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Effect of Sucrose Amounts on the Bacterial Cellulose Membrane's Breathability Properties

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Abstract
This study aims to produce a bacteria cellulose (BC) membrane. The critical parameter controlled in this study was the amount of carbon source during the production. The effects of carbon source concentrations on BC membrane properties, such as yield, thickness, and breathability, were evaluated. The BCs' yields and thicknesses were increased with the higher concentration of sucrose utilised during the production process. By weighing and observing the BC membranes, sample BCS10.0 possessed the highest yield and thickness. Nonetheless, the lowest yield and thickness of BC membrane (1.374%, 0.07mm) produced a high mean pore diameter (16.909Å) and increased permeability of water vapour (4242.23g/m^2/day).

Keywords: Acetobacter *xylinum*; bacterial cellulose membrane; breathability; porosity

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1.0 Introduction

So far, the primary industries that utilise bacterial cellulose pellicles and sheets are the food industry as a food base due to its soft texture and high fibre content, and also in the medical application as wound dressing because of its non-toxic and highly porous materials properties (Provin et al., 2021; Sharma, Mittal, Yadav & Aggarwal, 2023). Furthermore, bacterial cellulose also has some potential to be used in the mineral and oil recovery sectors (Liu et al., 2021), paper-making applications, the packaging sector and the textile industry due to its unique physical and mechanical behaviour (Kamiński, 2020; Gallegos, Carrera, Parra, Keshavarz, & Iqbal, 2016; Pathak & Prasad, 2014). Instead of using plant cellulose, one other way to produce cellulose fibres is via bacterial cellulose. BC structure also provides different and advanced properties from plant cellulose, such as nano-structure, high mechanical strength, high water holding capacity, high degree polymerisation and high crystallinity index (Cacicedo et al., 2016; Esa, Tasirin, & Rahman, 2014; Gallegos et al., 2016; Keshk, 2014).

A carbon source is needed during the fermentation of bacterial cellulose to convert it into cellulose microfibrils. Seddiqi et al. (2021) and Keshk (2014) stated that the composition of culture media and the variation of carbon sources had affected the surface area, porosity properties and production yield of BC membranes. Previously said that utilisation of sucrose as a primary carbon source at certain concentrations can provide higher water holding and porosity value instead of glucose, fructose and glycerol, however lesser in yield and thickness of BC membrane (Heydorn, Lammers, Gottschling, & Dohnt, 2023; Al-Shamary & Al-Darwash, 2013). In addition,

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Ruka, Simon and Dean (2013) claimed that sucrose could produce a consistently high yield and thicker BC membrane, indirectly affecting pore size, porosity and water-holding properties. Still, few investigations focus on utilising different carbon source concentrations and their effect on BC's breathability properties. Hence, this study needs to be investigated to contribute to a new finding on the breathability properties of BC membranes which can be applied as textile materials apart from cotton, rayon and polyester.

2.0 Materials and Method

2.1 Materials
Acetobacter *xylinum* used in this study was purchased from the Malaysian Agriculture Research and Development Institute (MARDI), Malaysia. In this study, carbon sources (sucrose) and ammonium sulphate were bought from BT Science Sdn. Bhd., Malaysia.

2.2 Inoculum Preparation and Culture Conditions
The coconut water-based culture medium for BC membrane formation consisted of the following: sucrose (4%, 8%, 10%), 0.5% of ammonium sulphate and 0.05% of acetic acid until pH4.5. The culture medium was autoclaved at 121°C for 15 minutes to remove any other bacteria. Then, the medium was left until cool to add 10% of inoculum bacteria (Acetobacter xylinum). The bacterial medium was left in a container (12 cm x 17 cm) at a static state for 7 days at room temperature. The samples produced with 40%, 80% and 100% sucrose were abbreviated as BC S4.0, BC S8.0 and BC S10.0, respectively.

2.3 Purification Conditions
The purification process of cellulose membranes was done by immersing the BC sample in an alkaline solution, sodium hydroxide (NaOH), at 1.0% for 24 hours at room temperature. After 24 hours of soaking, the cellulose membranes were washed using tap water and distilled water repeatedly until pH neutral gained. BC membranes were dried using an air dryer at 120°C for 3 hours or until a constant weight was obtained. The weight of the BC membrane before and after the drying process was measured to calculate the production yield of BC membranes.

