

# The effect of sodium hydroxide concentration on yield and properties of Bacterial Cellulose membranes

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**Abstract.** Bacteria cellulose (BC) derived from *Acetobacter xylinum* strain possess several advantages such as elevated water holding capacity, high porosity and excellent mechanical strength. BC is useful to replace vegetal plant and applied into various industrial field such as papermaking, packaging and textile. In this study, BC membranes were produced from coconut water based consists of 8.0% sugar, 0.5% ammonium sulphate and 1.0% acetic acid at pH 4.5, followed with the addition of 10.0% inoculum before subjected to static incubation for 7 days. This study evaluated the changes of BC membrane properties using different concentration of sodium hydroxide (NaOH) under room temperature within 24 hours. The morphological, physical, chemical structure and water vapour permeability (WVP) of BC were analysed using FESEM, XRD, FTIR and WVP analysis, respectively. Results show that BC is mainly consist of cellulose  $I_{\alpha}$  with high crystallinity and smaller crystallite sizes. The differences in NaOH concentrations varied the fibrils diameter of cellulose, in accordance to FESEM images. The use of higher concentration of NaOH ( $\geq 2.0\%$ ) gave a smooth cellulose structure with less porosity, thus reducing the WVP properties of BC.

## 1. Introduction

Bacteria cellulose (BC) is a unique biopolymer synthesized from Gram-negative bacteria such as *Acetobacter*, *Gluconacetobacter*, *Agrobacterium*, *Rhizobium* and *Sarcina* [1,2]. Currently, a significant amount of research has been devoted in the biosynthesis of BC membranes and its properties, as it is reported to have unique properties such as non-toxic materials [2], high crystallinity [1], high degree polymerization [3], good tensile strength [4] and excellent water holding capacity [5]. Besides, BC membranes are a great deal of interest for their potential to replace the utilization of vegetal cellulose, due to the similar chemical structure, with the absence of hemicellulose and lignin [1].

The properties of BC membranes can be modified by varying the culture conditions such as carbon and nitrogen source, the pH, the temperature, as well as purification process [1,3]. The purification process is crucial to remove any impurities and trapped microbe substances, in order to produce good quality BC membranes [6,7]. Several studies investigated the effect of type of alkaline used in the purification process on the resulting BC membranes. Al-Shamary and Darwash [8] reported that the porosity properties of BC membranes were reduced with the following use of  $K_2CO_3 > Na_2CO_3 > KOH > NaOH$ . Among reported studies, it is shows that the porosity of BC membranes does affect the



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transmission of oxygen throughout the membrane [7]. Hence, the aim of the present work is to evaluate the effect of using different concentration of sodium hydroxide (NaOH) solution in the purification process, in order to identify differences on morphological structure, physical and breathability properties of BC membranes.

## 2. Materials and methods

### 2.1. Culture medium and BC membrane formation

*Acetobacter xylinum* inoculum was purchased from Malaysian Agricultural Research and Development Institute (MARDI). The culture medium containing 1litre of coconut water, 8.0% (w/v) sugar, 0.5% ammonium sulphate and 1.0% acetic acid at pH4.5 were autoclaved before added into 10.0% (v/v) of inoculum. The production of BC membranes was conducted at room temperature using static batch for 7 days. After fermentation process, the BC membranes produced were harvested and washed with tap water repeatedly and the followed with purification process by soaking with different percentage of sodium hydroxide, NaOH; 0.0%, 0.5%, 1.0% and 2.0%. Each of BC membranes were immersed in NaOH solution at room temperature for 24 hours. The purified BC membranes then were washed repeatedly with tap water until the pH about 7 and then dried using air dryer at 120°C until constant weight obtained. The yield of purified BC membranes was calculated using the equation from Mohammadkazemi et.al [9].

