



DUAL SACCHARIDES: HOW DO THEY INFLUENCE BACTERIAL CELLULOSE PRODUCTION AND MECHANICAL PROPERTIES?

(Dwi-Sakarida: Bagaimana Ia Mempengaruhi Pengeluaran dan Sifat Mekanik Selulosa Bakteria?)

Nurul Nadhirah Ruzelan and Azila Adnan*

Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia

*Corresponding author: azila.adnan@umt.edu.my

Received: 12 March 2024; Accepted: 4 June 2024; Published: 27 August 2024

Abstract

Bacterial cellulose (BC) production has become more popular over the past few years and has been widely used as an alternative in diverse applications. The most prominently discussed is biomedical applications. Due to its popularity and high demand for biomaterials in that particular application, BC has been focusing on its production and mechanical properties that align with the industries' biomedical demands. This study aimed to investigate the effects of dual-saccharides on BC production and its mechanical properties. Monosaccharides and disaccharides such as glucose, fructose, sucrose, lactose and maltose were used as carbon source combinations and how these dual-saccharides affect the production and properties of BC. Bacterial *K. xylinus* can utilise dual-saccharides glucose+lactose to produce BC, based on the BC coefficient (g/g). The saccharide combinations of glucose+lactose gave the highest BC production with 0.15 (g) of final weight. Bacterial *K. xylinus* can fully utilise dual-saccharides glucose+lactose based on the BC coefficient, 0.007 (g/g). Upon drying, the purified BC was characterised using scanning electron microscope (SEM), Fourier transform infrared (FTIR) and X-ray diffraction (XRD) methods. Hence, combining dual-saccharides into the culture medium can enhance BC's production and mechanical properties for wound healing applications.

Keywords: bacterial cellulose, dual-saccharides, properties, biomedical applications, *Komagataeibacter xylinus*

Abstrak

Pengeluaran selulosa bakteria (SB) menjadi lebih terkenal sejak beberapa tahun kebelakangan ini dan telah digunakan secara meluas sebagai alternatif dalam pelbagai aplikasi. Aplikasi bioperubatan merupakan aplikasi yang kerap dibincangkan. Disebabkan populariti dan permintaan yang tinggi untuk biobahan dalam aplikasi tertentu, SB telah berfokus pada pengeluaran dan sifat mekanikalnya yang sejajar dengan permintaan industri bioperubatan. Kajian ini bertujuan untuk mengkaji kesan dwi-sakarida ke atas pengeluaran SB dan sifat mekanikalnya. Monosakarida dan disakarida seperti glukosa, fruktosa, sukrosa, laktosa dan maltosa digunakan sebagai gabungan sumber karbon dan bagaimana dwi-sakarida ini mempengaruhi pengeluaran dan sifat SB itu sendiri. Bakteria *K. xylinus* boleh menggunakan dwi-sakarida glukosa+laktosa untuk menghasilkan SB, berdasarkan pekali SB (g/g). Gabungan sakarida glukosa+laktosa menghasilkan SB tertinggi dengan 0.15 (g) berat akhir. Bakteria *K. xylinus* boleh menggunakan sepenuhnya dwi-sakarida glukosa+laktosa berdasarkan pekali SB, 0.007 (g/g). Selepas pengeringan, SB yang telah ditulenkan menjalani pencirian menggunakan kaedah mikroskop imbasan elektron (SEM), transformasi fourier inframerah (FTIR) dan kaedah pembelauan sinar-X (XRD). Oleh itu, penggabungan dwi-sakarida dalam medium kultur boleh meningkatkan pengeluaran SB dan sifat mekanikal untuk aplikasi penyembuhan luka.

Kata kunci: selulosa bakteria, dwi-sakarida, ciri-ciri, aplikasi biomedikal, *Komagataeibacter xylinus*

Introduction

Cellulose is an abundant polysaccharide on Earth. This polysaccharide is commonly derived from the plant cell walls and composed of repeated β -1,4 glycosidic bonds (shown in Figure 1) that link the glucose monomer together [1, 2]. Belongs to the group of carbohydrates, cellulose consists of numerous hydroxyl groups that exist as a linear polymer chain [3]. The capability of hydroxyl groups to hold the hydrogen bonds together is crucial for some properties such as microfibrillar structure, high crystallinity and high cohesive nature [4]. This biomaterial has recently drawn enormous interest among researchers due to its versatility and purely biotechnological processing approach aligned with the green chemistry principle [5]. Rather than plant cellulose, bacterial cellulose (BC) is most favourable as this polysaccharide offers excellent mechanical properties that meet the market demands. Hence, BC was chosen over plant cellulose due to its purity, which frees off contaminants such as pectin, lignin, and

hemicellulose. Previous studies have addressed that the *Komagataeibacter* genus can utilise various carbon sources such as sucrose, fructose, glucose, galactose, xylose and mannose to synthesise cellulose [6]. In this study, *Komagataeibacter xylinus* was chosen as the BC producer due to this strain's ability to synthesise BC at a high yield [7]. Carbon source becomes the main contributor that affects BC production. At present, several attempts have been made to improve BC productivity, including combining carbon sources into the fermentation media. Based on studies conducted by Anguluri et al. [8], combining two types of carbon sources can enhance BC productivity using strain *Gluconacetobacter persimmonis*. However, utilising dual-sugar has not yet been discovered to synthesise BC by strain *Komagataeibacter xylinus* and its mechanical properties on wound healing applications. According to Digel et al. [9], strain *Komagataeibacter* can utilise different types of carbon sources and can produce BC in high yield.

