

## Bacterial cellulose from young coconut husk fibre hydrolysate as sustainable biomaterial

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### Abstract

Young coconut husk (YCH) is considered one of the largest agro-industry wastes in Asian countries. This provides opportunities for its further utilisation, such as using YCH as a carbon source in fermentation media. The present work explores the utilisation of YCH hydrolysate as a fermentation medium to produce bacterial cellulose (BC) from *Komagataeibacter xylinus* strain. BC fermentation was conducted for 10 d, and the highest BC weight (776.55 mg/L) was observed at day 6 of fermentation. Reduction in glucose content (from 46.03 to 11.60 mg/mL) and protein concentration (from 3.77 to 0.77 mg/mL) indicated the utilisation of carbon and nitrogen sources for BC production. Water holding capacity (WHC) was assessed, resulting in 25.64 mg water/mg BC, providing the capacity to incorporate antimicrobial agents. Furthermore, the morphology of BC, BCPS, and BCSB was observed using FESEM micrographs, which revealed a dense and random arrangement of cellulose nanofibril networks, providing porosity and biocompatibility. Two antimicrobial agents, potassium sorbate (PS) and sodium benzoate (SB), were incorporated into BC at different immersion periods to assess the antimicrobial ability against *Salmonella Typhi*. Antimicrobial test resulted in larger inhibition for wet BC soaked with PS (BCPS) at 1,000 mg/mL; 48 h with maximum inhibition zone (25 ± 0.35 mm) compared to wet BC soaked in SB (BCSB) at 500 mg/mL, 24 h (16 ± 1.41 mm). Overall, the present work provided a new approach to reduce agro-industrial waste while exploring the potential of developing active food packaging with antimicrobial properties.

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### Introduction

Coconut (*Cocos nucifera* L.) is one of the important crops in Asian countries such as Malaysia, Indonesia, Thailand, and the Philippines that contribute about 90% of the coconut supply globally (Wan-Mohtar *et al.*, 2023). The significant increase in coconut yield, together with poor agricultural waste management, causes the disposal of 1.2 million tonnes of coconut waste, such as husks, shells, and coir, annually (Abd Wahid *et al.*, 2025). Hence, it is essential to redirect and utilise the abundant coconut waste for other applications.

Coconut waste, such as coconut husk, is usually used in biofuels and bioplastic production, while fibres in the husk are utilised in ropes, mats, and

biochar for enhancing soil condition (Abd Wahid *et al.*, 2025; D'Almeida and de Albuquerque, 2025). Recently, the trend of utilising young coconut husk (YCH) in producing bioproducts from fermentation has also received positive attention from researchers (D'Almeida and de Albuquerque, 2025). Since YCH is high in lignocellulose, it is suitable for use as a substrate in fermentation to produce bioproducts such as bioethanol, bacterial cellulose (BC), and lactic acid (Vieira *et al.*, 2024; Abd Wahid *et al.*, 2025; Chakane *et al.*, 2025). However, before YCH can be utilised for fermentation, it needs to be pre-treated with various methods, such as alkaline treatment, enzymatic hydrolysis, or with ionic liquid to break down lignocellulose into fermentable sugar (Din *et al.*, 2021; Anuchi *et al.*, 2022; Vieira *et al.*, 2024).

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The BC is an extracellular fibrous and gelatinous film produced with high efficiency by Gram-negative members of acetic acid bacteria, such as *Komagataeibacter* sp. (Horue *et al.*, 2020). Among the main advantages of BC are the purity and high crystalline structure compared to plant cellulose (*e.g.*, without other polymers such as xylans and lignins), high biocompatibility of BC that allows *ex situ* and *in situ* modifications, high mechanical strength, biodegradability, and high water holding capacity (Horue *et al.*, 2020; Urbina *et al.*, 2021; Infante-Neta *et al.*, 2024).

The BC can also be produced from waste hydrolysate, which provides promising results when conducted in optimum conditions. Some examples of waste hydrolysate used as a fermentation medium include apple waste pulp and stale bread for the valorisation of BC production. This results in BC with different features compared to control HS media, thus promising the ability to modify BC characteristics for specific applications (Esmail *et al.*, 2024). Another study successfully produced BC from sago liquid waste, and further developed BC into food packaging with the addition of carboxymethyl cellulose and glycerol (Yanti *et al.*, 2021). The results stated that modified BC from sago liquid waste demonstrated better physicochemical properties while maintaining the food quality of meat sausages for a longer period.

The high water holding capacity, biocompatibility, and biodegradability of BC offer promising characteristics for development in active food packaging with biodegradable properties. A study suggested that the addition of cerium oxide nanoparticles to BC composite enhanced the BC properties, such as antimicrobial and antioxidant activity, and thus can be developed as food wrapping when tested on bread slices (Mesgari *et al.*, 2024). Another study suggested that oxidised BC with a prodigiosin composite could be developed into active food packaging suitable for foods that require antimicrobial action (Amorim *et al.*, 2023). Chen *et al.* (2024) discussed in their paper that oil-infusion BC improved food-keeping performance of BC by reducing mouldiness and maintaining food freshness for a longer period. This was tested on foods such as strawberries, pork cut, and vegetables, while maintaining the high degradability of BC. Thus, it is proven that developing BC from agro-waste can be used in active packaging with many additional properties compared to conventional fermentation media.

Therefore, the objectives of the present work were to produce BC from YCH hydrolysate, and to determine its potential as a carrier for antimicrobial agents in food packaging applications.

## Materials and methods

### Materials

The YCH was obtained from a market in Selangor, Malaysia, and washed with distilled water before drying at 70°C for 24 h. The dried YCH was then ground and sieved using a pulveriser (GmbH Fritsch Pulverisette 19, Germany) into a 1 - 2 mm size, and stored in an airtight container at room temperature (25°C).

### Inoculum preparation

*Komagataeibacter xylinus* was purchased from Malaysian Agricultural Research and Development Institute (MARDI), Selangor, Malaysia. The inoculum was prepared by inoculating 5 mL of *K. xylinus* strain into 45 mL of sterilised MRS media (Merck, Germany) at a 1:10 ratio (v/v), and incubating at 30°C for 48 h in shaking conditions (150 rpm) (Zaki *et al.*, 2023).

