The Characteristics of Acetobacter xylinum Membrane Affected by pH of Culture Medium

Amira Syazwani Mustafa Kamal, Mohd Iqbal Misnon, Mohd Rozi Ahmad, Muhammad Ismail Ab Kadir, Sukirah Abdul Rahman

Abstract*: There are numerous previous studies working on biosynthesis, properties and applications of bacterial cellulose (BC) membrane from Acetobacter xylinum bacteria strain. However, there is less research examining the effect of initial pH level on BC membrane properties. Here, BC membranes were produced at different pH level (concentration of acetic acid) of coconut water-based culture medium which are 3.5, 4.0, 4.5, 5.0 and 5.5 for 7 days, statically. The effect of initial pH level during BC production on morphology, physical and characteristics were investigated. The Acetobacter xylinum membrane were studied its crystallinity using X-ray diffraction, FTIR analysis for chemical structure and FESEM for morphological analysis. In addition, the different of initial pH level does affect the membrane yield and breathability properties using an Upright Cup Method of water vapour permeability testing. Although certain suitable amount of acid had reduced the production yield, a breathable BC membrane was produced.

***Index Terms*: Cellulose membrane, pH level, breathability properties, water vapor permeability**

I. INTRODUCTION

Cellulose is commonly harvested from plants through rough acid treatment and alkaline processes in its extraction. It has been used in severalfield such as paper making, furniture, textiles, food applications and other industries to name a few. These pre-production treatment will enhance the global environmental issues especially waste water [1]. Besides, higher production cost also being discussed. Some bacteria are able to produce cellulose, and this potentially seems to create an alternative way other that traditional production methods. Bacterial cellulose (BC)can be produced from Gram-negative bacterium species for example Acetobacter, Agrobacterium, Rhibozium, Sarcina and Azotobacter [2].It possesses some great properties and characteristics such as high crystallinity, high degree of polymerization (DP), high tensile properties, non-toxic and biodegradable, differ with plant cellulose[1][3][4].

In BC production, the growth of membrane is depending on several factors in fermentation process such as temperature, carbon sources, medium types, pH level etc. As a fermentation process of BC progressing, the pH level rate will drop due to formation of gluconic acid [5][6]. Pourramezan et.al [7] stated that the different type of carbon sources will results to different rate of pH level and BC yield. The pH level rate in fermentation process is claimed to give different production yield, crystallinity index and degree of polymerization when applying different level of acidity [5][8]. Previously,Wang et.al [9] stated that growth rate of bacteria decreases when pH of culture medium is outside of 4<pH<7 range. Azeredo et.al [5] also mentioned that BC yield is 60% higher when pH of culture media being controlled rather than uncontrolled. This is just a few findings reported by other workers regarding the pH factors. Nevertheless, there is less study focusing on the effect of different initial pH level on the breathability characteristics of BC membrane. Thus, this work was done to fill up this gap.

For this purpose, this work was done to investigate the effect of pH levels in culture media to the cultivated BC membrane breathability characteristics. The BC membrane characteristics were examined for its water holding capacity, porosity and water vapor permeability. The investigation of its crystallinity, chemical structure and morphology of BC membranes produced were explored using X-ray diffraction, IR spectra and FESEM.

II. MATERIALS AND METHOD

A. Production of BC Membranes

The BC membrane was grown on 1L of coconut water based mediacontained 80g/L sugar, 5g/L ammonium sulphate. The culture media was prepared at various pH level (3.5, 4.0, 4.5, 5.0 and 5.5) by varying the amount of acetic acid. 10mL of stock culture (Acetobacter xylinum) was then added into each prepared culture media before they were left at room temperature using static batch for a week. After fermentation, all produced BC membranes were harvested and washed with tap water before undergo purification process by immersing samples in 1.0% of sodium hydroxide (NaOH) for 24hr at room temperature. Then, the purified BC membranes were washed repeatedly and weighed before and after drying process using air dryer at 120°C until constant weight was obtained.