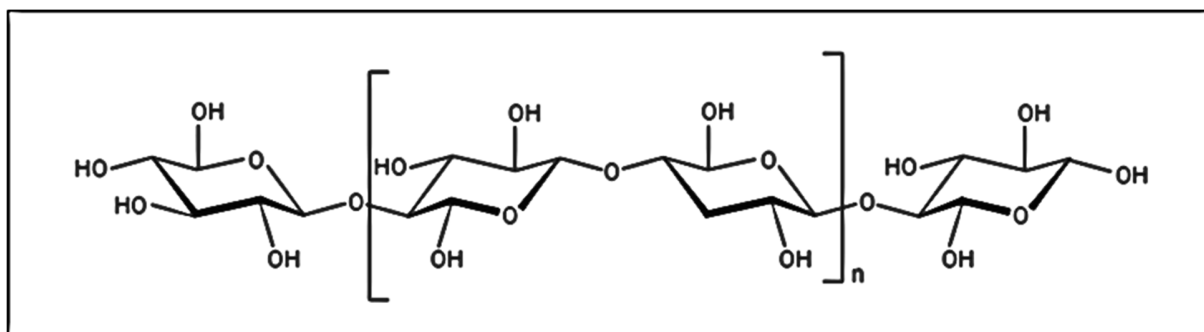


Figure 1. Chemical structure of cellulose made up of β -1,4 glycosidic bond. Adapted and modified from [10]

Materials and Methods

Microorganisms

Bacterium *Komagataeibacter xylinus* ATCC 53524 (American Type Culture Condition) strain was purchased and used as BC producer in this experiment owing to its capability to synthesise cellulose in a large amount [11, 12].

Culture media preparation

Culture media was prepared using the [13] method with some modifications. A standard Hestrin–Schramm (HS) medium, composed of 0.5% (w/v) yeast extract, 0.5% (w/v) peptone, 0.115% (w/v) citric acid, 0.2% (w/v) disodium hydrogen phosphate and filter-sterilised of 50% (v/v) glucose was used as fermentation medium to

synthesise BC. The pH value of the culture medium was adjusted until it dropped to 4-5. The overall procedure of BC biosynthesis is illustrated in Figure 2 below.

Preparation of inoculum

These methods followed [4] with slight modifications. The bacterial strain was initially grown on HS agar slant for five days and incubated at 30°C. A loop of newly developed bacterial strains was grown into a sanitised 250-mL conical flask of 75-mL of liquid growth media and incubated overnight in agitated conditions (150 RPM ~ 30°C). This process was run until the culture media became cloudy.

Bacterial cellulose biosynthesis

Approximately 10% of the inoculum was transferred into the fresh 75-mL HS media for fermentation. The

fermentation process was run in triplicates, in a static condition, for seven days until a layer of white pellicle was present on the upper layer of the media.

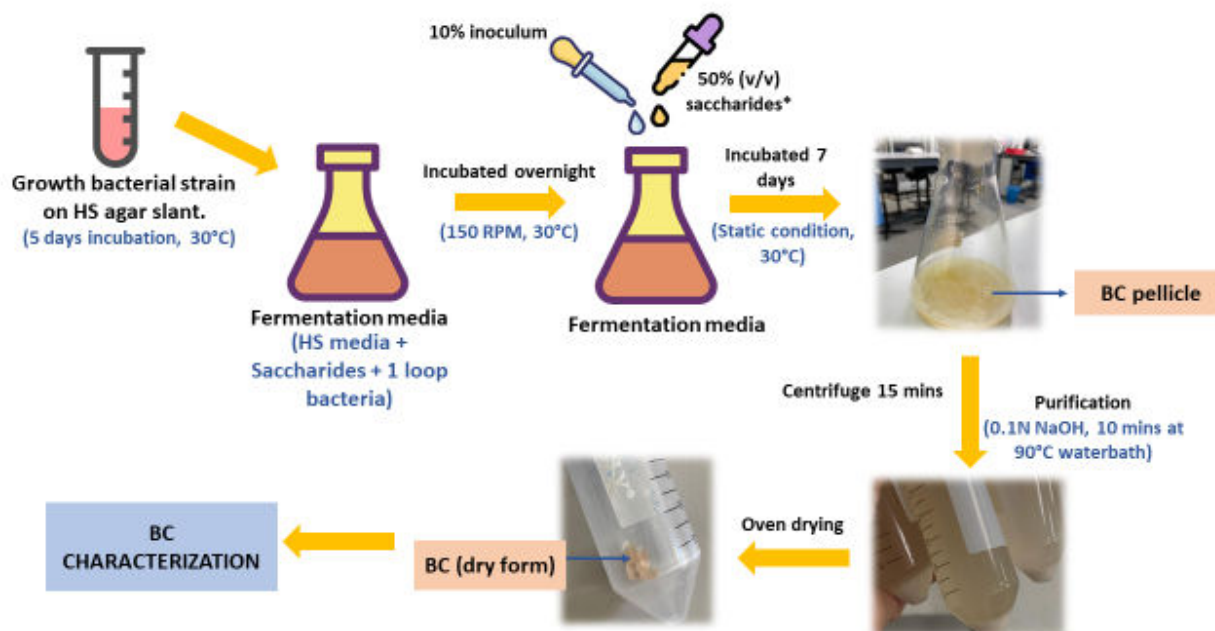


Figure 2. Overall illustration of BC biosynthesis produced by *Komagataeibacter xylinus*. (*) represent studied variables