### Production of YCH hydrolysate

YCH at 10% (w/v) was pre-treated with 5% sodium hydroxide (NaOH) at 121°C for 15 min (Din *et al.*, 2021). The solid was then recovered by centrifugation at 10,000 rpm for 20 min (4°C) and washed with distilled water until the pH was reduced to ~7. The pre-treated YCH was then dried and stored in a sealed container at room temperature (Zaki *et al.*, 2023).

A weight of 0.2 g pre-treated YCH was hydrolysed with 10% (w/v) Accellerase® 1500 at 50°C for 24 h in shaking conditions (300 rpm). The solution was then heated at 95°C for 10 min to deactivate the enzyme activities, followed by centrifugation at 10,000 rpm for 10 min to obtain the hydrolysate. Subsequently, the YCH hydrolysate was used for BC production (Din *et al.*, 2021).

### BC production from YCH hydrolysate

Yeast extract (Oxoid, France) was added to YCH hydrolysate at 1% (w/v) concentration before autoclaving (Hirayama HVE 50, USA) at 121°C for 15 min. *K. xylinus* inoculum (10%) was added to the sterile YCH hydrolysate and fermented for 10 d under static fermentation at 30°C. After 10 d, the BC

pellicle layer produced on the surface was harvested, then washed with distilled water. BC was then immersed in 0.1 M NaOH at 80°C for 2 h to remove any bacterial cells or other impurities. The pellicles were then rinsed with distilled water several times. The weight of wet BC was measured first, and dried BC was measured after drying at 60°C overnight (Zaki *et al.*, 2023).

#### *Determination of reducing sugar*

The determination of reducing sugar in fermentation media on days 2, 3, 6, 7, 9, and 10 was determined using the DNS method according to Zaki *et al.* (2023) with slight modifications. Accordingly, 1 g of DNS was dissolved in 20 mL of 2 M NaOH and stirred using a magnetic stirrer (Solution A). Meanwhile, 30 g of sodium potassium tartrate was dissolved in 50 mL of distilled water to produce Solution B. Slowly, 50 mL of Solution B was poured into Solution A and diluted to 100 mL with distilled water.

A volume of 1 µL of fermentation media and 1 µL of DNS solution were mixed in a 10 mL tube. Then, the tube was soaked in a water bath at 100°C for 5 min and left to cool at room temperature. A volume of 10 mL of distilled water was added and mixed well before reading the absorbance at a 540 nm wavelength using a spectrophotometer (InnovaTM 4200 New Brunswick Scientific, USA).

#### *Determination of protein content*

The protein content of the fermentation media was determined according to Yi *et al.* (2022) with slight modifications. A volume of 25 µL of fermentation media was added to 750 µL of Coomassie solution (Bradford) and incubated at 37°C for 10 min. Next, 25 µL of distilled water was used as a negative control to replace 25 µL of the fermentation media. Then, the solution was read at a wavelength of 595 nm using a spectrophotometer. The protein content in the sample was determined by comparing the absorbance value to the standard curve of the Bovine Serum Albumin (BSA).

#### *Determination of pH*

The pH meter of Mettler Toledo, Switzerland was used to determine the pH of the fermentation media.

#### *Water holding capacity (WHC)*

The WHC of BC was calculated using Eq. 1:

WHC =

$$\frac{\text{Bacteria cellulose wet weight} - \text{Bacteria cellulose dry weight}}{\text{Bacteria cellulose dry weight}} \quad (\text{Eq. 1})$$

#### *Morphology analysis*

BC was immersed with potassium sorbate at 1,000 mg/mL (BCPS) (Thong Sheng Food Technology, Penang) and sodium benzoate at 500 mg/mL (BCSB) (BDH Laboratory Supplies, Uganda) for 24 h. Wet BC was pre-dried by freeze-drying before being analysed, while dried BC was dried beforehand by oven drying at 60°C overnight. The morphology analysis of BC, BCPS, and BCSB was conducted using field emission scanning electron microscopy (FESEM) under 15,000× magnification.

#### *Disk impregnation with antimicrobial agent*

Antimicrobial agents were prepared in various concentrations of PS (100, 250, 500, 750, and 1,000 mg/mL) and SB (100, 250, 350, 450, and 500 mg/mL). The dried and wet BC was then soaked in PS and SB of different concentrations for 0, 2, 5, 24, and 48 h. Then, the BC was rinsed to remove excess antimicrobial agent before drying at 60°C for 5 h (Yi *et al.*, 2022).

#### *Antibacterial activity*

The antibacterial activities were determined using the disk diffusion method described by Isopencu *et al.* (2023) with modifications against *Salmonella* Typhi. Three to four colonies of *S. Typhi* were inoculated in the 2 mL nutrient broth and incubated until the growth in the broth was equivalent to the Mac-Farland standard (0.5%). About 1 mL of inoculum was placed on a Muller-Hinton agar plate and spread using a sterile cotton swab to produce an even bacterial lawn. The 6 mm diameter discs of wet and dried BC, BCPS, and BCSB were sterilised beforehand, and were placed aseptically on the nutrient agar containing *S. Typhi*. Ciprofloxacin was used as a positive control, while BC without an antimicrobial agent was used as the negative control. The plates were then incubated at 37°C for 24 h, and the zone of inhibition was measured afterward.

#### *Statistical analysis*

The data obtained were analysed using a One-way analysis of variance (ANOVA) with SPSS software. The data were expressed as mean value ± the standard deviation (SD). Significant differences

were determined using the Tukey's test at the mean confidence level of 95% ( $p < 0.05$ ).

