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Original Research Article

Preliminary Study: Antimicrobial Permeation and Release Rates of Biocellulose Wound Dressing Produced Using *Acetobacter Xylinum* 0416 in Pineapple Peel Waste Culture

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Abstract: Modern wound dressings are employed to hasten the healing of damaged skin. Alginate, foam, hydrogel, hydrocolloid and film are contemporary wound dressings. These contemporary wound dressings are effective for treating wounds because they keep moisture in the wound to help the skin of the damaged tissue heal. Another application for biocellulose (BC) is as a material for treating wounds. This is because biocellulose can absorb exudates from skin wounds and has a considerable water-holding capacity. Because pineapple peel waste has a high concentration of glucose and fructose, it can be used as a fermentation medium to make biocellulose. The manufacture of biocellulose also makes use of the bacterium *Acetobacter xylinum* 0416. The biocellulose fermentation process lasts five days at 30 °C in an incubator with static conditions. Utilising antimicrobial agents such as silver nanoparticle compounds, *Moringa oleifera,* or *Melastoma malabathricum* leaf extract will improve the antibacterial capabilities of biocellulose. The growth of *A. xylinum* 0416 indicates an exponential phase at 48 hours, where the number of bacterial cells increases, and the process of transferring 20% of the inoculum to the fermentation medium is carried out. BC fermentation in 6-well plates with 3 ml of pineapple peel extract volume at 30°C and pH 5.15 recorded the highest BC wet weight reading at 1.740g and an average of 1.393g. Low concentrations of silver nanoparticles and *Moringa* leaf extract at 0.02mg/ml, 5mg/l and 0.0125mg/l showed a good effect against the pathogen. The water absorption capacity of the raw BC shows a good absorption value of 144.84.

Keywords: Biocellulose; Pineapple peel; *Acetobacter xylinum* 0416; Wound Dressing; Antimicrobial

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1. Introduction

The human skin covers the entire human body and is the primary protector of the human body parts. Human skin plays an essential role in being a barrier for pathogens and bacteria to enter the human body (Zheng *et al.*, 2020). However, human skin can be injured when applying excessive pressure, such as friction on rough surfaces, being poked with sharp objects and more. When the human skin is injured, it has a mechanism to repair the damaged skin. Therefore, wound dressings are introduced as an initial treatment for wound areas on the skin. Modern wound dressings are often used because they can provide a moist environment for the wound-healing process to occur. In addition, modern wound dressings are also easy to degrade compared to traditional wound dressings. Modern wound dressings, including hydrogel, hydrocolloid, alginate, foam, and film, are often used in clinical treatment. Biocellulose-based (BC) wound dressings are also becoming more popular. This is because widely accessible raw materials can be used to make biocellulose. In addition, biocellulose possesses intriguing properties that protect human skin by enabling it to be used as a temporary skin substitute and as a wound dressing for treating burns. Biocellulose can hold onto moisture and is non-toxic, non-carcinogenic, and quickly biodegradable. As a result, biocellulose is used as a wound dressing since it can absorb exudate from wounded tissue and accelerate the granulation processes (Portela *et al.*, 2019). Biocellulose, a skinny fiber frequently used as synthetic skin, is made in a lab using a bacterium that converts glucose into cellulose. Bacteria from the families *Acetobacter, Rhizobium, Agrobacterium, Aerobacter, Achromobacter, Azetobacter, Salmonella, Escherichia*, and *Sarcina* are frequently utilised to generate biocellulose (Mohammad *et al.*, 2014.).

Depending on the intended use, biocellulose can be produced using static or agitated fermentation (Zhong, 2020). Due to its advantage of having a high water capacity, which makes it simple to absorb and release antimicrobial solutions slowly, biocellulose does not have antibacterial qualities (Savitskaya *et al.*, 2019). Antimicrobial substances like silver solution and Moringa Oleifera leaf extract are applied to create an antimicrobial wound dressing. A prior study found that 15 ppm of the silver solution was needed to produce antibacterial biocellulose. Because concentration values outside of that range cannot effectively kill bacteria and fungi on skin wounds, the concentration of the silver solution that is deemed adequate is in the range of 10 to 40 ppm (Lansdown, 2006). The study aims to determine the optimum ratio of *Moringa Oleifera* or *Melastoma* with silver solution

concentration on biocellulose utilising pineapple peel extract. Furthermore, this study is being conducted to determine the efficacy of the biocellulose wound dressing that has been created.

2. Materials and Methods

This project's research lab work took place at the Universiti Kebangsaan Malaysia (UKM). *Acetobacter xylinum* 0416 bacteria cultured in pineapple peel waste extract as part of the study's biocellulose synthesis procedure. In general, this research follows the plan shown in the attached flowcharts of Figure 1 and Figure 2.