### 2.2. Characterization

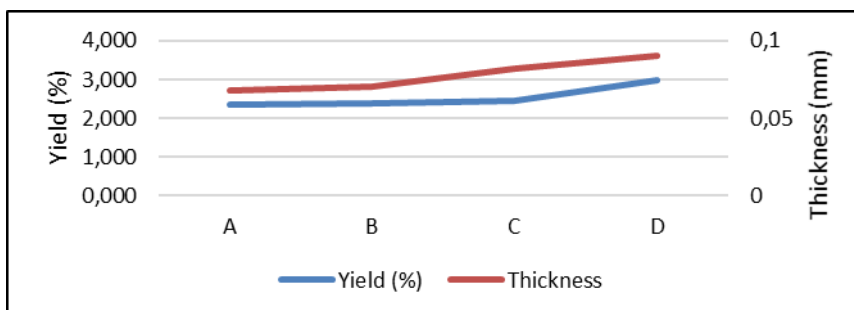
The crystallinity index of BC membranes and its crystallite sizes were determined using X-ray diffractometer (XRD) (Pan Analytical diffractometer, Netherlands), at  $2\theta$  range of 5°- 40° with a scanning speed of 5° per minute. Fourier transmission infrared (FTIR) spectroscopy primarily used to identify the chemical bonding of the BC membranes. The FTIR spectra of the membranes measured at wavelength ranging from 4000 to 500  $\text{cm}^{-1}$  using Nicolet (United States) SX-170 FTIR spectrometer. The field-emission scanning electron microscopy, FESEM (Leol 450 VP, Leo, Germany) observed the nanostructure of dried surface BC membranes at acceleration voltage of 15kV. The BC samples were gold coated using a sputter coater for 120s (SC500, BioRad, United Kingdom). Water vapour permeability test (WVPT) were performed to determine the breathability of BC membrane. Breathability test of BC membranes were measured using the Upright Cup Method principle on the SDL Atlas M21 Water Vapor Permeability Tester, in accordance with ASTM E96 in Textile Fabric Testing. The thickness of BC membranes was measured using digital thickness gauge at ten different positions and the average value was calculated.

## 3. Results and discussion

### 3.1. BC production yield

Figure 1 shows the production yield and thickness of dried BC membranes produced as affect from the usage of different NaOH concentration during purification process. The cellulose yield (%) and thickness (mm) of BC membranes were found to increase with the increment of NaOH concentration. The yield and thickness of BC sample increased in the following order of BCPu0.0 > BCPu0.5 > BCPu1.0 > BCPu2.0. Sample BCPu2.0 had been purified with the highest concentration of NaOH obtained 25% cellulose yield higher than untreated sample (BCPu0.0). Meanwhile, the membrane thickness of BCPu2.0 was increased approximately 32% more than sample BCPu0.0. It is suggested that the BC membrane thickness is related to the changes occurred on the morphology of the cellulose. Alkaline treatment was usually being applied in mercerization process during weaving which is responsible in the swelling of the cellulose microfibrils [10]. Therefore, the swelling of cellulose fibrils will eventually increase the BC membrane thickness upon the purification process. In addition, the color contrast of BC membranes immersed in different concentrations of NaOH solution is an indication of the removal of impurities, as resulted from the elimination of the remaining organic

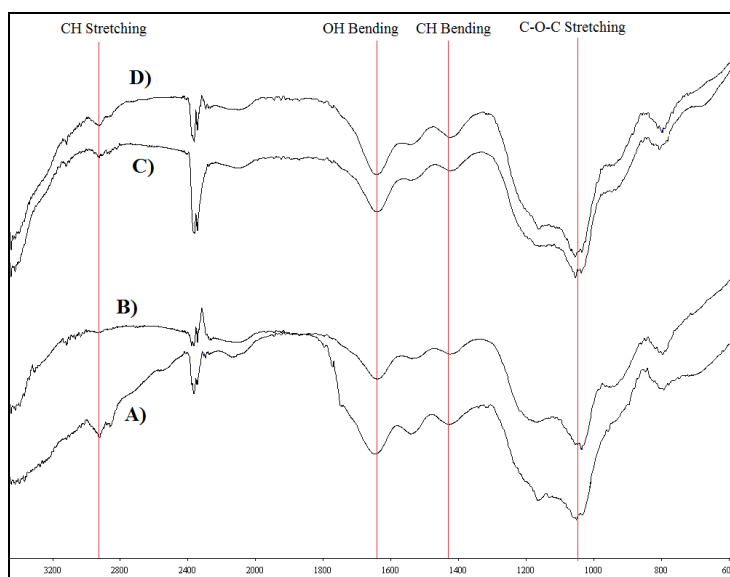
compounds, nucleic acids and proteins generated by microbes during the fermentation process. This finding has been supported by Meftahi et.al [6].