Effect of carbon source combinations on BC yield

This experiment was conducted to study the effect of dual-sugar on BC production. The inoculum was transferred into the 250 mL flask containing HS media as described previously, and 50% (v/v) of dual-sugar was filter-sterilised. Different combinations of sugar were used, such as 25% glucose + 25% fructose, 25% glucose + 25% maltose, 25% glucose + 25% lactose, 25% fructose + 25% lactose, 25% fructose + 25% maltose and 25% fructose + 25% sucrose were added into the fermentation media. The pH value of the broth was changed as previously described. The fermentation flasks were incubated at 30°C for seven days in static conditions.

Purifications of BC

After fermentation, the native BC pellicles formed were harvested and put into a sterile 50-mL Falcon tube and centrifuged at 4000 RPM for 15 mins to obtain the residue. The residue was immersed into 0.1 M NaOH and was placed in the 90°C water bath for 10 mins. This

process was conducted to remove the unwanted cells that were obtained during fermentation. Only purified BC was purified and dried in the oven at 60-70°C until constant weight was achieved. The weight of pure BC was measured.

Characterisations of BC: Scanning Electron Microscope (SEM) analysis

The dried BC obtained was cut into small pieces based on the desired cross-section. The dried pure BC surface morphology was accessed using the JEOL JSM6360LA (Japan) scanning electron microscope. The morphology of the BC structure was observed at an acceleration voltage of 10 kV. All BC samples were covered with gold sputter to enhance conductivity [14].

Fourier Transform Infrared Spectroscopy (FTIR) analysis

FTIR analysis was conducted according to Sing et al. [15] method using Bruker Invenio-S (Germany) to determine the functional groups and chemical bonds in

the dried BC membranes. The dried BC membrane was put onto the sample stage after being cleaned with acetone to avoid contaminations. The FTIR spectral analysis was then performed over the wavenumber range of 500-4000 cm⁻¹.

X-ray Diffraction (XRD) analysis

Upon drying process, the crystallinity of BC was analysed using X-ray diffraction techniques. The characterisations of the dried BC were analysed using Rigaku Smartlab SE (Japan). The XRD patterns were generated at a voltage of 40 kV at the filament emission of 30 mA and Cu K β radiation. The BC pellicles were characterised at the 2 θ range, between 5° and 60°.

Results and Discussion

Effect of dual-saccharides treatments on BC production

The effect of dual-saccharides on BC production (dry weight) is depicted in Figure 2 above. There were six types of dual-saccharides treatments utilised by *K. xylinus*, namely glucose+maltose, glucose+lactose,

glucose+lactose, fructose+maltose, fructose+sucrose, and fructose+lactose. [9] mentioned that the strain *K. xylinus* can utilise different types of carbon sources other than being able to synthesise BC in high yield. In this experiment, glucose and fructose were used as controls. According to the findings, the dual-saccharides treatments of glucose+lactose gave the highest yield (0.418 g), followed by fructose+lactose (0.132 g), glucose+fructose (0.083 g), fructose+sucrose (0.064 g), fructose+maltose (0.06 g) and glucose+maltose gave the lowest yield (0.022 g). This is because maltose is considered an inadequate carbon source since it does not lower the fermentation media's pH value. A higher pH indicates low BC production since *K. xylinus* works effectively in acidic conditions due to its nature (acid-tolerance bacteria) [16]. Glucose, which acts as a sugar control, gave the maximum yield of 0.127 g, while another sugar control, fructose, gave a yield of 0.092 g. Nevertheless, the findings were contradicted with [4] where BC can be produced at maximum yield when fructose is used as a carbon source.

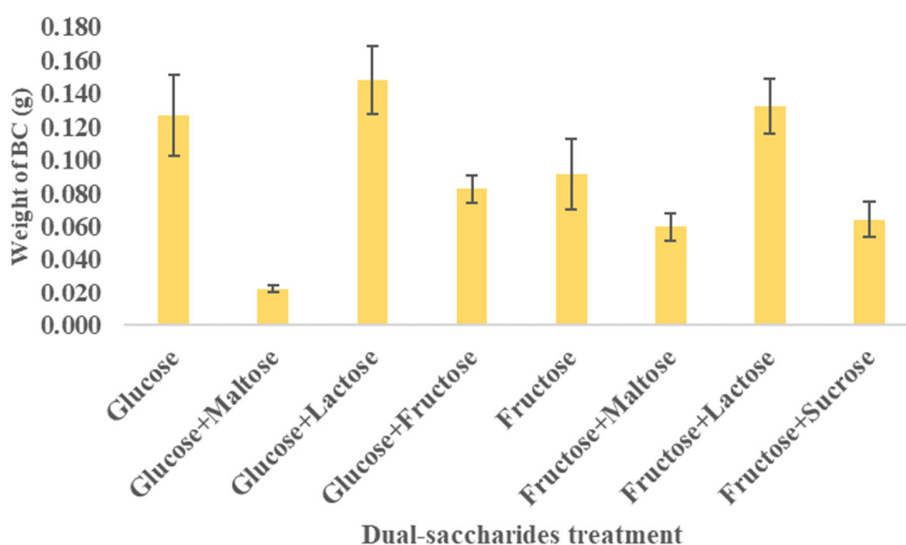


Figure 2. Graph effect of dual-saccharides treatments on BC weight (g)