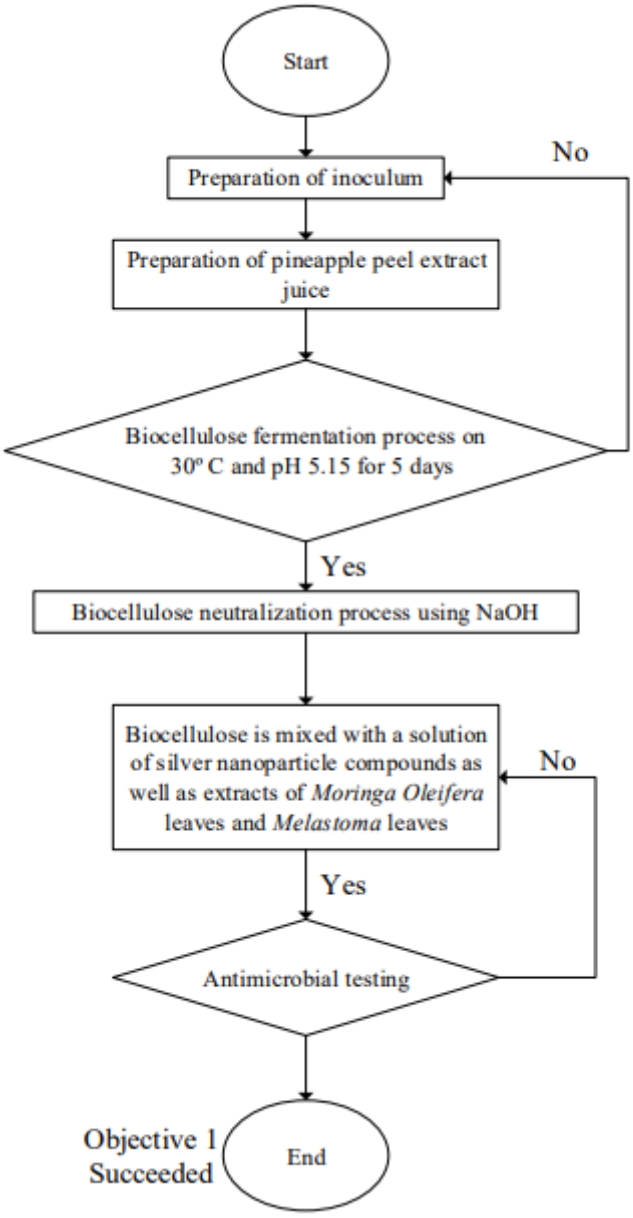

Figure 1. Flow Chart of Experimental Steps for Objective 1

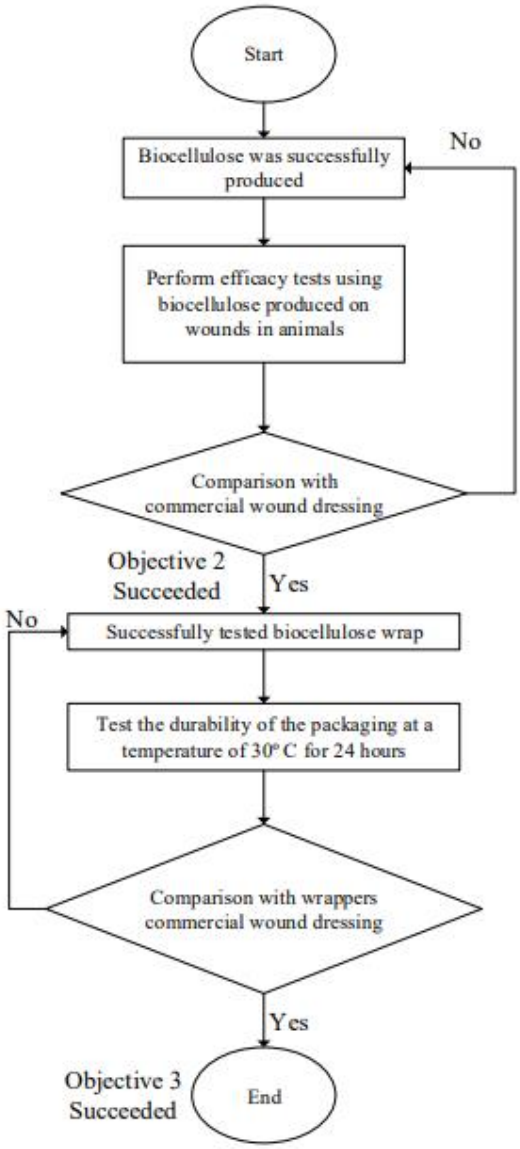

Figure 2. Flow Chart of Experimental Steps for Objective 2 and Objective 3

2.1. Materials

Acetobacter xylinum 0416, Distilled Water, Acetic Acid, Citric Acid, *Moringa Oleifera* Leaf Extract, *Melastoma* Leaf Extract, Yeast Extract, Glucose, Silver Nanoparticle Compound Solution, Sodium Dihydrogen Phosphate, Sodium Hydroxide. Drawtex Wound Dressing, Peptone, Pineapple Peel Residue.