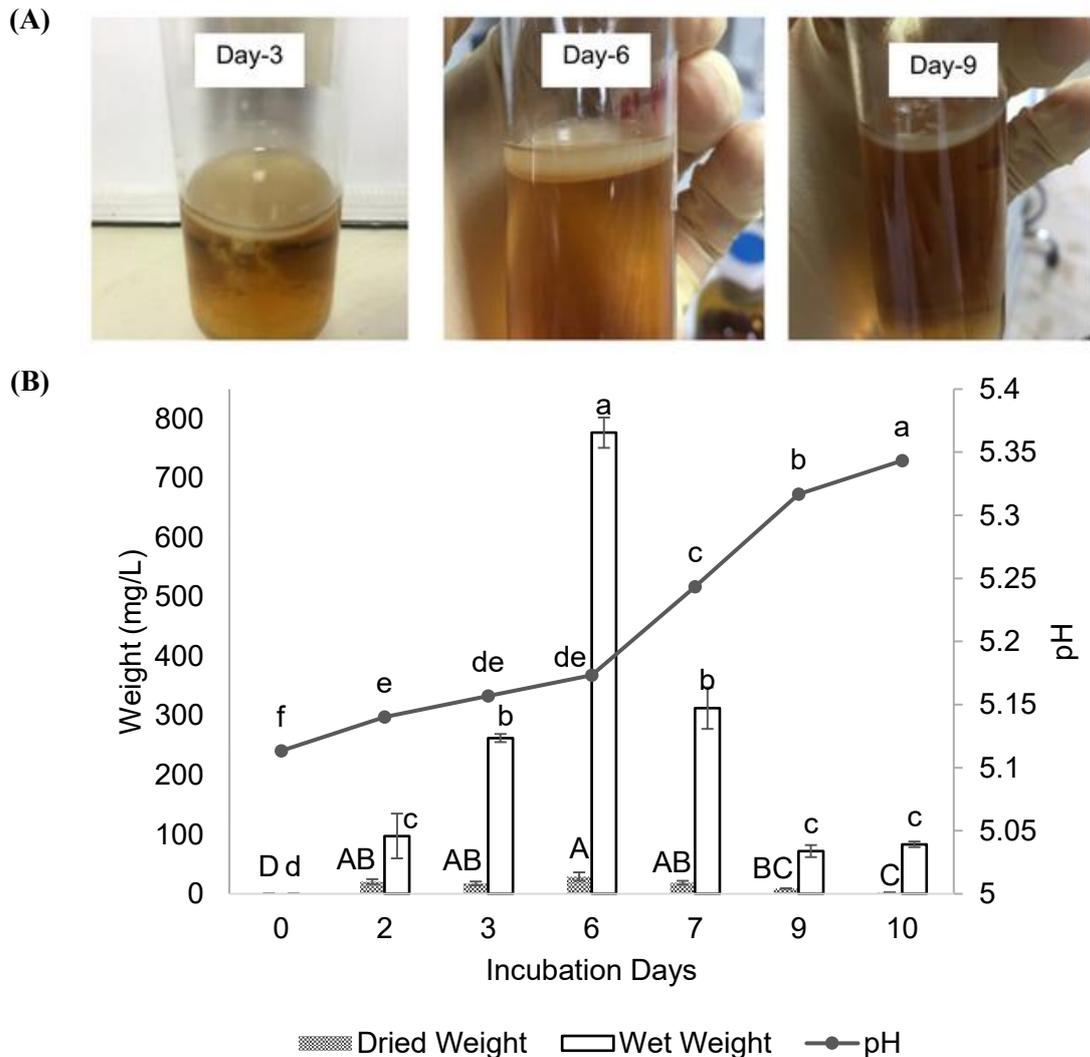
## Results and discussion

### BC production from YCH hydrolysate

The weight of wet and dry BC was measured during a 10-d fermentation process. BC produced on the surface of fermentation media is displayed in Figure 1A. Figure 1B illustrates the weight of wet and dry BC, and pH throughout the 10-d fermentation period.

The highest BC production was determined on day 6, with the highest BC weight recorded at 776.55 mg/L (wet BC) and 29.15 mg/L (dry BC) compared to the other days significantly ( $p < 0.05$ ). However, on day 7, the BC weight decreased to 312.7 mg/L (wet BC) and 18.8 mg/L (dry BC). A decrease in BC

weight on day 9 and 10 indicated the consumption of nutrients that were increasingly limited with a longer fermentation period. A study by Francis *et al.* (2022) stated that BC production from expired drinks was found to be optimum at day 6 to 10 of fermentation. Further fermentation process caused a decrease in BC weight due to acid shock from by-products of fermentation that lowered the pH of the media. Alemam *et al.* (2021) discovered that effective culture parameters play a vital role in BC production, where optimised conditions at day 8 of fermentation demonstrate better yield compared to non-optimised conditions. Another study by Rodrigues *et al.* (2019) discussed how inexpensive nutrients such as molasses, ethanol, corn steep liquor, and ammonium sulphate can provide optimum conditions to produce BC, which increases linearly with surface area, medium depth, and fermentation period.



**Figure 1.** (A): Formation of BC pellicle. (B): Weight of dried and wet BC produced, and pH values of fermentation media throughout 10-d static incubation at 30°C.

### Effects of BC production on pH, protein, and glucose

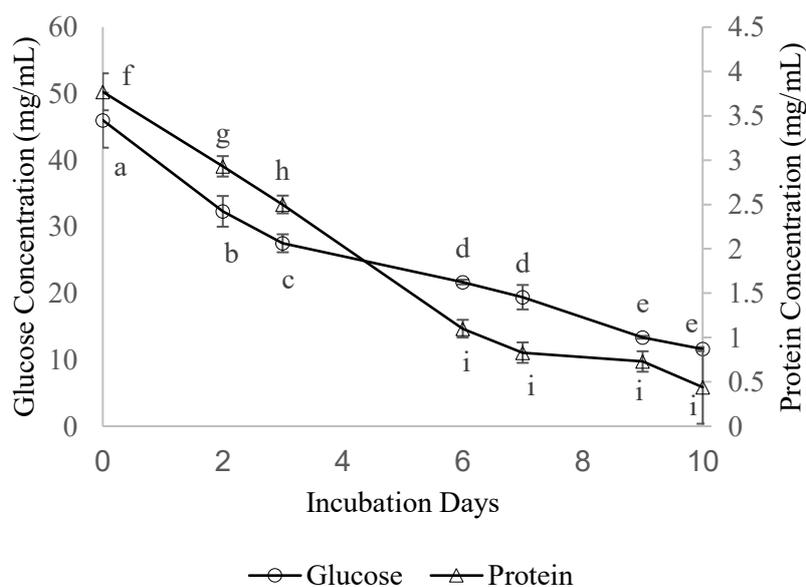
Figure 1B presents the weight of BC (wet and dry) and the pH of the medium throughout 10 d of fermentation. Days 2 to 6 recorded the increase in BC weight as the pH was maintained around 5.1. However, the weight of BC decreased after the pH slightly increased to 5.2 on day 7. The increase in pH was still maintained within a suitable pH range for optimum BC production, which is around pH 4 to 7, as pH outside this range could result in the disruption in BC production (Wang *et al.*, 2019). Usually, the fermentation process experiences a lower pH with the longer fermentation period due to the production of metabolites such as acetic or gluconic acid. As the fermentation occurs, carbon sources will be utilised for BC production and produce organic acids, such as acetic or gluconic acids. This reduces the pH below the optimal pH value, thus impairing BC production (El-Gendi *et al.*, 2022).