Effects of dual-saccharides treatments on BC coefficient

The following equation was used to determine the bacterial yield coefficient of *K. xylinus* in BC production.

$$\text{Growth yield coefficient} = \frac{\text{Dry weight of BC (g)}}{\text{Amount of C source consumed (g)}} \quad (1)$$

Bacteria *K. xylinus* has the capability to break down carbon sources to produce cellulose. This strain can also

produce cellulose by absorbing glucose and fructose as carbon sources [14]. In addition, the prospect of synthesising BC from carbon sources other than glucose has been discovered by researchers for a long time [17]. The findings in Figure 3 above depict the effects of various dual-saccharides treatment on the BC coefficient. Based on the results, the bacterium has utilised 20 g of carbon sources throughout the fermentation process to produce BC. The combined sugar of glucose and maltose proved that the bacteria *K.*

xylinus has the lowest efficiency in breaking down those sugar molecules. This is because when bacteria are grown in a combination of two distinct carbon sources, their growth behaviour is altered [18]. Maltose does not cause the pH of the media to be decreased at the end of the fermentation. Since this *K. xylinus* need acidic conditions to break down carbon sources to synthesise BC, the results indicated the strain was unable to utilise maltose to produce BC as it is known as a weak carbon source for the synthesis [16].

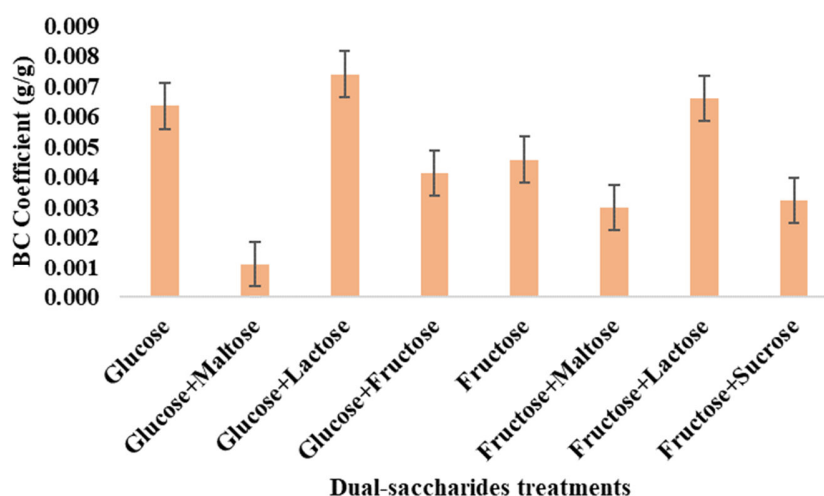


Figure 3. Graph effects of dual-saccharides treatments on BC coefficient (g/g)

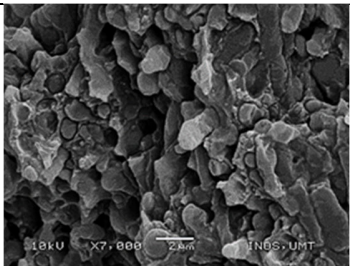
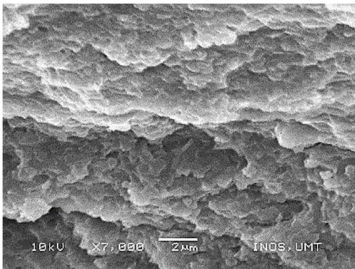
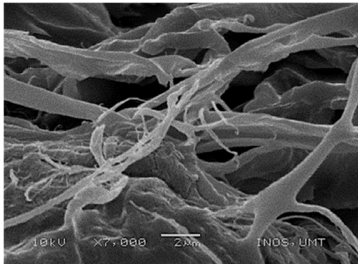
Characterisation of BC: SEM analysis

The intricate structure of BC morphology produced by *K. xylinus* using six different treatments of carbon sources are displayed in Figure 2 below. The SEM helps to provide the surface morphology of the BC [19]. The SEM images revealed that all the BC samples have microporous structures with smooth surfaces and highly open pores. The use of different dual-sugar treatments has an influence on BC morphology based on SEM images observed. Based on the surface morphology, it can be seen that BC with the combination of fructose + lactose [Table 1(A)] appeared relatively rigid and had blunt edges. The SEM images in Table 1 (B), (C), (D), (E) and (F) showed some differences in surface structures when sugar combinations were applied. The images in [Figure 5(B)] showed that it has a smudge-like structure and some fibrous-like structure [Table 1 (C)], while Table 1 (D), (E) and (F) have porous appearance. Since dual-sugar was implied into the fermentation media and incorporated into BC fibrils, the reticulated

structure may be assigned to the existence of different types of sugars in the broth. However, the findings above were compared with the morphology of the BC membranes when glucose and fructose were used as the only sources of carbon (Table 1 (G) and (F)). The SEM images from Figure 3 and Figure 4 revealed that glucose and fructose lead to a different interwoven pattern in BC. The results were determined from both the top and bottom surfaces of BC membranes. There was a difference in the morphology of BC in G and H, whereas G is more compact and denser, while H shows a fibrous-like structure. H exhibited BC morphology with the thinnest strand, higher porosity and more fibrous compared to G. Based on the results obtained, BC is composed of a nano-fibrillar network of pores, which helps in the absorption of wound exudates. This feature in BC accelerates the wound healing process since it provides a favourable temperature for the wound surface, which is vital in wound healing applications [13].