2.2. Apparatus

The apparatuses used were a beaker, incubator, conical flask, filter paper, oven, scale, blender, water bath, 6-well and 96-well plates, pH meter, glass rod and measuring cylinder.

2.3. Preparation of Pineapple Peel Juice Extract

The pineapple peels were purchased from suppliers. Weighed and blended, 300g of the remaining pineapple peel was used. Following grinding, 300 mL of distilled water was combined at a ratio of 1:1 with the leftover pineapple peel (Ch'ng *et al.*, 2020; Sayuti, 2022). The pineapple peel was sterilised in an autoclave at a temperature of 121ºC after being strained using a filter cloth to separate the juice from the peel.

2.4. Preparation of Inoculum

50 mL of distilled water, 1 g of glucose, 0.25 g of peptone, 0.25 g of yeast extract, 0.135 g of disodium phosphate, and 0.057 g of citric acid were combined to create the inoculum. The sample was next sterilised in an autoclave for 15 minutes at a temperature of 121°C (Zahan *et al.*, 2015). After that, a 5 mL Duran bottle was filled with the *Acetobacter xylinum* 0416 stock culture. The created culture media was kept in an incubator for three days at a constant temperature of 30°C. The fermentation process was initiated three days after the inoculum was ready (Zakaria, 2012).

2.5. Biocellulose Fermentation Process

50 mL of pineapple peel juice, 0.25g of yeast, 0.25g of peptone, 0.057g of citric acid, and 0.135g of disodium phosphate were combined in a 100 mL Duran container to start the fermentation process. This mixture is referred to as a fermentation medium. This fermentation medium was autoclaved for 15 minutes at 121°C. The fermentation media was combined with 0.06 mL of inoculum and placed into a 3 mL well plate. The well plates were kept in an incubator for five days under static circumstances at a temperature of 30°C. A biocellulose pellicle developed on the medium's top layer after five days. The biocellulose pellicle was steeped in sodium hydroxide at 90°C for an hour before being rinsed with distilled water until it appeared translucent (Sayuti, 2022).

2.6. Addition of Antimicrobe Agents

Melastoma and *Moringa Oleifera* leaf extracts were created by combining the two leaves into a powder and adding distilled water. The solution of the two leaves was extracted and then filtered via filter paper to produce another solution of the two leaves. Then, the concentration of the silver compound solution was diluted by dissolving the silver compound into distilled water following the desired concentration. The produced biocellulose was next immersed in the produced antimicrobial agent's solution. Using sterile forceps, the

biocellulose was inserted onto an agar plate populated with bacteria like *MRSA, E. coli, and S. aureus*, which are frequently seen in skin wounds. The agar plate was kept upside down and placed in an incubator for one day at a temperature of 37°C. In order to find the best concentration to utilise, the zone of inhibition for each concentration of the antimicrobial solution was compared to the results of the antimicrobial test.

The dry powder of *M. oleifera* leaves was extracted in an aqueous solution for three days. After three days, the aqueous solution of *M. oleifera* was filtered using filter paper. The dilution of the aqueous solution of *M. oleifera* was carried out in a centrifugal tube by adding distilled water to achieve a concentration of 5mg/ml and 0.0125 mg/ml. 6.7 ml of 150 ppm silver nanoparticles solution mixed into 18.3ml distilled water for a 20ppm silver nanoparticles solution dilution rate. Table 1 shows the solution ratio of silver nanoparticles and *M. oleifera*. The ratio between *M. oleifera* and the Ag nanoparticle solution is 1:1, respectively (Muhammad *et al.* 2013).

Table 1 Ratio of silver nanoparticles and *M. oleifera*

Run	Ratio	The Concentration of silver nanoparticles	The Concentration of aqueous *M. oleifera*
1	Control		
2	*M. oleifera*		5 mg/ml
3			0.0125 mg/ml
4	Ag	0.02 mg/ml	
5	Ag+ *M.oleifera*	0.02mg/ml	5mg/ml
6	1: 1	0.02mg/ml	0.0125mg/ml

3. Results

3.1. Analysis of Sugar Content in Pineapple Peel Extract Using DNS Method (3,5-Dinitrosalicylic Acid)

The glucose content in the pineapple peel extract medium is determined based on the standard linear graph of the plotted glucose. A study by Roha et al. in 2012 found that the fructose content of pineapples was higher in cores (2.24%), followed by peel (2.04%) and crown (0.87%). The glucose content was significantly higher in the core (2.56%), followed by the pineapple peel (2.18%) and crown (0.53%). The results showed that the content of glucose (1.30%) and fructose (1.07%) was slightly different compared to the simple sugar percentage analysed by Roha *et al.* (2013), and this range differs due to the ripeness of the pineapple peel used as shown in Table 2.