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Afterward, the BC samples were stored in plastic film for further testing.

B. Production Yield and Thickness of BC Membranes

The weight of dried BC membranes were calculated to calculate the production yield of BC according to Mohammadkazemi, et.al [10]. The dried cellulose sheets were measured its thickness using a digital micrometer at 10 random positions and the mean value was calculated.

C. Characterizations of BC Membranes

FTIR spectroscopy was used to identify the chemical structure of the membrane. The FTIR spectra of the membranes were measured at wave numbers ranging from 4000 to 500 cm^{-1} with a Nicolet (United States) SX-170 FTIR spectrometer.Next, X-ray diffraction analysis were conductedusing Pan Analytical diffractometer (Netherlands). Scanning were performed over the 5°-40°of 2θ range. The crystallinity index and crystallite size were calculated using method as mentioned in Mohammadkazemi, et.al [10], where the crystallinity index, C.I = $(S_c/S_t)\times100$; S_c and S_t is the area of the crystalline and total domains, respectively. Crystallite sizes, Crs = $K\lambda/\beta\cos\theta$; where K is the shape factor (0.9), λ is the x-ray wavelength (1.54Å), β is the line broadening at half the maximum intensity (FWHM) in radians and θis the Bragg's angle. Water holding capacity (WHC) of BC membranes were identified by weighing the wet (W_0) and dried BC membrane (W_1). The calculation to obtain WHC was taken from Xianchao Feng et.al [11] where the WHC (%) = $(W_0\text{-}W_1/W_1)\ \times100$.The porosity of BC membrane was calculated according to Tang et.al [12] where the porosity (%) = $((W_0\text{-}W_1)/(W_1\text{-}W_2))\times100$; W_2 is the weight of dried BC membranes immersed in deionized water for 24 hours at room temperature. The water vapour permeability (WVP) of BC membrane was obtained using SDL Atlas M21 Water Vapour Permeability Tester, in accordance with ASTM E96.

III. RESULTS AND DISCUSSION

Bacterial cellulose bio-polymers were produced in the form of pellicles at different pH level of coconut water media. After the drying process, a bacterial cellulose film (BCpH) was obtained to proceed with other testing. The production yield and thickness of dried BCpH differs from each other at every pH level. It was presumed that the pH level of culture media does not only affect yield and thickness, but also affect its water holding capacity, porosity and water vapor permeability characteristics.

A. BC Membrane Yield and Thickness

Table I1 shows the production yield, initial, final pH and acidity increment of every BC samples produced at different pH level for 7 days of fermentation. All these samples of BC were produced in a culture media containing the same concentration of glucose (80g), but with different initial pH level. The highest and the lowest yield and thickness of BC membrane were BCpH4.5 and BCpH3.5 samples respectively (**Fig.1**).The increment of acidity was measured by differentiating initial and final pH of medium culture in percentage. The acidity increment (%) of BC membrane from the highest to the lowest were BCpH5.0>BCpH3.5>BCpH4.0>BCpH5.5>BCpH4.5respectively. The reduction of pH from initialto end of fermentation process is due to the conversion of glucose to form gluconic acid [9]. The high reduction of pH level can reduce the BC growth and its production yield [6]. Therefore, BCpH4.5 possessed the highest cellulose yield and thickness as the lowest percentage of acidity increment was recorded. The increase in production yield can affect BC other properties and potential application of nano-cellulose films [5].

Table I1: BC membranes production yield, thickness and pH

Sample	Yield (%)	Dry thickness (mm)	Initial pH	Final pH	Acidity Increment (%)
BCpH3.5	1.65±0.24	0.03±0.008	3.5	2.9	17
BCpH4.0	3.29±0.25	0.07±0.011	4.0	3.4	15
BCpH4.5	5.59±0.25	0.12±0.011	4.5	4.2	6.7
BCpH5.0	3.16±0.16	0.06±0.015	5.0	3.9	22
BCpH5.5	4.80±0.37	0.09±0.011	5.5	4.9	11