**Figure 1.** The production yield and thickness of BC membranes (A)BCPu0.0, (B)BCPu0.5, (C)BCPu1.0 and (D)BCPu2.0.

### 3.2. FTIR analysis of BC membrane

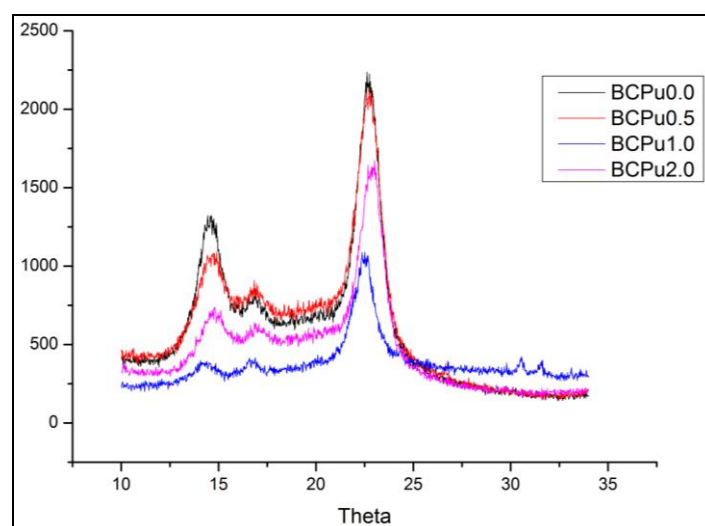
FTIR spectra in Figure 2 shows the functional group of BC membranes presence at the wavelength ranging from  $3200\text{cm}^{-1}$ – $800\text{cm}^{-1}$ . From the observation, all BC membrane samples shows the absorption peaks at  $2800$ – $2970$  and  $1023$ – $1024\text{ cm}^{-1}$  were derived from -CH stretching and C-O-C ring vibrations. Peaks for C-H<sub>n</sub> stretching indicated alkyl, aliphatic aromatic compound found at  $2890\text{cm}^{-1}$ , also supported by the CH bending vibrations at peaks  $1453\text{cm}^{-1}$  as mentioned in [11]. The O-H bending variation appeared at  $1670\text{cm}^{-1}$  wavenumber in Figure 2 represents the hydrogen bond of water absorption. Meanwhile, the peaks range from  $1060$ – $1031\text{cm}^{-1}$  represent C-O compound of carbohydrates as mentioned in Han et.al [12]. FTIR spectra in the region of  $1620$ – $1380\text{cm}^{-1}$  show that the band of the purified BC spectra has been slightly shifted upon purification [13]. The observed peak at  $1100$ – $1073\text{cm}^{-1}$  region assigned as C-O-C stretching at the  $\beta$ -(1→4)-glycosidic bond/linkage in cellulose has confirmed that the cellulose I structure remain intact as BC membrane treated with 2% of NaOH solution.



**Figure 2.** FTIR spectra of the BC membranes (A)BCPu0.0, (B)BCPu0.5, (C)BCPu1.0 and (D)BCPu2.0.

### 3.3. X-ray diffraction analysis of BC membrane

X-ray diffraction patterns of BC membranes of different NaOH concentration were shown in Figure 3. According to x-ray diffraction pattern of BC membranes, the presence of broad diffraction peaks around  $14^\circ$  and  $22^\circ$  were observed, which correspond to the crystallographic planes, characteristic interplane distances of cellulose  $1\alpha$  and  $1\beta$  phases [11]. These two major peaks are representing the cellulose type I structure [12], which are revealed in the XRD patterns of all BC samples. As observed from the highest peak appeared  $22^\circ$  of BCPu1.0 on the XRD pattern (Figure 3), it shows the high crystallinity index of cellulose I. The highest crystallinity index of cellulose I was at BCPu1.0 (79.28%), followed with BCPu0.0, BCPu0.5 and BCPu2.0 range from 70% to 67%, respectively. The removal of impurities by purification using NaOH suggesting a change in the orientation of crystallite BC fibre structure and provide high degree order of polymer, which increased the relative crystallinity [5,12]. Besides, a sharp peak indicates of highly ordered of cellulose arrangement, which is depends on many factors such as its molecular composition and crystallite sizes.



**Figure 3.** Crystallinity changes in X-ray diffraction pattern of BC membranes; (A)BCPu0.0, (B)BCPu0.5, (C)BCPu1.0 and (D)BCPu2.0