Figure 2 illustrates the protein and glucose contents in the fermentation media throughout 10 d of fermentation. The protein content decreased from 3.77 mg/mL on day 0 to 0.77 mg/mL on day 10, indicating the use of nitrogen as energy to repair and build new cell tissue to aid the BC production. The addition of yeast extract as a nitrogen source in fermentation media increased BC production more efficiently. As discussed by Aswini *et al.* (2020), the BC produced using yeast extract yielded 522 mg/mL BC, while only 100 mg/mL BC was produced when using peptone.

The decrease in glucose content from 46.03 mg/mL on day 0 to 11.60 mg/mL on day 10 was also observed. A previous study reported that carbon sources such as glucose and mannitol were reported to be efficient in producing higher BC yield compared to other carbon sources (Alemam *et al.*, 2021). The assimilation of glucose produces UDP-glucose, which will be polymerised into a chain of  $\beta$ -1,4-glucan, thus forming ribbons of cellulose chains (Aswini *et al.*, 2020). A study by Din *et al.* (2021) presented findings that YCH hydrolysate produces 89.49% sugar recovery with 20.05 g/L glucose content after being treated with 10% (w/v) enzyme loading for 24 h. This suggests that high glucose content in YCH hydrolysate can provide a good carbon source for BC production. This is supported by Gorgieva and Trček (2019), who stated that glucose is the best energy source for BC production, as glucose is used as a cellulose precursor.

Thus, glucose obtained from the hydrolysate media of YCH in the present work proves that agricultural waste, *i.e.*, YCH, has the potential to be used as an alternative carbon source in the production of BC.

The BC production from YCH hydrolysate was demonstrated to produce the highest yield on day 6 of fermentation. The decrease in glucose and nitrogen contents proves the use of carbon and nitrogen sources for BC formation, indicating a success in BC production from YCH hydrolysate.



**Figure 2.** Concentrations of glucose and protein in fermentation media throughout 10-d static incubation at 30°C.

### Water holding capacity (WHC) of BC

BC is widely documented to have high WHC, as the structure of a highly porous network allows more water molecules to be trapped with hydrogen bonds (Potivara and Phisalaphong, 2019; Infante-Neta *et al.*, 2024; Sozcu *et al.*, 2024). From Figure 1B, the weights of wet BC (776.55 mg/L) and dry BC (29.15 mg/L) were used to calculate the WHC. The WHC of BC calculated was 25.64 mg of water/mg BC. This indicates that BC produced in the present work can expand about 25 times in weight when absorbing water. The larger the surface area and pore size, the greater the amount of water that can be absorbed and trapped in the BC matrix. The three-dimensional fibril network formed by the entangled nanofibril contributes to the absorption and retention properties of water in BC (Corzo Salinas *et al.*, 2021). The trapped water molecule is held strongly by the hydroxyl group in the cellulose chain, providing the hydrophilic characteristic of BC that allows it to hold water more than 100 times its dried weight, even stronger than plant-produced cellulose. A study by Esmail *et al.* (2024) discovered that BC produced from apple pulp and bread hydrolysate has a WHC value of  $87 \pm 2$  g water/g BC and  $75 \pm 8$  g water/g BC, respectively. Another study by Amorim *et al.* (2023) using a tea mixture and apple waste produces BC with high WHC of  $148.20 \pm 0.87$  and  $115.50 \pm 2.61$  g/g, respectively. This suggests that BC production from other waste materials can also produce high-quality BC that can retain more water compared to using commercial fermentation medium.

Furthermore, the WHC of BC depends on the size and arrangement of the fibrils and the surface area of BC. *In situ* modification during BC production might change the WHC, as reported by Szymańska *et al.* (2022), where the addition of silicone polyether surfactant in HS medium managed to increase WHC by 1.4 times compared to the control condition. A study by Corzo Salinas *et al.* (2021) discussed that the arrangement of the fibril network was influenced by the incubation period. Longer incubation days cause the membranes in BC to become less shafted and the structure of the nanofibril network to be denser, which results in less WHC.

### Morphological analysis of BC from YCH hydrolysate

Table 1 displays wet and dry BC, BCPS, and BCSB surface micrographs at 15,000 $\times$  magnification with BC nanofibril diameter distribution. The results revealed that the structure of all BC in both wet and

dry conditions consisted of three-dimensional fibrils stacked in random order. Table 2 illustrates the average diameter of BC nanofibrils, with the largest diameter being dry BCPS ( $53.57 \pm 12.16$  nm), and the smallest diameter was dry BC ( $41.80 \pm 11.11$  nm). The diameter of BC nanofibril recorded in the present work was found to be in accordance with the average BC nanofibril diameter reported by Cazón and Vázquez (2021), which is around 40 to 70 nm.

The dry BCs membrane structure shows larger pores compared to wet BCs because wet BCs fibrils form a dense reticulated structure and are stabilised by extensive hydrogen bonding (Vasconcellos and Farinas, 2018). Morphological changes in BCSB indicated the formation of crystals in the membrane. The images obtained in the present work align with the surface of the BC-based hydrogel membrane supplemented with silver nanoparticles, as reported by Mokhtarom and Lazim (2018). The authors explained that during the absorption activity, BC releases energy and heat through the transition phase of solids by recrystallising the fibrils into a different order. The curved fibrils on wet BC in Table 1 are likely to indicate that cellulose fibrils have expanded during the absorption of antimicrobial agents (Gedarawatte *et al.*, 2021).