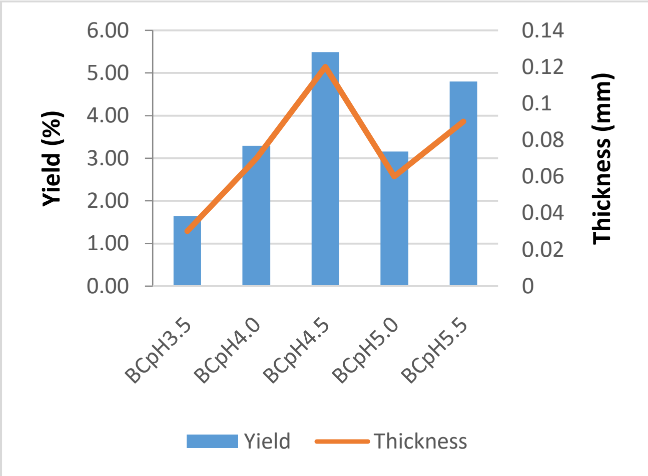

Fig.1: Bacterial cellulose yield and thickness produced after 7 days fermentation

XRD Analysis

Fig.2shows the X-ray diffraction pattern of BC with different pH level of culture media. The two major peaks appear between 14°- 23° correspond to the (1 1 0) and (2 0 0) reflexion plane of cellulose I structure on bacterial cellulose [13]. The XRD pattern obtained at the peaks around 14°, 16° and 22°, confirms that the pure BC produced were semi-crystalline respectively. However, the intensity of (2 0 0) plane of every BC membrane is different. The crystallinity index and crystallite sizes of BC membrane obtained from XRD data was tabulated in Table . The crystallite sizes (nm) of BCpH3.5 recorded to be the smallest and BCpH5.5 obtained the biggest. It was also found that BCpH4.0 obtained the highest C.I (%), while BCpH3.5 was the lowest as compared to other BC membranes. The higher crystalline structure of BC can perform better mechanical properties in comparison to lower crystalline structure due to the formation of a denser and orderly arranged of cellulose structure [14].

Table II: Crystallinity index and crystallite sizes for BC membrane

Samples	Theta (°)	Crystallite sizes (Å)	Crystallinity index (%)
BCpH3.5	22.387	4.49	71.5
BCpH4.0	22.438	5.33	84.5
BCpH4.5	22.506	6.05	75.7
BCpH5.0	22.744	5.81	76.4
BCpH5.5	22.608	6.67	74.2

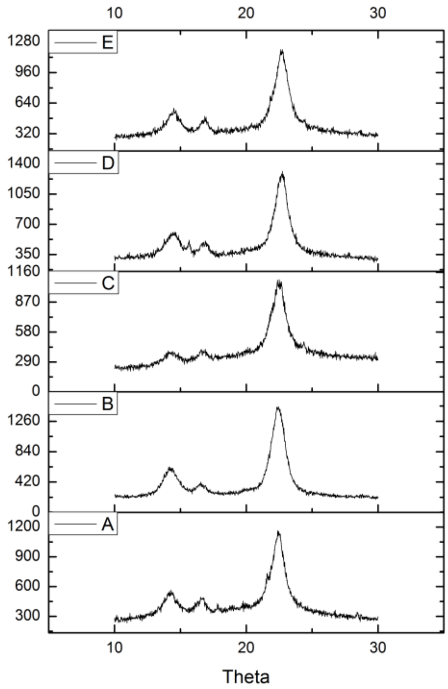

Fig.2: XRD spectra of BC membrane A) BCpH3.5, B) BCpH4.0, C) BCpH4.5, D) BCpH5.0 and E) BCpH5.5