Table 2 The total content of simple sugar elements of glucose and fructose

Element	Pineapple peel extract	Young coconut water
Glucose (mg/ml)	0.00651 (1.30%)	0.00089 (0.18%)
Fructose (mg/ml)	0.00535 (1.07%)	0.00072 (0.14%)

3.2. Determination of the Optimal Growth Phase of A. xylinum 0416 for Inoculum

The optimal determination of the growth of *A. xylinum* is recorded using a spectrophotometer at an optical density of 600nm. The optical density of 600nm is not detrimental to culture and is suitable for use against bacteria. Figure 2 shows the graph of bacterial growth during culture. The different growth phases start at the lag phase, exponential or logarithm phase (log), stationary phase and death phase. The growth of *A. xylinum* reaches the exponential phase after 36 hours of culture.

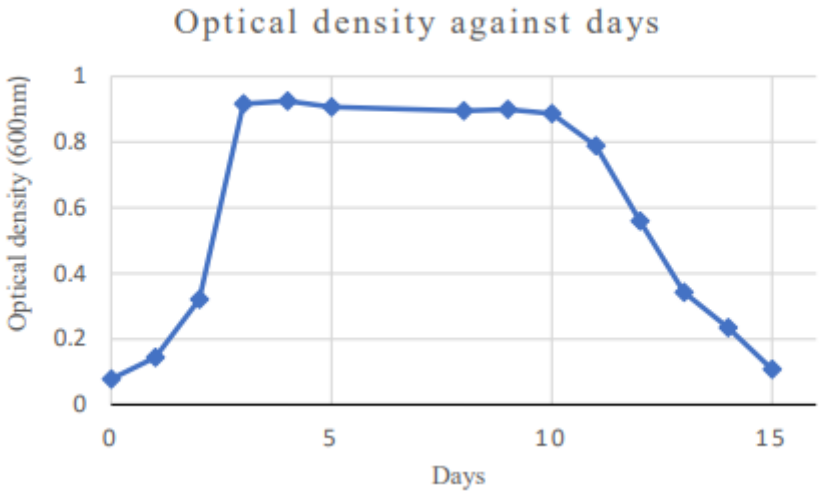

Figure 2. The graph of optical density against days for *A. xylinum* 0416 in HS medium

3.3. Production of BC From the Medium of Pineapple Peel Extract (Josephine Type)

BC fermentation in the pineapple peel extract medium is performed statically in the six-well plate. Figure 5 shows the results of static fermentation of BC. Different media types for BC fermentation are used to compare the higher BC weight yields in the HS medium, young coconut water without additives, young coconut water with additives and pineapple peel extract without additives at optimum pH and temperatures of 5.15 and 30°C, respectively. Figure 5 shows the graph of BC's wet and dry weight at different types of medium fermentation.

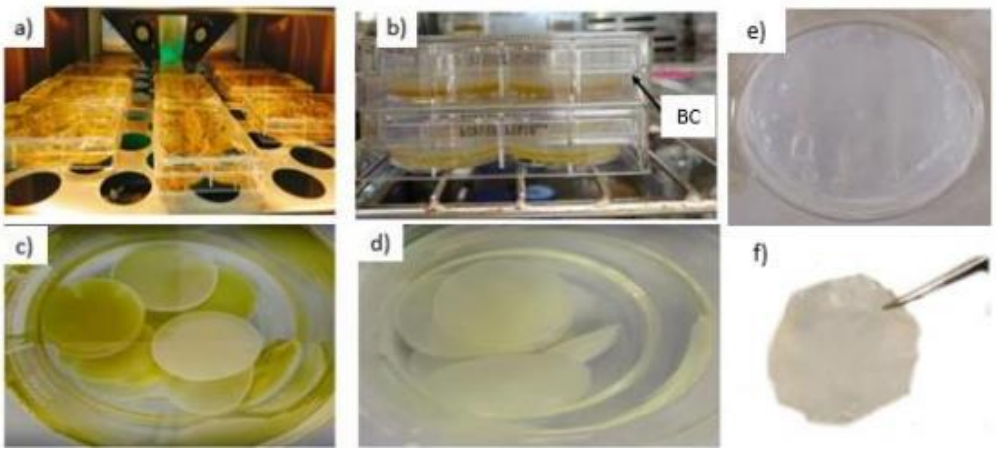

Figure 5. The result of BC fermentation in a static state: (a) Fermentation of BC is carried out in an incubator, (b) BC pellicles form a layer on the surface of the medium, (c) Uncleaned BC pellicles, (d) BC pellicles after cleaning in 1% NaOH, physical morphology(e) wet BC pellicles and (f) dry BC pellicles