Table 1. SEM morphology of cellulose produced by *K. xylinus* under six different treatments of dual-sugar

Carbon Sources	SEM Images	
Fructose+Lactose	A	
Fructose+Maltose	B	
Fructose+Sucrose	C	
Glucose+Lactose	D	
Glucose+Fructose	E	

Carbon Sources	SEM Images
Glucose+Maltose	F 
Glucose	G 
Fructose	H 

The SEM helps to provide the surface morphology of the BC [19]. The SEM images revealed that all the BC samples have microporous structures with smooth surfaces and highly open pores. The use of different dual-sugar treatments has an influence on BC morphology based on SEM images observed. Based on the surface morphology, it can be seen that BC with the combination of fructose + lactose [Table 1(A)] appeared relatively rigid and had blunt edges. The SEM images in Table 1 (B), (C), (D), (E) and (F) showed some differences in surface structures when sugar combinations were applied. The images in [Figure 5(B)] showed that it has a smudge-like structure and some fibrous-like structure [Table 1 (C)], while Table 1 (D), (E) and (F) have porous appearance. Since dual-sugar was implied into the fermentation media and incorporated into BC fibrils, the reticulated structure may be assigned to the existence of different types of sugars in the broth. However, the findings above were

compared with the morphology of the BC membranes when glucose and fructose were used as the only sources of carbon (Table 1 (G) and (F)). The SEM images from Figure 3 and Figure 4 revealed that glucose and fructose lead to a different interwoven pattern in BC. The results were determined from both the top and bottom surfaces of BC membranes. There was a difference in the morphology of BC in G and H, whereas G is more compact and denser, while H shows a fibrous-like structure. H exhibited BC morphology with the thinnest strand, higher porosity and more fibrous compared to G. Based on the results obtained, BC is composed of a nano-fibrillar network of pores, which helps in the absorption of wound exudates. This feature in BC accelerates the wound healing process since it provides a favourable temperature for the wound surface, which is vital in wound healing applications [13].

FTIR analysis

FTIR spectra represent compound and functional groups present in bacterial cellulose (BC). The purified BC formed when dual-sugar used as carbon sources were determined. The FTIR spectra were scanned, ranging from 500 to 4000 cm^{-1} (Figure 5). The FTIR analysis of BC pellicles formed from various dual-saccharides treatments were compared to the standard sugar, glucose, and fructose. According to the findings in Figure 5 below, the peaks obtained by BC are almost similar in each dual-saccharides treatment and sugar control. Fructose and glucose act as carbon source controls in this experiment. All the peaks were determined in the same position at a similar intensity. Generally, the native BC has plenty of hydroxyl groups, causing the molecule to be hydrophilic [9]. Owing to the plenty of -OH groups present in BC and nanofibril network makes this biopolymer suitable for the current

wound-dressing material since it forms strong intramolecular and intermolecular hydrogen bonds that have a significant impact on tensile strength and elastic modulus of BC [20, 13]. According to Choi et al. [19], -OH groups might help in water absorption, and the nanofibril itself can provide a good skin environment for wound healing. Hence, the presence of hydroxyl groups was proven by the FTIR analysis. The FTIR spectra formed also proved that the material produced by strain *K. xylinus* was cellulose [14]. Functional groups obtained from the BC are listed in Table 2 below. Generally, BC itself possessed plenty of hydroxyl groups and oxygen atoms, summarised in Table 2. These create the inter and intra of hydrogen bonding in BC, which helps in improving the mechanical strength of the biopolymer [21], which might be favourable in the wound healing process.