B. Chemical Structure of BC Membranes

Fig.3shows the FTIR spectrum of bacterial cellulose produce by various pH level of culture medium. It was observed that all the samples have the same wave number during FTIR analysis (**Fig.3**). The band occurred between 3420cm^{-1} and 3390cm^{-1} are corresponding to intramolecular hydroxyl group with O-H stretching for cellulose. BC pH4.5 and BC pH5.0 obtained the same band for hydroxyl group which at 3418 cm^{-1}. Based on **Table** adapted from Khalid et.al [15], all BC samples produced at different pH level also possess the same band ranging at 2367cm^{-1} - 2375cm^{-1} and 1632cm^{-1}-1637cm^{-1} that are assigned to be CH stretching and O-H bending. The existing band occurred during FTIR analysis represented there is no effect of pH level of culture media to the functional group of cellulose bacteria.It just brought a different intensity of absorption waves showing that the amount of functional group at each molecular bond ranges were different.

Table III: FTIR band assignment of BC [15]

Wave number (cm^{-1})	Assignment
3300 -3200	Hydroxyl group, OH stretch
2890 - 2360	Methylene, CH_2 stretching
1678-1637	OH bending, water molecules
1450	CH bending
1060	Primary amine, C-O-C stretching

C. Breathability Properties

The water holding capacity (WHC) and porosity properties are crucial in application of BC membrane. It is stated that the porosity of BC membranes is related to the water holding capacity [16]. As shown in **Fig.4**, the WHC values of BC membranes reduced with the reduction of porosity values. Sample BCpH3.5 obtained the highest WHC and porosity values, which is 849.1g/g and 99.47%, while BCpH4.5 obtained the lowest values that are 339.6g/g and 97.66%. From previous results of BC yield and thickness, BCpH3.5 acquired the lowest yield and thickness, whilst BCpH4.5 obtained vice versa. The variation of yield and thickness of BC had attributed in the WHC and porosity. Same goes with BCpH5.0 that obtained higher WHC values with low yield and thickness, meanwhile the WHC values of sample BCpH5.5 decrease due to high yield and thickness. UI-Islam et.al [16] also mentioned that a compact and denser structure of BC had inhibit the penetration of water into the materials thus will reduce the WHC of BC membrane. Meanwhile, a low yield of BC membrane contained less fibrils and more pores on the structure in which can help them to absorb and penetrate more water. The loose and compact fibrils arrangement of sample BCpH3.5 and BCpH4.5 were showed at **Fig.5**. The pores available in **Fig.6**A) help BCpH3.5 sample to gain high WHC and porosity as compared to BCpH4.5 sample in **Fig.6**B).

Water vapor permeability (WVP) testing were performed to estimate the moisture transfer from cellulose membrane to the surroundings, which will provide the significant information on breathability properties of BC membranes. All five different samples of BC were performed with WVP analysis for three replicatesat standard laboratory conditions of 65 ± 2% relative humidity and 20 ± 2°C temperature [17]. After 5 hours of WVP testing on samples, the weight of water in the test cup were reduced. From**Fig.5**, it shows that sample BCpH4.5 has the lowest value of WVP in percentage (%). Meanwhile, BCpH5.0 obtained the highest value of WVP. Jinka et.al [17] studied on the breathability of non-woven fabrics and they found that WVP of the spun-bonded non-woven fabrics were 707.85 g/m^2/day. As a result, this paper finding proved that WVP readings of BC membranes produced with different pH level of culture medium were higher than WVP value of spun-bonded non-woven fabric, where sample BCpH4.5 obtained the lowest WVP (1046.17 g/m^2/day) among other BC membranes.

According to Varsha et.al [18], the surrounding temperature are crucial to the permeability of water vapor. Other than temperature and humidity, WVP of BC membranes also was related to its available pores and thickness properties. Jinka et.al [17] also stated that the rate of moisture vapor transmission are related to the porosity of membranes or fabrics; where the higher available pores in a fabrics structure increased the rate of water vapor diffusion. As shown in **Fig.4**, sample BCpH4.5 has the lowest value of porosity (%) with the highest yield and thickness(**Table I1**), thus harder the permeability of water vapor from inside test cup to the surrounding.The different pH level of culture medium during production of BC membrane helped to produce high and low production yield of BC, besides had created a distinct value of WVP results, where it can help to obtain a breathable BC fabric. Hence, it can be said that water vapor permeability properties are related to the yield, thickness properties and pore structure of BC dried samples.