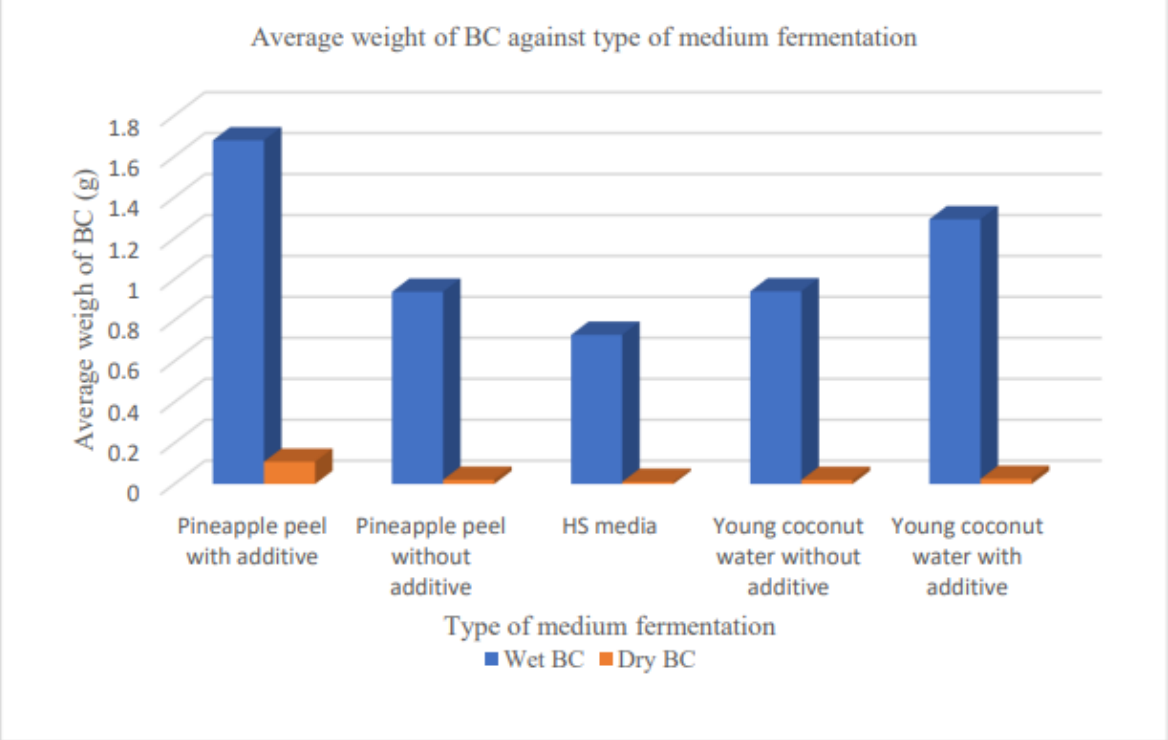

Figure 6. The average weight of BC against the fermentation medium in a static state

Based on Figure 6, pineapple peel extract with an additive mixture recorded the highest average reading of wet weight and dry weight BC. The second-highest average weight was recorded using a young coconut water medium with additives. A comparison of pineapple peel residue and young coconut water without additives was also carried out, where the dry weight of BC produced by the pineapple peel extract medium without additives was

0.09% higher than that of young coconut water. The lowest BC weight is produced in the HS medium.

3.4. FESEM Analysis on BC and Commercial Product Drawtex™

The morphology of the surface structure BC pellicles and the Drawtex™ wound bandage was analysed using FESEM at Crim Laboratory, UKM. The BC surface consists of many intertwined ropes that produce an aggregate structure. Figure 7 indicates the network of thin layers of the BC structure and the BC cross-section at different magnifications. The BC fibrous network is a structured three-dimensional nanofiber, forming a hydrogel sheet with a high surface area and porosity.

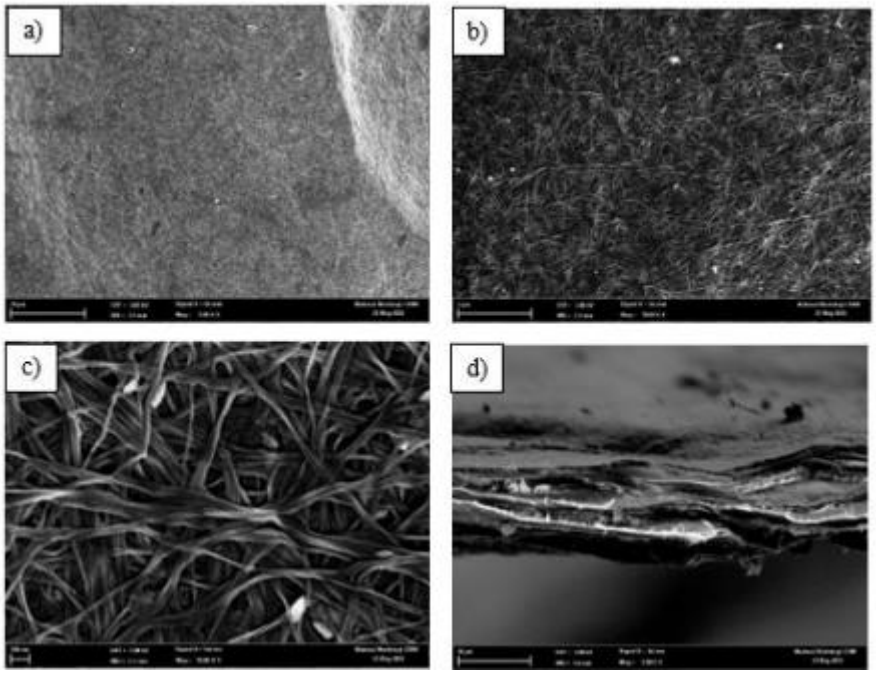

Figure 7. The morphology of the BC structure surface on the magnification (a) 2 K, (b) 10K, and (c) 50K, the morphology of the BC cross-section on the magnification (d) 2K