2.3 Characteristics of BC Membranes
The Production Yield of BC Membranes - The production yield of BC samples was obtained from the dry cellulose weight within the medium volume in a litre (gL-1). The dry cellulose sheets were measured, and the yield of BC production yield was calculated as Equation 1:

$$Yield\ (\%) = \left(\frac{m_0}{C}\right) \times 100 \quad (1)$$

Where, m_0, is the dry weight of cellulose in grams and C, is the weight of carbon source in grams.

Water Holding Capacity (WHC) and Water Release (WR) Analyses - The weight of samples was weighed in wet form (W_0) and dry form (W_1). The samples were dried using an air dryer at 120°C until constant weight. Then, dried BC samples were soaked in deionised water for 48 hours to get the samples' rewetting weight (W_2). The water holding capacity and water absorption rate were calculated using Equation 2 as mentioned in Feng, Ullah, Wang, Sun, Li, Bai, Chen and Li (2015):

$$WHC\ (\%) = \left(\frac{W_0 - W_1}{W_1}\right) \times 100 \quad (2)$$

Next, the WR of cellulose bacteria was determined using the method according to Ul-Islam, Khan and Park (2012). The weight of samples was measured from wet until a constant weight was achieved, continuously at different time intervals. The wet samples were stored under ambient temperature without any pressure attached to them.

Porosity (%) - The dried BC membranes were immersed in deionised water for 24 hours at room temperature. The weight in (W_2) of BC samples was measured using Mancini, Berndt, Sun and Kucuk (2001) method. The percentage porosity of the BC membrane was calculated using Equation 3.

$$Porosity\ (\%) = \left(\frac{W_0 - W_1}{W_0 - W_2}\right) \times 100 \quad (3)$$

BET Surface Area and Pore Size - BET analysis was carried out to calculate the pore size of the various BC membranes using Belsorp II mini, Surface Area and Porosity Analyser (Japan). The samples were placed in a sample cell and heated to 200°C for 3 hours to remove moisture. The samples were cooled down before undergoing BET testing through N_2 adsorption at 77K.

Water Vapour Permeability (WVP) - The water vapour permeability of the BC membrane was measured using the Upright Cup Method principle on the SDL Atlas M21 Water Vapour Permeability Tester in accordance with ASTM E96. First, the BC sample was cut according to the standard sampling method. The cup was filled with 50ml of distilled water before attaching the sample to it sealed. After attaching all the BC samples to the dish and placing them on the dish holder, the WVP tester was run for 30 minutes. The whole cup dish with the sample was weighed. The testing was rerun for the next 5 hours, and the whole cup dish with the sample was weighed.

The water inside the cup dish was not attached to the sample during the weighing process. Then, the weight of the cup dish and sample after 30 minutes test was subtracted from the sample after 5 hours test to obtain the weight change (G). The water vapour permeability rate was calculated based on Jinka, Behrens, Korzeniewski, Singh, Arunachalam, Parameswaran, Coimbatore, Kendall, Wolf and Ramkumar (2013):

$$WVP = \left(\frac{24\times G}{A\times t}\right) \times 100 \quad (4)$$

Where G is a weight change in grams, A is a test area (0.005413m^2), and t is the time taken for the testing to occur (5 hours).

3.0 Results and Discussion

3.1 Production Yield and Thickness of BC Membranes

The cellulose production yield and its thickness were measured and evaluated, as shown in Table 1. The production yield of sample BC S10.0 obtained the highest value (1.553%), while sample BC S4.0 acquired the lowest value (1.374%). Besides, the highest thickness value of the BC membrane sample is BC S10.0, while the lowest is sample BC S4.0. The yield and thickness of the BC membrane increased with the addition of sucrose concentration in the culture media.

Maintaining carbon supply during BC production is crucial. The different concentrations of sucrose ranging from 7% to 19% in Kombucha tea broth affected the production yield of BC membranes, as reported in AL-Kalifawi and Hassan (2014). The addition of sucrose in culture media gave a higher yield of wet BC membranes (63.58g/L). However, it declined after adding too much sucrose (≥130g/L). In this investigation, sucrose at 100g/L (BC S10.0) also was the most suitable amount for BC membrane production due to the highest cellulose yield (%) produced.

Sucrose is a disaccharide, a combination of two monosaccharides; glucose and fructose. The growth of bacteria was limited when sucrose was used as a carbon source, probably due to the long pathway to convert sucrose into UDP-glucose. Some authors claimed that sucrose gave insignificant progress to cellulose production (Heydorn, Lammers, Gottschling, & Dohnt, 2023). However, the variation of sucrose amount differentiated the BC membrane growth, thickness and water-holding capacity properties (AL-Kalifawi et al., 2014; Jagannath, Kalaiselvan, Manjunatha, Raju, & Bawa, 2008). Similarly, the inclination of bacteria secretion in the form of cellulose helps to increase the cellulose membrane thickness and fibrils compactness.