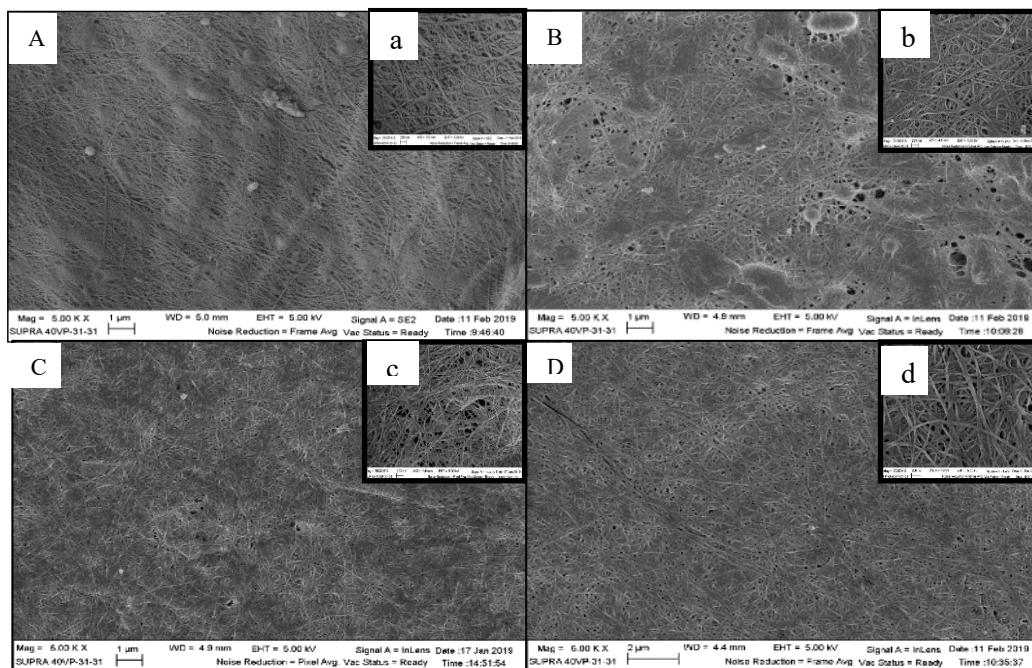
### 3.4. Morphology of BC membrane

Figure 4 A, B, C, and D shows the FESEM images with 5.0K magnification for untreated BC and purified BC with 0.5%, 1.0% and 2.0% alkaline solution. While Figure 4 a, b, c and d shows the morphology images at 30.0K magnification. The FESEM image of BC without purification (BCPu0.0) shows a compact structure with microfibrils interweaved in a random arrangement (Figure 4 A and a). As shown in Figure 4 (B) and (b), the fibrils arrangement of BCPu0.5 was in a loose structure arrangement with the present of interconnected pores but with incomplete impurities removal. While morphology images of BCPu1.0 in Figure 4 (C) and (c) shows the completely removal of impurities along the cellulose structure. The functions of NaOH was to remove impurities and other microbes by dissolving non-cellulosic components (NCC) which can expand pores on the cellulose surface [6,10,12]. The FESEM images for sample BCPu2.0 in Figure 4 (D) and (d) showed an effective impurities removal with drastically fibres structure enlargement. Previously, Tang et.al mentioned that higher swelling rate increased the micro fibrils diameter hence will reduce the pores size [7]. Suryanto et.al also reported that purification treatment with higher concentration of NaOH had altered the OH bonding and decreasing the mechanical strength of BC films [10]. Therefore, this study found that

purification with 1.0% of NaOH solution has provided better results in the effective removal of impurities with minimum changes on the microfibrils morphological structure.

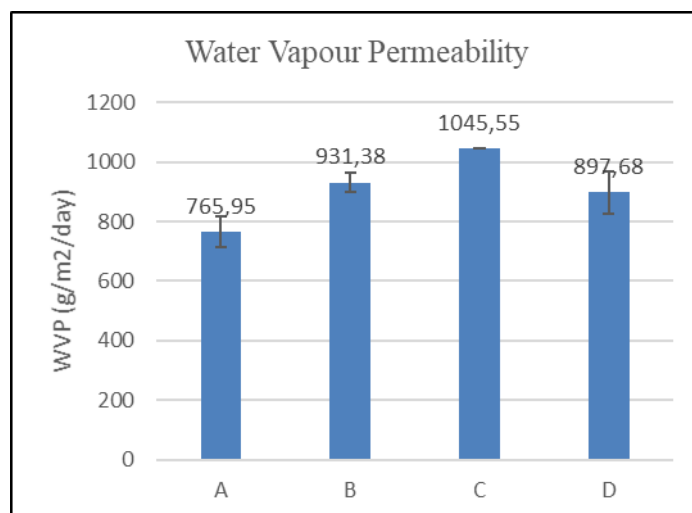
### 3.5. Water vapour permeability analysis