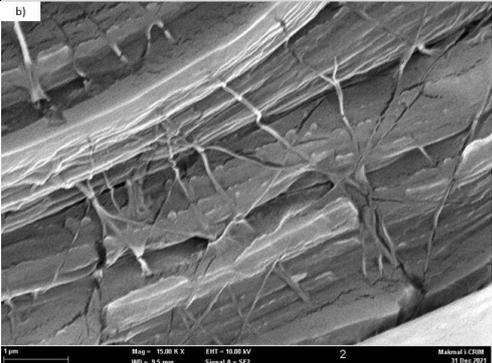
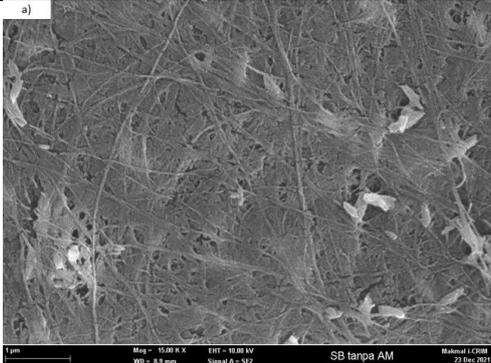
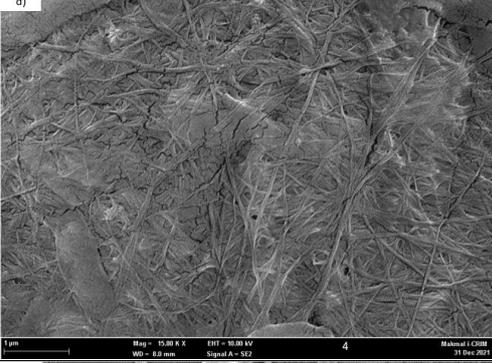
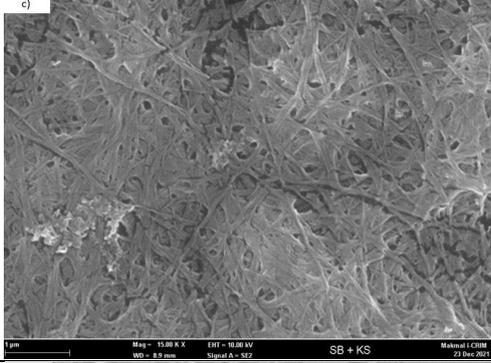
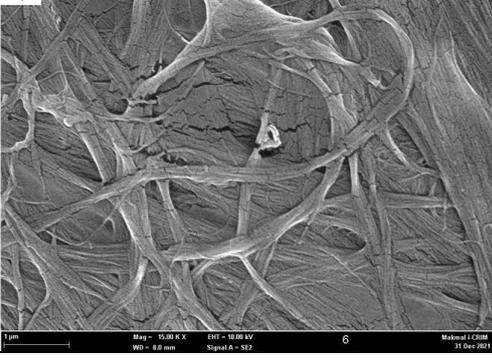
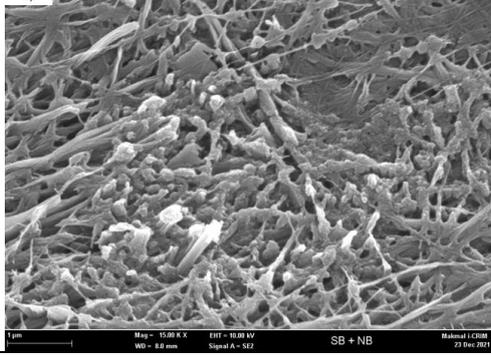
In addition, BC produced under different incubation conditions, such as agitated and static, results in different morphologies of BC. BC produced in static conditions resulted in a uniform film that neatly thickened over time, while under agitated conditions, it formed BC pellets in a spherical shape that tangled and clumped into irregular masses (Cazón and Vázquez, 2021).

### Antimicrobial activity of BC

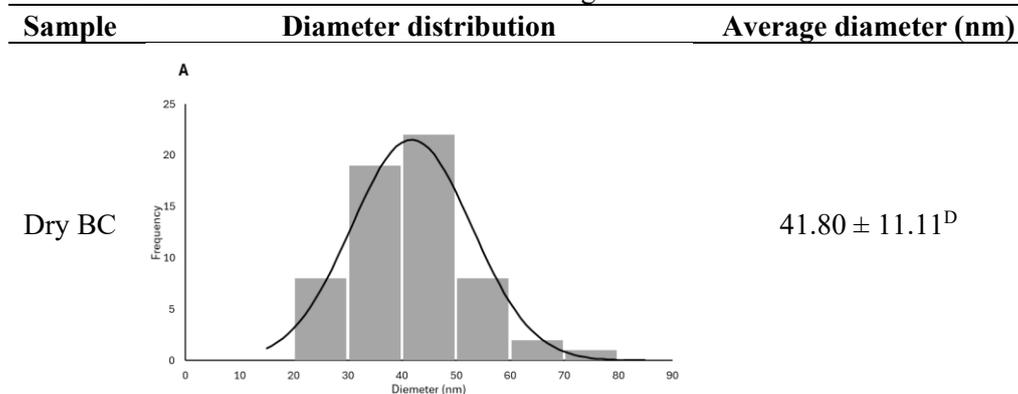
BC has the potential to become an active food packaging by incorporating an active ingredient through several techniques, such as immersion, spray, wrapping, and others. The present work used immersion techniques at room temperature using different concentrations of PS (100, 250, 500, 750, and 1,000 mg/mL) and SB (100, 250, 350, 450, and 500 mg/mL) for 2, 5, 24, and 48 h to compare the effectiveness of antimicrobial activity. Note that 1,000 mg/mL SB was not used as the maximum concentration since 1,000 mg/mL exceeded the solubility value of SB in water at room temperature, and achieving complete solubility was not possible.