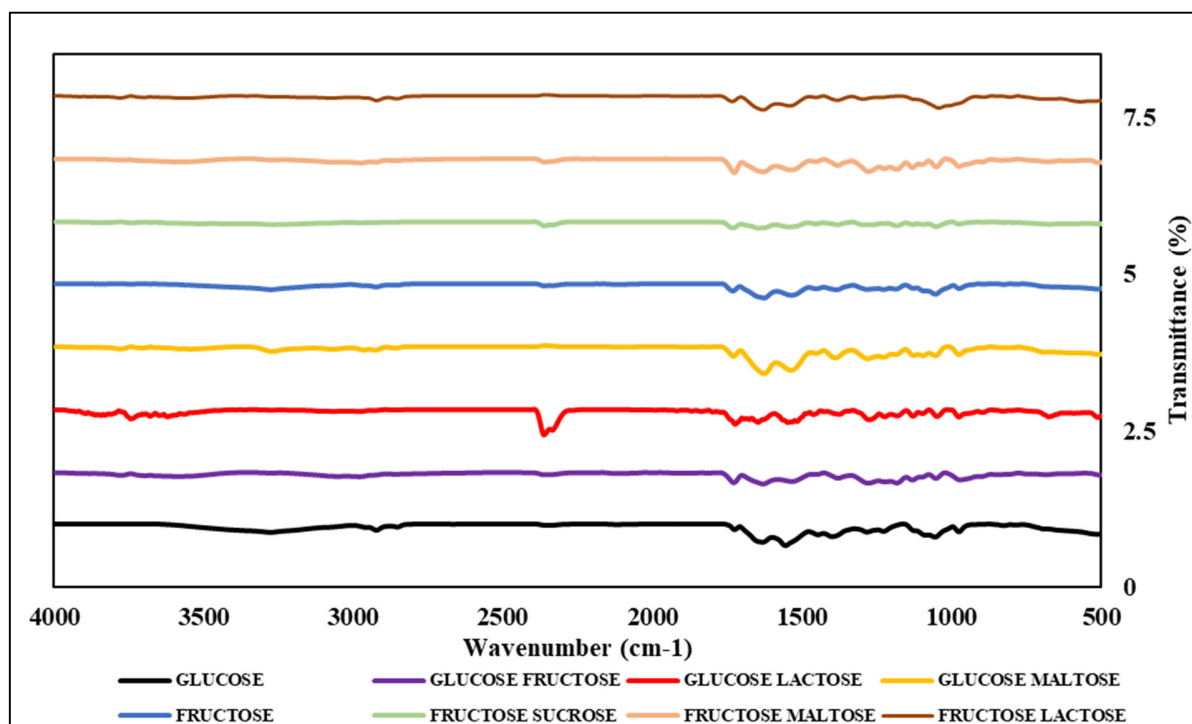


Figure 5. FTIR spectra of BC when dual-saccharides treatments were used

Table 2. FTIR spectra for dried pure BC after different treatments of sugar were applied

Sugar Treatment	Peaks (cm ⁻¹)	Peak Details	Functional Group Obtained	Class
Glucose	~1052; ~1557; ~1630	Strong; medium; medium	C-O-H bonding; C=C, C-H stretching	Polysaccharide; alkene;
Glucose+Fructose	~1728; ~1635; 970	Strong; medium	C=O stretching; C=C stretching; OH bonding	Carbonyl; alkene; alcohol
Glucose+Lactose	~2360; ~1724; ~1535	Strong; medium	C=C; C=O; C-O-H stretching	Alkene; carbonyl; carboxyl
Glucose+Maltose	~1627; ~1534; ~1383	All strong	O-H bending; C-O-H and O-H stretching	H ₂ O [14]; carboxyl; and alcohol
Fructose	~1627; ~1527; ~1399	Strong, medium, strong	O-H bond; C-O-H bending; O-H stretching	Water; carboxyl; and alcohol
Fructose+Sucrose	~1731; ~1628; ~1053	All medium	C=O stretching; C-H stretching [13]; C-O-C stretching [13]	Carbonyl; aldehyde and ester
Fructose+Maltose	~1723; ~1533; ~1277	Strong, medium	C=O stretching [13]; C-O-H stretching; and O-H bending	Acetyl, carboxyl and alcohol
Glucose+Maltose	~1728; ~1626; ~1387	Strong and medium	C=O, C=C bending and C-H stretching	Aldehyde, alkene and aldehyde

XRD analysis

It is reported by [22] that BC is a homogeneous polycrystalline macromolecular compound that consists of ordered crystalline and minimum ordered amorphous regions. In order to determine the crystalline structure of cellulose produced from *K. xylinus*, all BC samples were analysed using XRD. Figure 6 shows the XRD pattern of BC under the treatment of various dual-saccharides, and peaks of BC were determined at 2 θ angles ranging from 0° to 60°. The findings show that the native BC has

a crystalline structure [19]. Analysis of XRD is used to identify the characteristics of cellulose Ia since BC is composed of cellulose Ia [11]. According to Figure 6, BC pellicles from dual-saccharides fructose+lactose, fructose+sucrose, fructose+maltose, glucose+fructose and glucose+lactose had distinct peaks at 2 θ angles in the range of 28° to 30° while glucose+maltose formed prominent peaks at range 10° to 30°. The control sugar glucose formed three distinct strong intensity peaks [22], which shows that the typical cellulose produced

from *K. xylinus* contains Ia cellulose. All the results obtained indicate that BC implemented with dual-saccharides has high crystallinity. BC pellicles formed from fructose proved that BC has high crystallinity, although the peaks were produced with low intensity. The crystallinity of the dried BC membranes determined under the various dual-saccharides treatments was reported in Table 2. In general, BC has higher crystallinity than plant cellulose [23], and the crystallinity value can be up to 90% [20]. Based on

findings in Table 2, the results indicate that BC with dual-saccharides treatments gave higher crystallinity, parallel to the native BC except for fructose+sucrose and fructose+maltose. The higher crystallinity of BC indicates that the biopolymer has a high degree of polymerisation, making the properties suitable for wound healing material [13]. According to the observations, the unique feature of BC favoured in wound healing material has been proven.