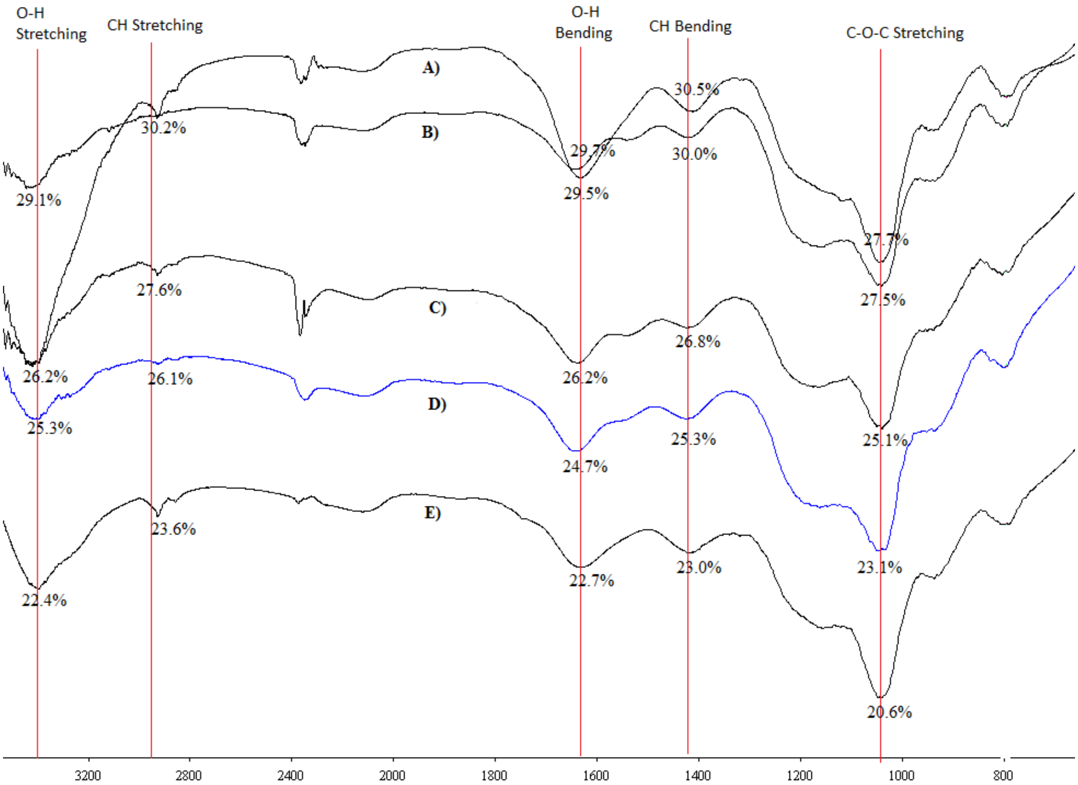

Fig.3: FTIR spectra analysis of BC membranes; A) BCpH4.5, B) BCpH3.5, C) BCpH5.0, D) BCpH4.0 and E) BCpH5.5