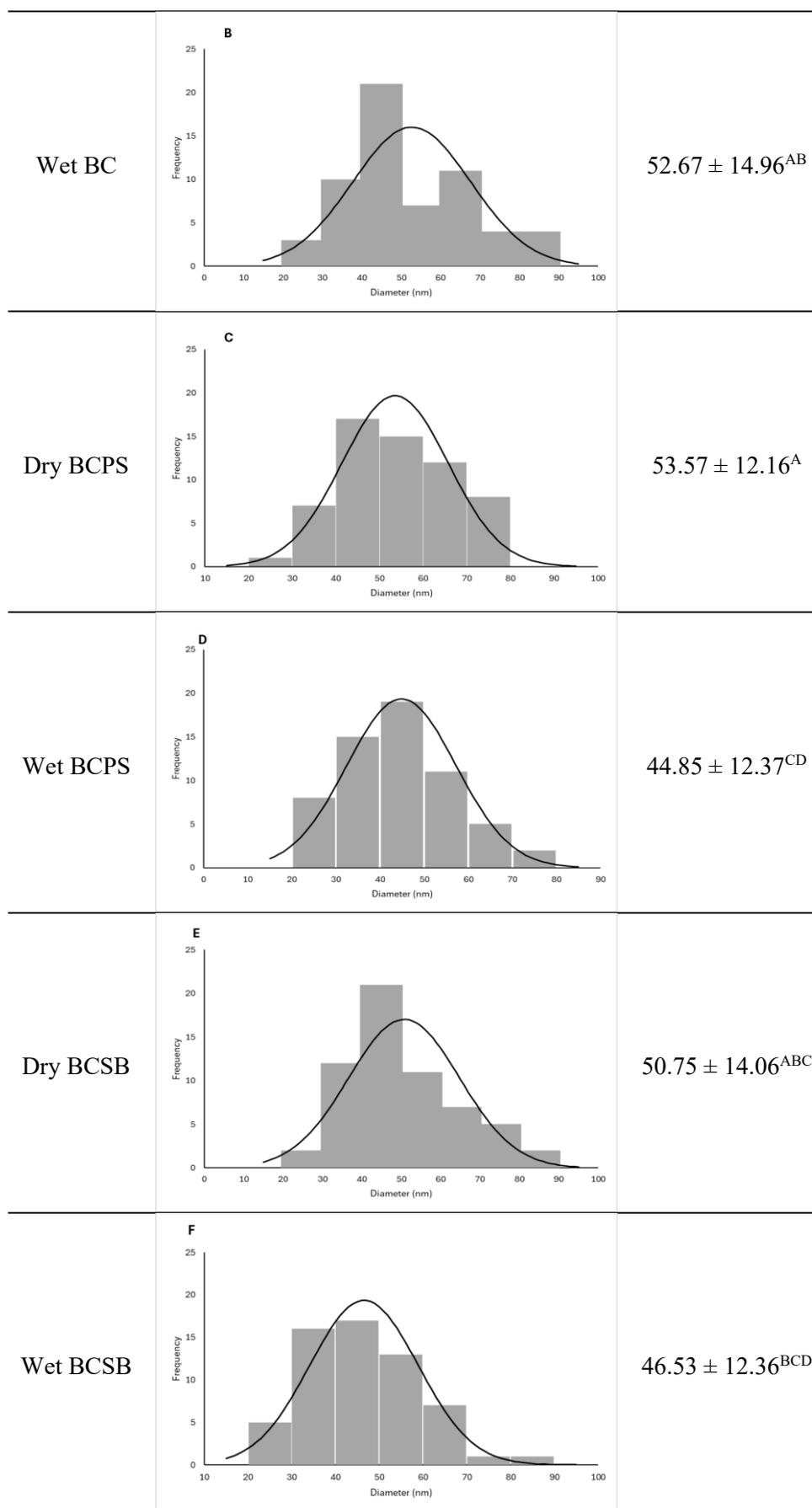
Table 3 presents the diameter of the inhibition zone after wet and dry BC were immersed in various

**Table 1.** Micrograph of wet and dry BC surfaces at 15× magnification in different conditions.

	Wet BC	Dry BC
Without antimicrobial agents		
BCPS		
BCSB		

**Table 2.** Diameter distribution and average diameter of BC nanofibrils.





**Table 3.** Inhibition zone against *S. Typhi* after soaking wet and dry BC in various concentrations of PS and SB for 0, 2, 5, 24, and 48 h.

Soaking time (h)	BC	Ciprofloxacin, 10 mg (Positive control)	Diameter inhibition zone (mm)									
			Concentration of PS solution (mg/mL)					Concentration of SB solution (mg/mL)				
			100	250	500	750	1000	100	250	350	450	500
0 (Negative control)	Wet		0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	Dried		0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
2	Wet		0 ± 0	13.25 ± 0.35 <sup>GH</sup>	14.5 ± 0.71 <sup>FGH</sup>	17.2 ± 0.35 <sup>EF</sup>	19.5 ± 0.71 <sup>CDE</sup>	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	Dried		0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
5	Wet		0 ± 0	14.5 ± 0.71 <sup>FGH</sup>	16.25 ± 0.35 <sup>EFG</sup>	19 ± 0 <sup>DE</sup>	21 ± 0 <sup>CD</sup>	0 ± 0	0 ± 0	0 ± 0	11.25 ± 0.35 <sup>c</sup>	12.75 ± 1.06 <sup>bc</sup>
	Dried	40 ± 0 <sup>Aa</sup>	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
24	Wet		0 ± 0	15.25 ± 0.35 <sup>FG</sup>	17.25 ± 0.35 <sup>EF</sup>	19.2 ± 1.06 <sup>DE</sup>	23 ± 1.06 <sup>BC</sup>	0 ± 0	0 ± 0	12.5 ± 0.71 <sup>c</sup>	13.5 ± 0.71 <sup>bc</sup>	16 ± 1.41 <sup>b</sup>
	Dried		0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
48	Wet		0 ± 0	16.0 ± 0 <sup>EFG</sup>	19 ± 0.71 <sup>DE</sup>	21 ± 0.71 <sup>CD</sup>	25 ± 0.35 <sup>B</sup>	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	Dried		0 ± 0	5.5 ± 2.12 <sup>J</sup>	9 ± 1.42 <sup>IJ</sup>	11.0 ± 1.42 <sup>HI</sup>	16 ± 1.42 <sup>EFG</sup>	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

concentrations of PS and SB at different soaking times (0, 2, 5, 24, and 48 h). As expected, ciprofloxacin, which was used as a positive control, produced a uniform inhibition zone of 40 mm, and BC without any antimicrobial agent did not demonstrate any inhibitory zone. Dry BCPS only presented an inhibition zone on *S. Typhi* after 48 h of immersion. Results revealed that wet BCPS had a better antimicrobial effect on *S. Typhi* compared to dried BCPS. Wet BCPS showed a positive reaction at every immersion hour with a minimum inhibition zone of  $13.25 \pm 0.35$  mm in 2 h (250 mg/mL), while the maximum inhibition zone of  $25 \pm 0.35$  mm was shown after 48 h (1,000 mg/mL). For dried BCPS, the antimicrobial effect only occurred at 48 h for concentrations 250 mg/mL ( $5.5 \pm 2.12$  mm), 500 mg/mL ( $9 \pm 1.42$  mm), 750 mg/mL ( $11 \pm 1.42$  mm), and 1,000 mg/mL ( $16 \pm 1.42$  mm). A smaller inhibition zone was obtained for dried BCPS due to the structure of dried BC with a fragile fibril structure and less surface area from the shrinkage of the fibril during the drying process in the oven, which limited antimicrobial agent absorption (Bodea *et al.*, 2021).