Table 1. The yield and thickness of produced bacterial celluloses.

Sample	BC Yield (%)	Thickness (mm)
BC S4.0	1.374	0.07
BC S8.0	1.526	0.10
BC S10.0	1.553	0.11

3.2 Water Holding Capacity and Porosity Analysis of BC Membranes

Fig. 1 shows the water holding capacity and porosity values of BC membranes produced in the coconut-based medium at different sucrose concentrations. It shows that adding sucrose in media culture during the fermentation process increased the water holding capacity and reduced the porosity value of BC membranes. Based on the result obtained, samples of BC membrane produced at 40g/L sucrose had the lowest water holding capacity (7274.94%) and the highest porosity (99.78%). While BC membrane produced at 80g/L and 100g/L obtained 27.5% and 29.8% (increments) of WHC value higher than sample BC S4.0, respectively. The percentage of porosity for these three different BC membranes was increasing in the range of BC S4.0>BC S8.0>BC S10.0.

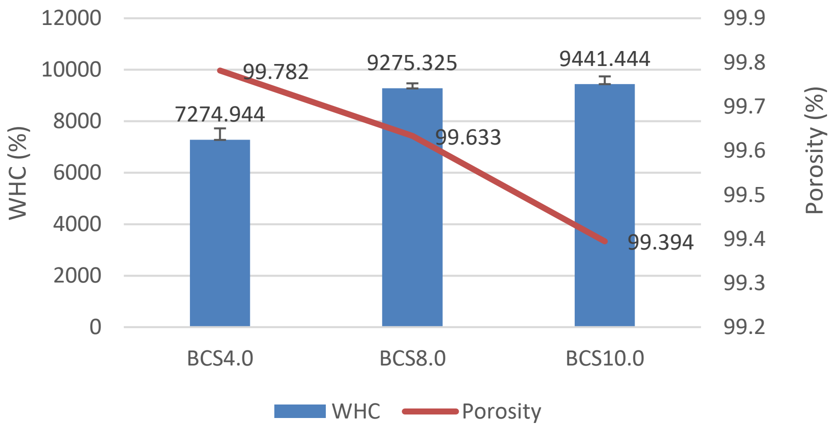

Fig. 1: Results of water holding capacity and porosity of BC membranes

Ul-Islam et al. (2012) investigated the variations of single sugar α-linked glucuronic acid-based oligosaccharide (SSGO) in culture media (0%, 1%, 2% and 4%) on BC membrane water holding and porosity properties. The addition of SSGO in media culture reportedly changed the physical and other properties of the final BC produced. The thickness of BC with high SSGO values was increased, whilst the water holding capacity (%) value was decreased. In this experiment, the thicker BC membranes can hold more water due to the plenty of OH bonding in the microfibrils on the BC compact structure. Besides, the loose arrangement of BC structure probably helps BC to obtain high porosity (%); however, it might have fewer microfibrils with OH bonding which can help to hold more water molecules.

3.3 Water Release Analysis of BC Membranes

Fig. 2 shows the water release (WR) for all samples produced with different concentrations of sucrose. The WR of BC membrane samples was determined for more than 200 hours of evaporation. From the graph, the weight of BC S4.0, BC S8.0 and BC S10.0 were reduced by 21.05%, 15.24% and 14.25%, respectively, from their initial weight at the first 1-hour evaporation. Besides, it was observed that there was 99.23% water reduction for the BC S4.0 sample after 200 hours of evaporation, 97.71% and 96.38% for BC S8.0 and BC S10.0 samples, respectively. The higher weight percentage reduces, resulting in a high water release value. This means that the BC membrane's range of water release produced at different sugar concentrations is BC S4.0 > BC S8.0 > BC S10.0. Even though BC membrane S4.0 obtained a high WR after 200 evaporation, it stated that the average WR for BCS4.0 is lower than BCS10.0, based on Fig. 2. The same goes for the BCS10.0 membrane, and the sample obtained a high value of average WR than the BCS4.0 and BCS8.0 samples. These results of the overall water release differed with the water reduction after 200 hours of evaporation may be due to the undetermined amount of water on the surface of the cellulose membrane that evaporated to the air surrounding. Also, the changes in the water release value of every different BC sample caused due to the variety of sucrose concentrations. As its primary carbon source during BC production, sucrose probably produces a BC sample with different yields and thicknesses, as referred to in Section 3.1, thus affecting its porosity and water release value.