The water vapour permeability (WVP) results of BC samples with various NaOH concentration shown in Figure 5. WVP testing were performed to calculate the moisture transfer from cellulose membrane to the surroundings that will give the information of breathability properties of BC membranes. Previous research mentioned that BC membranes is considered as a breathable when they possess high value of water vapour permeable [13]. According to the bar graph, the WVP of BC samples increased upon purification process from 0.0% to 1.0% that is 765.95g/m<sup>2</sup>/day to 1045.55g/m<sup>2</sup>/day, respectively. With the usage of 0.5% of NaOH solution, the WVP increased up to 21.59% as compared to the untreated sample (BCPu0.0). While, 1.0% of NaOH gave the highest value of WVP, which is 36.5% more than the untreated BC sample (BCPu0.0). However, the vapour permeability for BCPu2.0 decreased 14.14% as compared to BCPu1.0 samples. From the FESEM images of BC in Figure 4, the addition of NaOH concentration up to 1% had reduced some impurities on BC structure, which had increased the pores availability. Thus, it had improved the vapour permeability. Nevertheless, the addition of NaOH concentration also had increase the thickness and swellness of fibrils as recorded in Figure 1. Tang et.al also explained that due to the swelling of BC microfibrils structure, the pore sizes reduced and the water vapour transmission rate throughout the membrane decreased [7].



**Figure 4.** Surface morphology images of BC membrane at 5.0K and 30.0K magnification, respectively: (A) (a) BCPu0.0, (B) (b) BCPu0.5, (C) (c) BCPu1.0 and (D) (d) BCPu2.0





**Figure 5.** Transmission rate of water vapor permeability (A)BCPu0.0, (B)BCPu0.5, (C)BCPu1.0 and (D)BCPu2.0 of BC membrane

#### 4. Conclusion

Purification treatment using different concentration of NaOH had affected the yield, thickness and water vapour permeability properties of BC membranes. The usage of higher concentration of NaOH causes the increment on the yield and thickness of BC membranes, while reducing the water vapour permeability of BC membrane. However, high concentration of NaOH also remove some impurities from the remaining organic compounds, nucleic acids and proteins generated by microbes during the fermentation process which can help to produce a breathable BC membrane to be applied in textile application.

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#### References

- [1] Esa F, Tasirin S M and Rahman N A 2014 Overview of bacterial cellulose production and application *Agriculture and Agricultural Science Procedia* **2** 113 – 119
- [2] Gallegos A M A, Carrera S H, Parra R, Keshavarz T and Iqbal H M N 2016 Bacterial cellulose: A sustainable source to develop value-added products - A review *BioResources* **11** 5641 – 5655
- [3] Chawla P R, Bajaj I, Survase S and Singhal R 2009 Microbial cellulose: fermentative production and applications *Food Technology & Biotechnology* **47**
- [4] Costa A, Rocha M and Sarubbo L 2017 Bacterial cellulose: an ecofriendly biotextile *Int. J. Text. Fashion Technol* **7** 11 – 26
- [5] Saibuatong O and Phisalaphong M 2010 Novo aloe vera–bacterial cellulose composite film from biosynthesis *Carbohydrate Polymers* 455 – 460
- [6] Meftahi A, Khajavi R, Rashidi A, Rahimi M and Bahador A 2015 Effect of purification on nano microbial cellulose pellicle properties *Procedia Materials Science* **11** 206 – 211
- [7] Tang W, Jia S, Jia Y and Yang H 2010 The influence of fermentation conditions and post-treatment methods on porosity of bacterial cellulose membrane *World Journal of Microbiology and Biotechnology* **26** 125
- [8] Al-Shamary E E and Al-Darwash A K 2013 Influence of fermentation condition and alkali

- treatment on the porosity and thickness of bacterial cellulose membranes *The Online Journal of Science and Technology* **3** 2 194 – 203
- [9] Mohammadkazemi F, Azin M and Ashori A 2015 Production of bacterial cellulose using different carbon sources and culture media *Carbohydrate Polymers* **117** 518 – 523
- [10] Suryanto H, Muhajir M, Sutrisno T, Zakia N and Yanuhar U 2019 The mechanical strength and morphology of bacterial cellulose films: the effect of NaOH concentration *IOP Conference Series: Materials Science and Engineering* **515**
- [11] Khalid A, Khan R, Ul-Islam M, Khan T and Wahid F 2017 Bacterial cellulose-zinc oxide nanocomposites as a novel dressing system for burn wounds *Carbohydrate Polymers* **164** 214 – 221
- [12] Han J, Shim E and Kim H R 2018 Effects of cultivation, washing, and bleaching conditions on bacterial cellulose fabric production *Textile Research Journal* 1 – 11
- [13] Jinka S, Behrens R, Korzeniewski C, Singh V, Arunachalam A, Parameswaran S, Coimbatore G, Kendall R, Wolf R and Ramkumar S 2013 Atmospheric pressure plasma treatment and breathability of polypropylene nonwoven fabric *Journal of Industrial Textiles* **42** 501-514