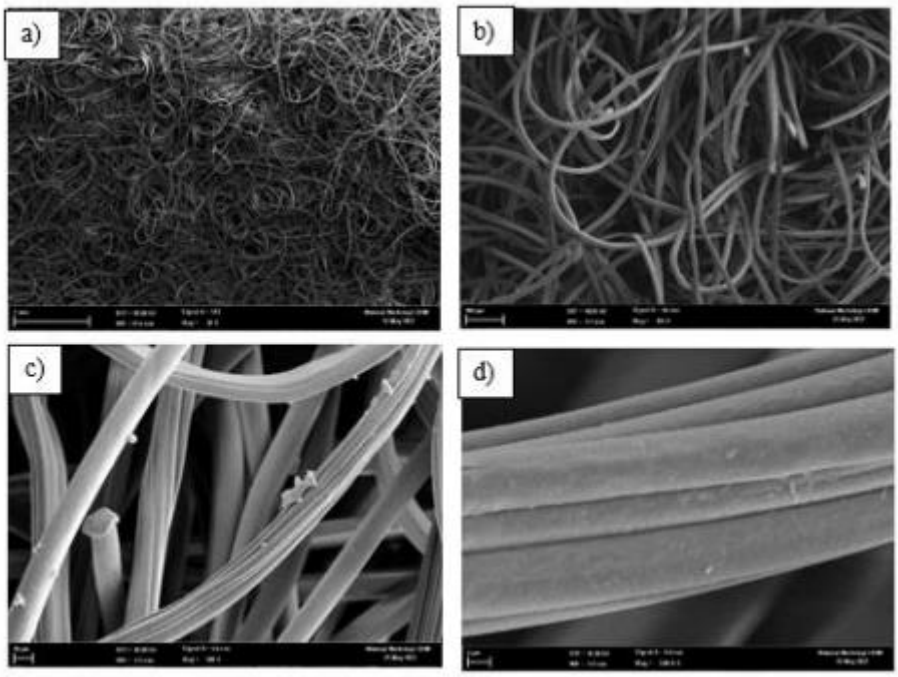

Figure 8. The morphology of the Drawtex surface structure at magnification (a) 20, (b) 100, (c) 500, (d) 3K

3.5. Antibacterial Analysis on BC Pellicle

This study observed antimicrobial effects after incubating petri dish plates for 24 hours at a room temperature of 30°C. Figure 9 shows antimicrobial activity against BC pellicles with different ranges of silver nanoparticles and *M. oleifera* solutions and distilled water used as a control. Antimicrobial activities based on the diameter of the bacterial inhibition zone were formulated in this study. Figure 9 shows an excellent bacterial inhibition zone against a range of 1:1solution of silver nanoparticles and *M. oleifera* at concentrations of run 5. This suggests that low doses of the antimicrobial range have the potential to be applied in the production of BC as wound bandages. Table 3 shows the incubation zone of bacteria against BC.