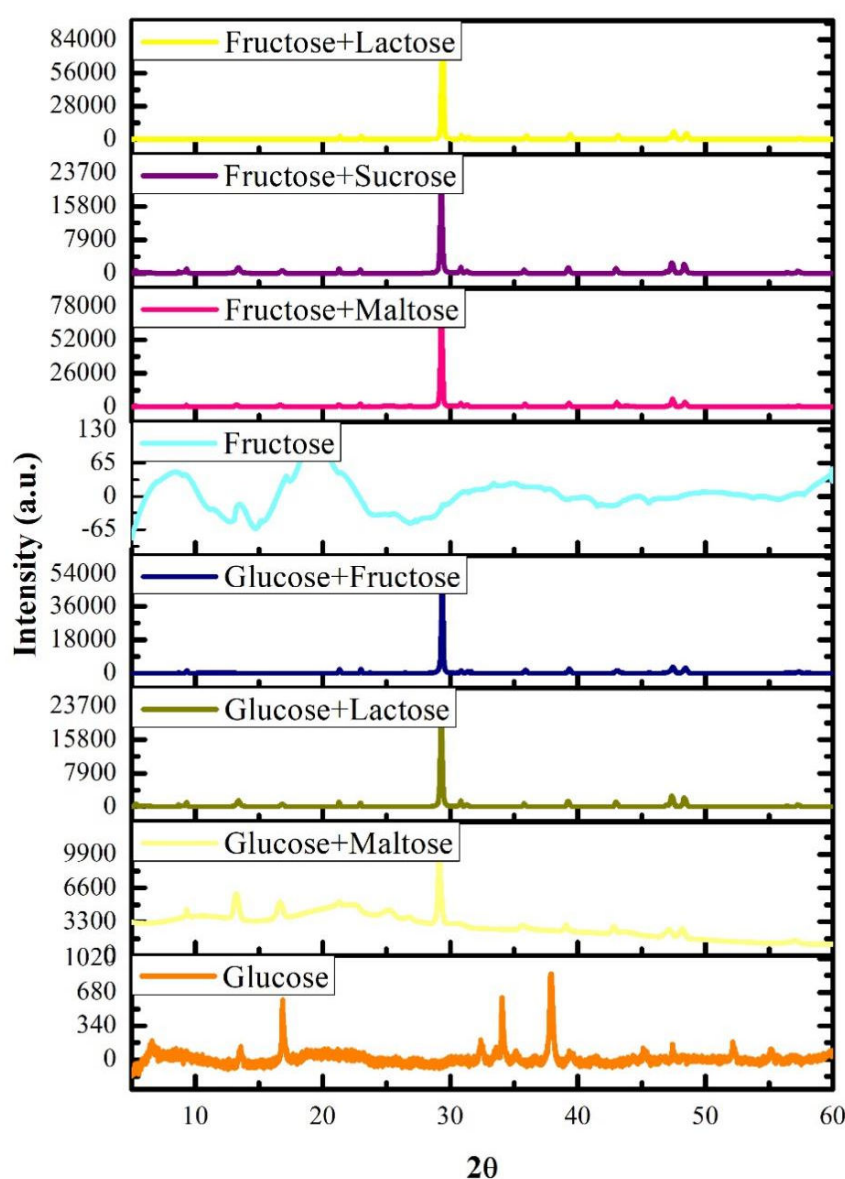


Figure 6. XRD patterns of BC when dual-saccharides treatments were applied

Table 2. Crystallinity % for dried BC after different dual-saccharides treatments were applied

Dual-Saccharides Treatments	%Crystallinity
Glucose	100
Glucose+Fructose	58.8
Glucose+Maltose	1.58
Glucose+Lactose	12.1
Fructose	100
Fructose+Sucrose	0
Fructose+Maltose	0
Fructose+Lactose	2.2

Conclusion

This study demonstrates that the combination of dual-saccharides, specifically glucose and lactose, significantly enhances the production and mechanical properties of bacterial cellulose (BC) produced by *K. xylinus*. The optimal combination of glucose and lactose yielded the highest BC production, confirmed by a BC coefficient of 0.007 g/g. Characterization of the purified BC using SEM, FTIR, and XRD further validated its suitability for biomedical applications, particularly in wound healing. This finding highlights the potential of dual-saccharides to improve BC production, aligning with the increasing demand for high-performance biomaterials in the biomedical field.

Acknowledgements

We would like to express our acknowledgement to the Ministry of Education of Malaysia for the Fundamental Research Grant Scheme (FRGS) 59584 (FRGS/1/2019/STG05/UMT/02/5) and Universiti Malaysia Terengganu for research support and facilities.