Based on the statistical analysis conducted, it was observed that there was a significant difference ( $p < 0.05$ ) between the concentration of PS at 250 and 1,000 mg/mL, while 500 and 750 mg/mL presented no significant difference ( $p > 0.05$ ). It can be concluded that the longer the wet BC was immersed in a higher concentration of PS, the greater the inhibition zone produced. A study by Santiesteban-López *et al.* (2019) discussed their findings that PS at 7,000 ppm can cause sublethal damage to bacterial cells, such as a collapsed cell wall and dense DNA in *E. coli*, while SB with the same concentration barely resulted in any damage to bacterial cells. PS was also proven to prevent the growth of yeast in food, as discussed by Awaad *et al.* (2023), where PS alone or mixed with chitosan was proven to inhibit the growth of yeast in cheese more efficiently compared to control conditions.

The positive effects of the antimicrobial characteristics of BC using SB in the present work were found only in wet BCSB. Results by SB suggested that there was an inhibition zone by wet BCSB for 24 h in 350, 450, and 500 mg/mL;  $12.5 \pm 0.71$ ,  $13.5 \pm 0.71$ , and  $16 \pm 1.41$  mm, respectively, while at other concentrations and immersion hours demonstrated a negative reaction to *S. Typhi* with no significant difference ( $p > 0.05$ ) of all the inhibition zones.

Based on the results obtained, wet and dry BC displayed antimicrobial activity after immersion in PS and SB. This could have been due to the high WHC of BC, which can effectively absorb antimicrobial agents. Thus, BC from YCH hydrolysate has the potential to be used as a carrier for applications as an active food wrapper. However, the present work produced limited results on antimicrobial activities as it was tested against only one bacterial strain, *S. Typhi*. Further studies can be planned on exploring the antimicrobial action of BC produced from YCH hydrolysate on different bacterial strains and a possible yeast strain.

## Conclusion

The present work successfully produced BC from YCH hydrolysate, achieving promising BC morphology results comparable to previous studies. It was also observed that BC incorporated with PS could inhibit the growth of *S. Typhi* in wet and dried forms better than SB, suggesting its antimicrobial activities. However, there were some limitations in the present work, such as the antimicrobial activity was only assessed on one bacterial strain. This would provide opportunity for further exploration of BC production from YCH for food applications, such as testing the antimicrobial activities on more bacterial strains, and exploring natural antimicrobial agents. The optimisation of BC fermentation can also be explored by adjusting different parameters, such as pH, glucose, and protein contents for future upscaling objectives.

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## References

- Abd Wahid, M. Z. A., Abidin, M. N. Z., Mokmin, A. I. M. and Sayuti, M. S. 2025. Preliminary insights into sustainable management of coconut waste from smallholders in Bagan Datuk, Malaysia: Sustainable management of coconut waste from smallholders. *Bioresources and Environment* 3(1): 57-68.
- Alemam, A. M., Shaheen, T. I., Hassan, S. E.-D., Desouky, S. E. and El-Gamal, M. S. 2021.

- Production enhancement of bacterial cellulose nanofiber using local *Komagataeibacter xylinus* SB3.1 under static conditions. *Egyptian Journal of Chemistry* 64(4): 2213-2221.
- Amorim, L. F. A., Li, L., Gomes, A. P., Fangueiro, R. and Gouveia, I. C. 2023. Sustainable bacterial cellulose production by low cost feedstock: Evaluation of apple and tea by-products as alternative sources of nutrients. *Cellulose* 30(9): 5589-5606.
- Anuchi, S., Campbell, K. and Hallett, J. 2022. Effective pretreatment of lignin-rich coconut wastes using a low-cost ionic liquid. *Scientific Reports* 12: 6108.
- Aswini, K., Gopal, N. O. and Uthandi, S. 2020. Optimized culture conditions for bacterial cellulose production by *Acetobacter senegalensis* MA1. *BMC Biotechnology* 20(1): 46.
- Awaad, S. S., Sherief, M. A., Mousa, S. M., Orabi, A. and Abdel-Salam, A. B. 2023. A comparative study on the antifungal effect of potassium sorbate, chitosan, and nano-chitosan against *Rhodotorula mucilaginosa* and *Candida albicans* in skim milk acid-coagulated (Karish) cheese. *Veterinary World* 16(9): 1991.
- Bodea, I. M., Beteg, F. I., Pop, C. R., David, A. P., Dudescu, M. C., Vilău, C., ... and Cătușescu, G. M. 2021. Optimization of moist and oven-dried bacterial cellulose production for functional properties. *Polymers* 13: 3821.
- Cazón, P. and Vázquez, M. 2021. Bacterial cellulose as a biodegradable food packaging material: A review. *Food Hydrocolloids* 113: 106530.
- Chakane, A., Nakamura, Y. and Asada, C. 2025. Total utilization of components contained in coconut husk by microwave-assisted thermal hydrolysis and deep eutectic solvent treatment. *Waste and Biomass Valorization* 16(4): 1899-1910.
- Chen, G., Wang, K., Chen, P., Cai, D., Shao, Y., Xia, R., ... and Yu, Y. 2024. Fully biodegradable packaging films for fresh food storage based on oil-infused bacterial cellulose. *Advanced Science* 11(23): 2400826.
- Corzo Salinas, D. R., Sordelli, A., Martínez, L. A., Villoldo, G., Bernal, C., Pérez, M. S., ... and Foresti, M. L. 2021. Production of bacterial cellulose tubes for biomedical applications: Analysis of the effect of fermentation time on selected properties. *International Journal of Biological Macromolecules* 189: 1-10.
- D'Almeida, A. P. and de Albuquerque, T. L. 2025. Coconut husk valorization: Innovations in bioproducts and environmental sustainability. *Biomass Conversion and Biorefinery* 15(9): 13015-13035.
- Din, N. A. S., Lim, S. J., Maskat, M. and Aqilah, N. 2021. Bioconversion of coconut husk fibre through biorefinery process of alkaline pretreatment and enzymatic hydrolysis. *Biomass Conversion and Biorefinery* 11: 815-826.
- El-Gendi, H., Taha, T. H., Ray, J. B. and Saleh, A. K. 2022. Recent advances in bacterial cellulose: A low-cost effective production media, optimization strategies and applications. *Cellulose* 29(14): 7495-7533.
- Esmail, A., Morais, M., Yilmazer, U., Neves, L. and Freitas, F. 2024. Bacterial cellulose production through the valorization of waste apple pulp and stale bread. *Biomass Conversion and Biorefinery* 15: 14587-14602.
- Francis, F., Zaki, Z. A. and Zaini, N. A. M. 2022. Production of bacterial cellulose from expired cordial beverages and their potential use as anti-bright cellulose gel. *Sains Malaysiana* 51(5): 1399-1410.
- Gedarawatte, S. T. G., Ravensdale, J. T., Al-Salami, H., Dykes, G. A. and Coorey, R. 2021. Antimicrobial efficacy of nisin-loaded bacterial cellulose nanocrystals against selected meat spoilage lactic acid bacteria. *Carbohydrate Polymers* 251: 117096.
- Gorgieva, S. and Trček, J. 2019. Bacterial cellulose: Production, modification and perspectives in biomedical applications. *Nanomaterials* 9(10): 1352.
- Horue, M., Cacicedo, M. L., Fernandez, M. A., Rodenak-Kladniew, B., Torres Sánchez, R. M. and Castro, G. R. 2020. Antimicrobial activities of bacterial cellulose – Silver montmorillonite nanocomposites for wound healing. *Materials Science and Engineering C* 116: 111152.
- Infante-Neta, A. A., D'Almeida, A. P. and Albuquerque, T. L. 2024. Bacterial cellulose in food packaging: A bibliometric analysis and review of sustainable innovations and prospects. *Processes* 12(9): 1975.