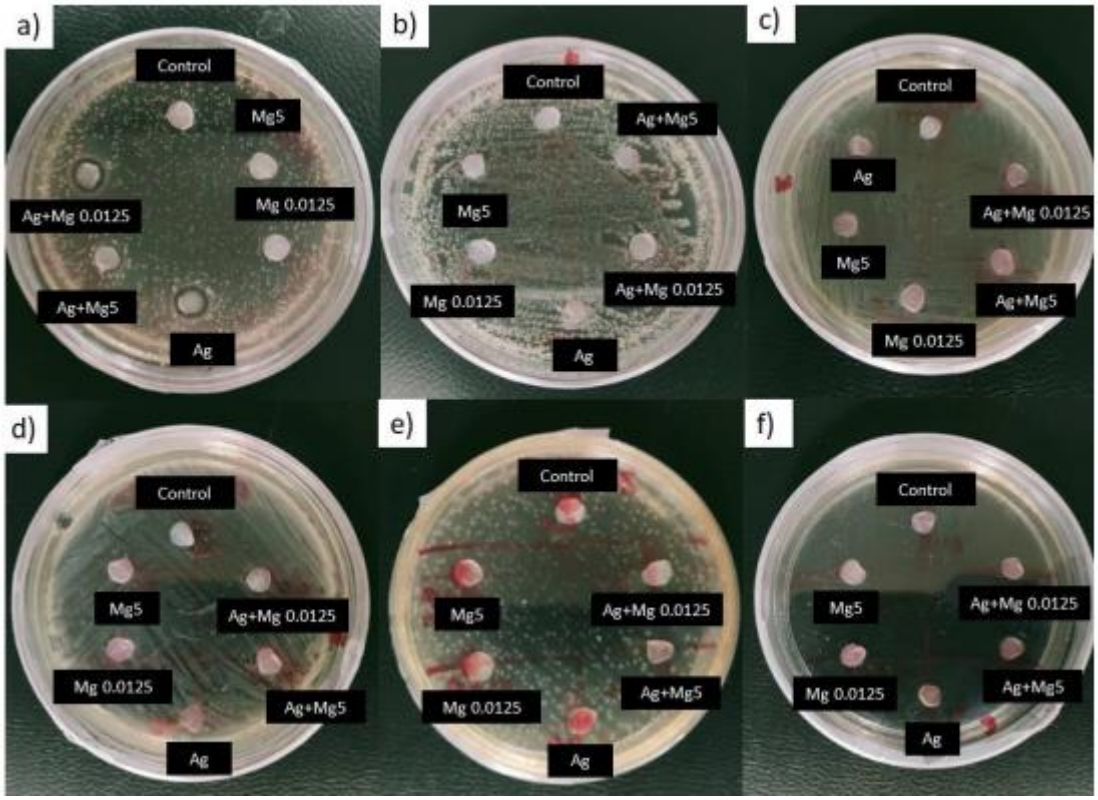

Figure 9. Antimicrobial activity against (a) *A. xylinum* 0416 sp., (b) Bacillus sp., (c) *Klebsiella pneumoniae sp*., (d) *E. coli sp*., (e) MRSA (f) *Staphylococcus aureus sp.*

Table 3. Inhibition zone of bacteria against BC pellicle

Type	*A.xylinum*	*Bacillus*	*MRSA*	*K. pneumonia*	*S. aureus*	*E.coli*
Control	0	0	0	0	0	0
Mg 5 mg/ml	0	0	0	0	0	0
Mg 0.0125mg/ml	0	0	0	0	0	0
Ag 0.02mg/ml	3 mm	0	<1mm	0	0	0
Ag+Mg 5mg/ml	1mm	0	<1mm	0	0	0
Ag+Mg 0.0125mg/ml	2 mm	<1 mm	<1mm	0	0	0

4. Discussion

4.1. Production of BC From the Medium of Pineapple Peel Extract (Josephine Type)

The results of this study show that pineapple peel waste is capable of producing BC with high yield compared to BC in other mediums and has the potential to be used as a medium for the growth of *A. xylinum* and able to replace the use of expensive glucose in the HS medium. In static fermentation, gelatine pellets BC form on the surface of the medium. *A. xylinum* cultured in static fermentation represents a higher genetic stability to continue to

produce BC. Static fermentation is more suitable for producing raw materials that require constant geometry, tensile strength and high water absorption ability, including nata de coco, wound bandage and face mask (Zakaria & Kah Weng, 2012).

The BC fibrous network is a structured three-dimensional nanofiber, forming a hydrogel sheet with a high surface area and porosity. The protofibril of the glucose chain is secreted through the cell wall of the bacteria, forming a network of cellulose ribbons of nanofibril during the synthesis process. The increased concentration of carbon sources leads to higher production of fibrils. The nanofibril network builds a highly porous matrix structure on the surface of the BC. Based on Figure 8, Drawtex^{TM} consists of a network of random fibres of the same size. There are three variations of fibres: smooth striped, fine striped, and large. Robson *et al.* 2012, study of the analysis of Drawtex wound bandages found that the formation of fibre strips with average diameter fibres was smooth, 16.5μm; fine, 21.5μm; and large, 25μm.

Based on Figure 7 and Figure 8, the morphological structures of the BC and Drawtex are different. There are three variations of fibre: smooth striped, fine striped, and large for Drawtex. The BC pellicle pores are neater with strong hydrogel ties, while Drawtex has a random mesh of the same size (Edward-Jones *et al.*, 2014).

The water absorption capacity (WAC) of BC is calculated based on equation (2) by Zakaria and Kah Weng (2012).

$$WAC\ (\%) = \left[\frac{(Wh-Wd)}{Wd}\right] \times 100\% \quad (2)$$

Where,
WAC = Water absorption capacity
Wh = Wet weight BC (g)
Wd = Dry weight BC (g

The water absorption capacity of the raw BC calculated using equation (2) shows a good absorption value of 144.84. Zakaria ***et al.*** (2012) study showed the highest water absorption capacity determined from BC synthesised from a medium containing a mixed carbon source of 20% glucose and 80% fructose with 122.22. BC from the medium with fructose only has a water absorption capacity of 107.15. This suggests that a mixture of fructose and glucose at a specific ratio can produce BC with a strong structure and a good distribution of microbial fibrils, leading to a high surface area for more water absorption. The

typical nano morphology on BC with the size of fibril is 100 times smaller than plant cellulose, giving the incredible water absorption ability for BC Zakaria ***et al.*** (2012).