The water molecules that evaporate from the BC membrane to the environment are related to the arrangement of cellulose microfibrils (Shezad, Khan, Khan, & Park, 2010). Besides, the BC membrane's pore size and porosity (%) also influenced the water release trend, as Ul-Islam et al. (2012). As stated in Fig. 1 above, BC S4.0 obtained the highest porosity value, while BC S10.0 obtained the lowest value. This porosity value can explain the water release trend on every different BC membrane sample. In conclusion, the loose arrangement of microfibrils with high porosity helps to evaporate water molecules trapped in the cellulose structure, which also can increase the WR.

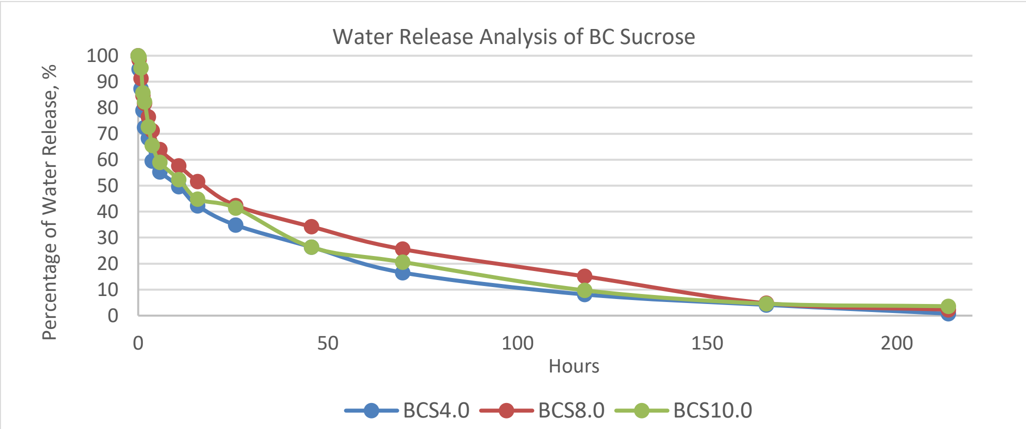

Fig. 2: The water release of BC membranes with different concentrations of sucrose as a carbon source

3.4 Effect of Sucrose Concentration on Pore Size, Pore volume and Surface Area of BC Membranes

Brunauer Emmett Teller analysis was done to measure the materials' pore diameter and surface area. Table 2 shows the total surface area (m2/g), pore volume (cc/g) and average pore diameter (Å) of all fermented BC Sucrose samples. The results of total surface area and pore volume were decreased with the addition of sucrose concentration in the culture medium. The total surface area of BCS4.0 is 21.703 m2/g, decreased to 10.527 m2/g for BCS8.0, while BCS10.0 obtained the lowest (5.0486 m2/g). Similar to pore volume, BCS4.0 obtained the highest (0.086 cc/g), followed by BCS8.0 (0.042 cc/g), while BCS10.0 obtained the lowest pore volume (0.021 cc/g). The mean pore diameter gradually decreased from 16.9 Å to 15.8 Å due to the addition of sucrose concentration from 40g/L, 80g/L to 100g/L. The highest mean pore diameter was found by the BCS4.0 samples, whereas it decreased to 16.025Å for BCS8.0 and 15.814Å for the BCS10.0 sample. The BET analysis's overall results followed a decreasing trend with the increasing sucrose concentration in the culture medium in order of BCS4.0 > BCS8.0 > BCS10.0.

These trends were reported to be similar to Ul-Islam et al. (2012), where the pore size and pore volume decreased with the increased carbon source concentration, SSGO, in the culture medium. The dense and compact BC membrane obtained from the highest concentration of SSGO (4.0%) provided the lowest mean pore diameter (49.48Å) compared to BC with the lowest SSGO source. Similarly, the highest concentration of sucrose (10.0%) also provides the lowest average pore diameter (Table 6.3). As stated by Ul-Islam et al. (2012) and Gao, Li, Bao, Hu, Nian, Feng, An, Li, Xian and Zhang (2019), the porosity and surface area are related to the fibrils arrangement of cellulose membrane, where a closely arranged of cellulose membrane obtained lower porosity and surface area.