- Isopencu, G., Deleanu, I., Busuioc, C., Oprea, O., Surdu, V.-A., Bacalum, M., ... and Stoica-Guzun, A. 2023. Bacterial cellulose—Carboxymethylcellulose composite loaded with turmeric extract for antimicrobial wound dressing applications. *International Journal of Molecular Sciences* 24(2): 1719.
- Mesgari, M., Matin, M. M., Goharshadi, E. K. and Mashreghi, M. 2024. Biogenesis of bacterial cellulose/xanthan/CeO<sub>2</sub>NPs composite films for active food packaging. *International Journal of Biological Macromolecules* 273: 133091.
- Mokhtarom, M. and Lazim, A. M. 2018. Study on bacterial cellulose (BC)-based hydrogels and the effect of combining them with silver nanoparticles as antibacterial agents. *Malaysian Applied Biology* 47(6): 53-60.
- Potivara, K. and Phisalaphong, M. 2019. Development and characterization of bacterial cellulose reinforced with natural rubber. *Materials* 12(14): 2323.
- Rodrigues, A. C., Fontão, A. I., Coelho, A., Leal, M., da Silva, F. A. G. S., Wan, Y., ... and Gama, M. 2019. Response surface statistical optimization of bacterial nanocellulose fermentation in static culture using a low-cost medium. *New Biotechnology* 49: 19-27.
- Santiesteban-López, N. A., Cerón-Carrillo, T. G., Carmona-Silva, J. L. and Castro-Rosas, J. 2019. Electron microscopic studies in *Escherichia coli* on mode of action of sodium benzoate and potassium sorbate. *Biotechnology* 4(4): 82-86.
- Sozcu, S., Frajova, J., Wiener, J., Venkataraman, M., Tomkova, B. and Militky, J. 2024. Effect of drying methods on the thermal and mechanical behavior of bacterial cellulose aerogel. *Gels* 10(7): 474.
- Szymańska, M., Hoppe, J., Dutkiewicz, M., Sobolewski, P., Palacz, M., Janus, E., ... and Drozd, R. 2022. Silicone polyether surfactant enhances bacterial cellulose synthesis and water holding capacity. *International Journal of Biological Macromolecules* 208: 642-653.
- Urbina, L., Corcuera, M. Á., Gabilondo, N., Eceiza, A. and Retegi, A. 2021. A review of bacterial cellulose: Sustainable production from agricultural waste and applications in various fields. *Cellulose* 28(13): 8229-8253.
- Vasconcellos, V. and Farinas, C. 2018. The effect of the drying process on the properties of bacterial cellulose films from *Gluconacetobacter hansenii*. *Chemical Engineering Transactions* 64: 145-150.
- Vieira, F., Santana, H. E., Meirielly, J., Mata, F., Pires, P., Vaz-Velho, M., ... and Ruzene, D. 2024. Comparative study of pretreatments on coconut fiber for efficient isolation of lignocellulosic fractions. *Sustainability* 16: 4784.
- Wang, J., Tavakoli, J. and Tang, Y. 2019. Bacterial cellulose production, properties and applications with different culture methods - A review. *Carbohydrate Polymers* 219: 63-76.
- Wan-Mohtar, W. A. A. Q. I., Khalid, N. I., Rahim, M. H. A., Luthfi, A. A. I., Zaini, N. S. M., Din, N. A. S. and Mohd Zaini, N. A. 2023. Underutilized Malaysian agro-industrial wastes as sustainable carbon sources for lactic acid production. *Fermentation* 9(10): 905.
- Yanti, N. A., Ahmad, S. W., Ramadhan, L. O. A. N., Jamily, Muzuni, Walhidayah, T. and Mamangkey, J. 2021. Properties and application of edible modified bacterial cellulose film based sago liquid waste as food packaging. *Polymers* 13(20): 3570.
- Yi, T. J., Francis, F., Mutalib, S. A. and Mohd Zaini, N. A. 2022. Antimicrobial activity of bacterial cellulose from *Komagataeibacter xylinus* using expired commercial sweet drinks as a source of carbon. *Sains Malaysiana* 51(8): 2695-2711.
- Zaki, Z. A. A., Francis, F. and Zaini, N. A. M. 2023. Bacterial cellulose production from oil palm empty fruit bunch (OPEFB) hydrolysate using *Komagataeibacter xylinus* strain. *Biomass Conversion and Biorefinery* 13(16): 14629-14640.