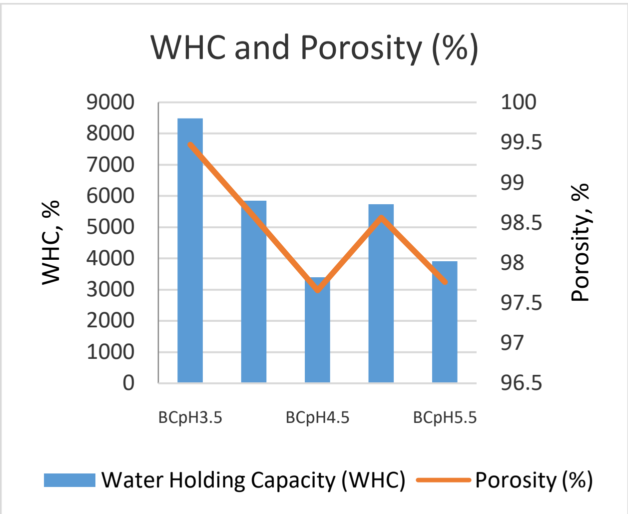

Fig.4: Water holding capacity and porosity value of BC membrane

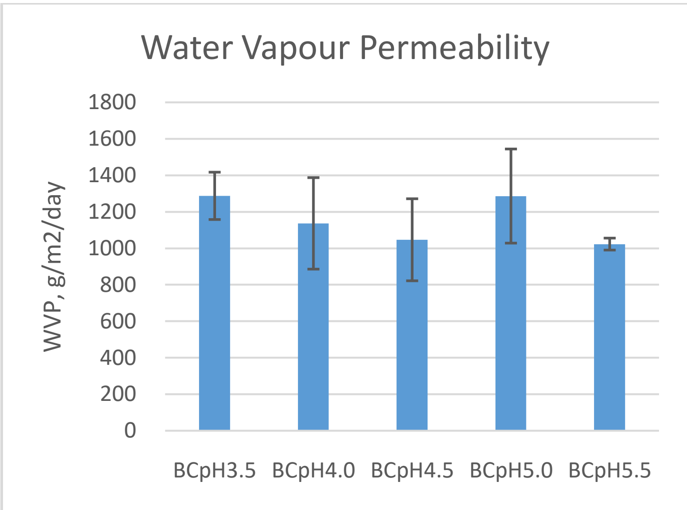

Fig.5: Water vapor permeability of BC membrane produced at different pH level

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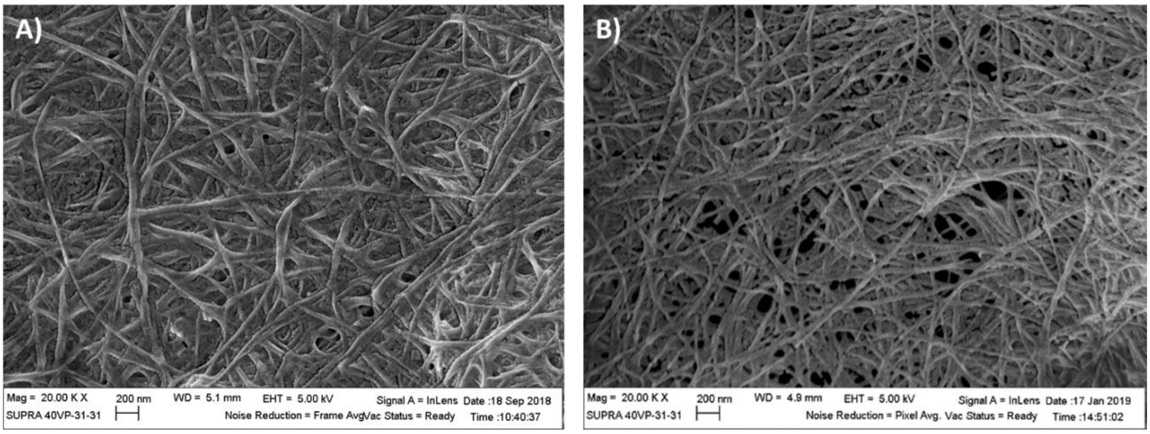

Fig.6: FESEM images of BC membrane A) BCpH3.5 B) BCpH4.5

IV. CONCLUSION

In this study, the different initial pH level of culture medium during production of BC membrane had provided some variations in the BC membrane properties such as water holding capacity, porosity and water vapour permeability. The changes of pH medium culture also responsible to their production yield and thickness. The highest yield and thickness value of BCpH4.5 in which contained compact fibrils arrangement had reduced the breathability properties such as WHC, porosity and WVP. Meanwhile, a loose structure with the present of pores had improved the breathability properties. Hence, some applications of BC membrane in textile industry as breathable materials is expected.

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