Edwards-Jones *et al.* (2014) showed that Drawtex could absorb eight times its weight in a liquid over time and offer a 90% reduction in bacteria over 24 hours in the isolation experiment. A 5x5cm piece of Drawtex wound bandage with an average weight of 1.61g shows rapid absorption with a capacity of more than five times its weight at 7.87ml in just 15 minutes. The fluid absorbed into the wound bandage has increased by up to eight times its weight, with 14.03ml of liquid absorbed within 24 hours at room temperature.

4.2. Antibacterial Analysis on BC Pellicle

Antimicrobial activity based on the diameter of the bacterial inhibition zone is formulated in this study in Table 7. The highest bacterial inhibition zone was recorded by Ag concentration at 0.02mg/ml, and the second highest was by mixing silver nanoparticle solution (run 4) with the bacteria *A.xylinum*. In this study, BC for (control sample), *K. Pneumonia, S. aureus* and *E.coli* did not show any antibacterial activity where continuous bacterial growth over Muller-Hinton (MHA) and no inhibition zone were observed based on Figure 9. This study used six different bacterial strains, and a broader analysis of inhibitory zones could be studied compared to Ibrahim *et al.* (2021), who only examined the antibacterial effects of AgNPs and *M.oleifera* on *S. aureus* and *E.coli*. The results of this study of antibacterial effects on BC compared with the study of Ibrahim *et al.* (2021) in terms of concentration of antimicrobial agents showed that as the concentration of Mg increased, antibacterial activity also increased.

The concentration of AgNPs and *M. oleifera* solutions at 0.10mg/ml and 3mg/ml indicate the inhibitory zones of *S. aureus* (16mm) and *E. coli* (13.5mm). Percent reduction in cotton fabric bacteria treated with silver nanoparticles (AgNPs) and *M. oleifera* extract (Mg) reached above 99.9% for both *S. aureus* and*. coli,* indicating that the treated fabric has a higher antibacterial activity by Ibrahim *et al.* (2021). However, there was no antibacterial inhibition zone in this study using *S. aureus* and *E.coli*. This is due to the difference in the film used, which has different antimicrobial agent solution absorption rates on cotton fabrics and BC differences in antimicrobial test techniques through excess diffusion solution for non-uniform soaking BC, resulting in no recorded incubation zone.

The results of this study are also supported by the research of Gothai *et al.* (2016) where the results showed that lower concentrations (0.0125 mg/ml, 0.025 mg/ml, and 0.050 mg/ml) of *M. oleifera* extract showed high proliferative and migratory effects on normal

human dermal fibroblast. Fibroblasts are one of the most common types of cells found in the stroma. Fibroblasts have many functions and organise a basic framework for tissues and organs. The fractional concentration of the antimicrobial solution *M. oleifera* is lower (0.0125 mg/ml), leading to cell growth for faster wound healing than higher concentrations (0.050 mg/ml). Although the rate of cell migration increased at 0.050 mg/ml, the morphology in size and shape has changed, showing evidence of toxicity to skin cells Ibrahim *et al.* 2021 also showed that mixing Ag and low M.oleifera extracts is effective in wound treatment. AgNPs from leaf extracts with a concentration of 0.050mg/ml showed high antibacterial potential against V. cholera and S. aureus with a zone diameter of 21.7±4.7 mm and 21.7±1.5 mm, respectively. This is also explained by researchers Greulich *et al.* (2012), who found that silver ions show different antimicrobial effects depending on the dose tested against the bacterial strain. The differences in physical properties in gram-negative bacteria such as *E. coli* and gram-positive *S. aureus* to silver acetate indicate a low antibacterial effect on S. aureus because gram-positive bacteria have ten times thicker cell walls with multiple layers of murein and teichoic acid protects cells from silver ions Greulich *et al.* (2012).

5. Conclusions

BC is an attractive biopolymer due to its unique and superior properties, which makes it a versatile biomaterial for applications in the fields of biomedical and pharmaceuticals. This study shows thatBC pellicles can be an alternative to commercial and Drawtex wound bandages. The pineapple peel residue has a low acidity and a high sugar concentration. These properties make the pineapple peel extract practical for use as a fermentation medium for the production of BC. The glucose and fructose content of the pineapple peel extract is analysed using the DNS method and optical density at 550nm. The glucose content is 6.51 mM, and fructose is 5.35 mM in pineapple peel extract, which was studied and is suitable for carbon sources for BC synthesis by *A. xylinum*.

The growth of *A. xylinum* in this study showed a change in the lag phase to the exponential phase after 36 hours of culture in the HS medium with an optimal temperature and pH at 30°C and 5.15, respectively. Based on this study, BC production from pineapple peel extract was determined by forming a transparent gel of BC pellicle on the surface of fermentation medium pineapple peel with the highest wet weight recorded at 1.74g and an average of 1.393g. The composition of the solution ratio of Ag nanoparticles (0.1wt%) and *M. oleifera* (0.1wt%) showed a low value of the mixture *M.oleifera* run four and run five recorded the composition of Ag at 0.8wt% and *M. oleifera* at 1.4wt%. The use of *M.oleifera*

also positively impacts the antimicrobial properties of BC. The limitation of this research was that the clinical trial had not been conducted yet.

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