment at high temperature, 90°C showed an increase in the crystalline content compared to treatment at low temperature, 70°C [13]. Wave number at 1800 – 1500 cm$^{-1}$ correspond to the amine and carbonyl functional group associated with lipid, protein and nucleic acid as stated by Fuller et al [14].

**Figure 2. Morphology of BC from FESEM analysis**
3.4 Analysis on the loss of biomass

3.4.1 Purification of BC at different NaOH concentration. The loss of biomass of BC via first purification method is shown in Figure 4. Higher percentage of biomass loss can be obtained at 0.5 M concentration of NaOH. However, 1.0 M is chosen to be the best concentration to purify the BC as the colour is whiter compared to the others.
3.4.2 Purification of BC at different temperature. The loss of biomass of BC via second purification method is shown in Figure 5. The result revealed that higher percentage of loss of biomass can be observed at high temperature. This is because at high temperature, NaOH solution can penetrate better into the membrane of the cellulose to hydrolyse the bacterial cell and remove the impurities present in the membrane [15]. As shown in Figure 5, both 80°C and 90°C show high percentage of removal which are 11.45% and 10.07%. However, 90°C is chosen to be the best temperature to purify the BC as the colour is whiter compared to other temperature.

![Figure 5. Purification treatment of BC at different temperature](image)

3.4.3 Subsequent treatment of BC. The loss of biomass of BC via third purification method is shown in Figure 6. From the figure, it can be observed that higher percentage of biomass loss can be obtained by heating the earlier treated BC in distilled water. Higher removal of biomass, 22.03 % was achieved when the BC was heated at 90°C. Therefore, it can be concluded that subsequent treatment of BC at 90°C was the most effective to purify the BC.

![Figure 6. Subsequent purification treatment of BC by using distilled water heated at different temperature](image)
4. Conclusion
The BC pellicle has been successfully treated from the proposed purification methods. Findings showed that higher percent loss of biomass, 22.03% was obtained by purifying the BC at 1.0 M NaOH at 90°C and further treated with distilled water at 90°C. Chemical composition of pure cellulose was observed through FTIR analysis with higher intensity at higher temperature. FESEM analysis showed the morphology of the BC are free from any impurities in the membrane matrix.

5. References

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