References

1. Naeem, M. A., Alfred, M., Saba, H., Siddiqui, Q., Naveed, T., Shahbaz, U., and Wei, Q. (2019). A preliminary study on the preparation of seamless tubular bacterial cellulose-electrospun nanofibers-based nanocomposite fabrics. *Journal of Composite Materials*, 53(26–27): 3715-3724.
2. Przygodzka, K., Chareza, M., Banaszek, A., Zielińska, B., Ekiert, E., and Drozd, R. (2022). Bacterial cellulose production by *Komagataeibacter xylinus* with the use of enzyme-degraded oligo- and polysaccharides as the substrates. *Applied Sciences*, 12(24): 2673.
3. Ghazali, M., Meliana, Y., and Chalid, M. (2021). Synthesis and characterization of bacterial cellulose by *Acetobacter xylinum* using liquid tapioca waste. *Materials Today: Proceedings*, 44: 2131-2134.
4. Choi, S. M., Rao, K. M., Zo, S. M., Shin, E. J., and Han, S. S. (2022). Bacterial cellulose and its applications. *Polymers*, 14(6): 1080.
5. Singh, R., Mathur, A., Goswami, N., and Mathur, G. (2016). Effect of carbon sources on physicochemical properties of bacterial cellulose produced from *Gluconacetobacter xylinus* MTCC 7795. *e-Polymers*, 16(4): 331-336.
6. 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(13): 2088.
7. Ghazali, M., Meliana, Y., and Chalid, M. (2021). Synthesis and characterization of bacterial cellulose by *Acetobacter xylinum* using liquid tapioca waste. *Materials Today: Proceedings*, 44: 2131-2134.
8. Anguluri, K., La China, S., Brugnoli, M., Cassanelli, S., and Gullo, M. (2022). Better under stress: Improving bacterial cellulose production by *Komagataeibacter xylinus* K2G30 (UMCC 2756) using adaptive laboratory evolution. *Frontiers in Microbiology*, 13: 994097.
9. Digel, I., Akimbekov, N., Rogachev, E., and Pogorelova, N. (2023). Bacterial cellulose produced by *Medusomyces gisevii* on glucose and sucrose: biosynthesis and structural properties. *Cellulose*, 30(18): 11439-11453.
10. Fatima, A., Ortiz-Albo, P., Neves, L. A., Nascimento, F. X., and Crespo, J. G. (2023). Biosynthesis and characterization of bacterial cellulose membranes presenting relevant characteristics for air/gas filtration. *Journal of Membrane Science*, 674: 121509.

11. Rongpipi, S., Ye, D., Gomez, E. D., and Gomez, E. W. (2019). Progress and opportunities in the characterization of cellulose – an important regulator of cell wall growth and mechanics. *Frontiers in Plant Science*, 9: 1-28.
12. He, W., Wu, J., Xu, J., Mosselhy, D. A., Zheng, Y., and Yang, S. (2021). Bacterial cellulose: functional modification and wound healing applications. *Advances in Wound Care*, 10(11): 623-640.
13. Gorgieva, S., Jančić, U., Cepec, E., and Trček, J. (2023). Production efficiency and properties of bacterial cellulose membranes in a novel grape pomace hydrolysate by *Komagataeibacter melomenus* AV436T and *Komagataeibacter xylinus* LMG 1518. *International Journal of Biological Macromolecules*, 244: 125368.
14. Azmi, S. N. N. S., Asnawi, A. S. F. M., Ariffin, H., and Abdullah, S. S. S. (2023). The production and characterization of bacterial cellulose pellicles obtained from oil palm frond juice and their conversion to nanofibrillated cellulose. *Carbohydrate Polymer Technologies and Applications*, 5: 100327.
15. Singh, R., Mathur, A., Goswami, N., & Mathur, G. (2016). Effect of carbon sources on physicochemical properties of bacterial cellulose produced from *Gluconacetobacter xylinus* MTCC 7795. *e-Polymers*, 16(4): 331-336.
16. Hungund, B., Prabhu, S., Shetty, C., Acharya, S., Prabhu, V., and Gupta, S. G. (2013). Production of bacterial cellulose from *Gluconacetobacter persimmonis* GH-2 using dual and cheaper carbon sources. *Journal of Microbiology Biochemical Technology*, 5(2): 31-33.
17. Thongwai, N., Futui, W., Ladpala, N., Sirichai, B., Weechan, A., Kanklai, J., and Rungsirivanich, P. (2022). Characterization of bacterial cellulose produced by *Komagataeibacter maltaceti* P285 isolated from contaminated honey wine. *Microorganisms*, 10(3): 528.
18. Jittaut, P., Hongsachart, P., Audtarat, S., and Dasri, T. (2023). Production and characterization of bacterial cellulose produced by *Gluconacetobacter xylinus* BNKC 19 using agricultural waste products as nutrient source. *Arab Journal of Basic and Applied Sciences*, 30(1): 221-230.
19. Choi, S. M., Rao, K. M., Zo, S. M., Shin, E. J., and Han, S. S. (2022). Bacterial cellulose and its applications. *Polymers*, 14(6): 1080.
20. Lahiri, D., Nag, M., Dutta, B., Dey, A., Sarkar, T., Pati, S., ... and Ray, R. R. (2021). Bacterial cellulose: Production, characterization, and application as antimicrobial agent. *International Journal of Molecular Sciences*, 22(23): 12984.
21. Volova, T. G., Prudnikova, S. V., Sukovatyi, A. G., and Shishatskaya, E. I. (2018). Production and properties of bacterial cellulose by the strain *Komagataeibacter xylinus* B-12068. *Applied Microbiology and Biotechnology*, 102: 7417-7428.
22. Betlej, I., Krajewski, K. J., Boruszewski, P., and Zakaria, S. (2021). Bacterial cellulose-properties and its potential application. *Sains Malaysiana*, 50(2): 493-505.
23. Singhania, R. R., Patel, A. K., Tsai, M. L., Chen, C. W., and Di Dong, C. (2021). Genetic modification for enhancing bacterial cellulose production and its applications. *Bioengineered*, 12(1): 6793-6807.