The BET results also show that all BC Sucrose samples' surface and internal matrix contained mesopores due to the pore sizes ranging between 2~50nm. This shows that the BC membrane produced with a particular sucrose concentration is mesoporous.

Mesoporous materials, also classified as nanoporous materials, are advantageous in many applications, such as drug delivery and adsorbent, due to their low toxicity and biocompatibility properties. Therefore, it has been found that this mesoporous BC Sucrose sample can be applied in various potential industries.

Table 2. An example of a table

Sample	Total surface area (m^2/g)	Total pore volume (cc/g)	Mean pore diameter (Å)
BC S4.0	21.703	0.085803	16.909
BC S8.0	10.527	0.042174	16.025
BC S10.0	5.0486	0.021342	15.814

3.5 Water Vapour Permeability of BC Membranes

Based on Fig. 3, the water vapour permeability rate of BC membranes decreased with the addition of sucrose concentration during the fermentation process (BCS4.0 > BCS8.0 > BCS10.0). Sample of BC S8.0 and BC S10.0 obtained 16.32% and 33.55% of WVP value less than the BC S4.0 sample. These three different BC samples obtained three different thickness values, which is indirectly related to the water vapour permeability rate. As reported in previous research, the breathability of a membrane or fabric can be related to the membrane structure and thickness. The higher value of water vapour permeability rate indicates a high rate of vapour penetrating in and out through the membranes, which indirectly can produce a breathable membrane (Jinka et al., 2013). Saibuatong and Phisalaphong (2010) reported that BC membrane with the addition of aloe vera gel (30%) obtained a high water vapour transmission rate of up to 2029.5g/m2/day due to the looser fibrils network structure.

In this study, a BC with a loose arrangement of cellulose microfibrils helps to increase the porosity of the membrane, hence will eventually increase the water vapour permeability rate where it is reported that a thicker sample, BCS10.0 with less porosity value (99.4%) and pore diameter (15.814 Å) obtained a decrease in WVP value (2818.99g/m2/day). At the same time, BCS4.0, with the highest porosity value and pore diameter (99.78%, 16.909 Å), can possess a high WVP value of up to 4242.23g/m2/day).

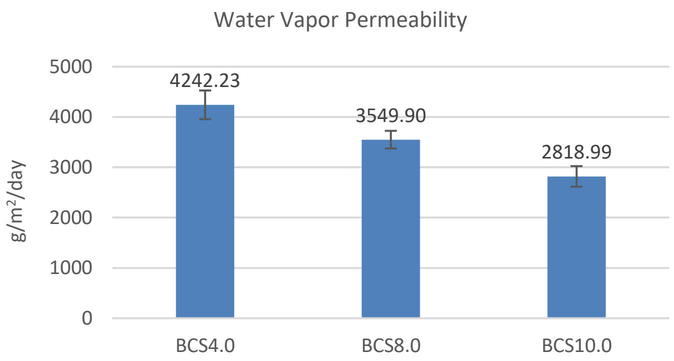

Fig. 3: The water vapour permeability rate of BC membranes produced with different sucrose concentrations

4.0 Conclusion

The modifications of BC membranes by the variations of sucrose concentration in the culture medium during fermentation of Acetobacter xylinum, gram-negative bacteria, had provided certain advantages. The advanced properties of water holding capacity, porosity, water release, water absorption and water vapour permeability rate were obtained through certain samples. The sample of BCS4.0 with lower cellulose yield (1.37g/L), thickness (0.07mm) and WHC value (7274.94%) obtained a high surface area (21.703 m2/g), pore diameter (16.909 Å), porosity (98.78%) and water vapour transmission rate (4242.23 g/m2/day). Besides, the water release and absorption after 200 hours of the BCS4.0 sample were the highest among other samples, which are 99.23% and 80.54%, respectively. However, BCS10.0 samples reported otherwise. A breathable BC membrane can be achieved through the high value of water vapour permeability rate. The BC membrane properties are reported to give good future implication in textile sectors. However, there are some other properties necessary and need to be investigated for BC membrane to be used as commercialized materials in textile market such as comfort ability, durability and biodegradability. Besides, the physical condition of bacterial cellulose itself as a membrane may limit the usage of it as textile material. Therefore, future research on the BC production other than membrane should be investigate for example by using agitation method to produce bacterial cellulose fibre. The continuation of studies is extremely important which can replace the existing natural materials in textile industry, besides to create an environmental-friendly product.

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Paper Contribution to Related Field of Study

The paper contributes to the study field of textile materials, bacterial cellulose membrane production and breathability characterisation.

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