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Research article

Production of Bacterial Cellulose by *Komagataeibacter xylinus* InaCC B404 Using Different Carbon Sources

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Keywords

Komagataeibacter xylinus;

bacterial cellulose;

carbon source;

lactose

Abstract

Recently, microorganism-based hydrogel has been attracting attention for its applications in the revegetation of marginal land and in crop production on dry land. *Komagataeibacter xylinus* is a bacterium that can biosynthesize bacterial cellulose, the material for microorganism-based hydrogel. Nonetheless, various factors influence the production of bacterial cellulose by *K. xylinus*, and one of them is the substrate nutrient used. Therefore, we investigate various types of carbon sources for bacterial cellulose synthesis by our strain collection *K. xylinus* InaCC B404. Five simple sugars were selected as the main carbon source, namely glucose, lactose, mannitol, xylose, and sucrose. The results showed that the type of carbon source affected bacterial cellulose production yield. Lactose was the carbon source that produced the highest yield of bacterial cellulose (8.7 g/l). Moreover, sugar consumption by the isolate was also the lowest in the lactose broth (25% w/w). However, the swelling capacity of dried natural bacterial cellulose produced was relatively similar for all carbon sources, and it was between 2.5-3.1 times per gram. Physical observation based on scanning electron microscope (SEM) micrographs showed that the appearances of bacterial cellulose derived from lactose and glucose were more rugged than other types of bacterial cellulose. On the other hand, chemical structure analyses using Fourier-Transform Infrared Spectroscopy (FTIR) revealed that the chemical composition of bacterial cellulose was relatively similar for all carbon sources. This was revealed by the presence of the characteristic bands of cellulose. This study points to the value of the use of lactose as a carbon source in the production of bacterial cellulose as a hydrogel for further application.

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1. Introduction

Various strategies have been proposed to overcome the effect of low water availability on marginal land. Due to its effectivity and efficiency, the application of hydrogel have attracted attention worldwide and has been the subject of intensive study in order to cope with the problem of water scarcity on drought land [1-3]. A hydrogel is composed of hydrophilic polymeric materials that have the capability to hold a large amount of water in their three-dimensional networks. Because of their water retention properties, hydrogels are able to provide the water needs for plants and other organisms in surrounding soils in drought conditions for a period of time [4]. Depending on its polymer origin, a hydrogel can be grouped as a synthetic and natural hydrogel. Both types of hydrogels are applicable for various purposes. On average, synthetic hydrogels have a higher swollen capacity than their natural counterparts, but their components are considerably less environmentally friendly, particularly for long-term use in soils [5, 6]. Moreover, the cost of hydrogel production, particularly synthetic hydrogel, is still quite high. Furthermore, the presence of recalcitrant such as boric acid during the synthesis of the hydrogel, is dangerous for the soil ecosystems [7]. Therefore, efforts to optimize the swelling capacity of natural hydrogels are considerably preferred.

Some microorganisms, especially bacteria, are reported to produce cellulose as extracellular polymeric substances (EPSs) [7]. One of the examples is *Komagataeibacter xylinus.* The bacterium itself is gram-negative that biosynthesizes and extracellularly secretes cellulose as part of its metabolism process. It is non-pathogenic and commonly found in soil. Traditionally, the bacterium has been used to make healthy edible cellulolytic fibrils from coconut water, which are the main ingredient of nata de coco. But recently, due to its purity and distinct characteristic, and also due to economic as well as environmental considerations, natural bacterial cellulose has been viewed as a good alternative replacement for plant-based cellulose, particularly for synthesizing hydrogel for various purposes [8]. Though the swelling capacity of the microorganism-based hydrogel is less than chemical-based hydrogel, and though other problems such as cost and complexity of production process exist, a number of promising applications of this hydrogel, particularly in agriculture, is worthy of further investigation.

One important factor to be optimized in the synthesis of bacterial cellulose is the compatibility of nutrients, in this particular case carbon sources, which are the main energy source required for the formation of extracellular matrix biomolecules such as cellulose. Moreover, bacterial strains, even identified in one species, have been shown to be able to use a variety of carbon sources to survive or produce specific secondary metabolites [9, 10]. For application purposes, the selection of an appropriate carbon source utilized by the bacterial strain will effectively produce bacterial cellulose with desirable yield and characteristics. Therefore, the aim of this study was to evaluate the carbon sources for the effective production of bacterial cellulose by *K. xylinus* InaCC B404. We selected 5 simple sugars, namely glucose, lactose, mannitol, xylose, and sucrose as the main carbon sources.

2. Materials and Methods

2.1 Microorganism

Komagataeibacter xylinus InaCC B404 was obtained from Indonesian Culture Collection (InaCC). The previous name was *Gluconacetobacter xylinus* and due to taxonomic reclassification, it was renamed as *K. xylinus* [11]. The culture was characterized based on morphological appearances and simple biochemical tests such as Gram staining, catalase testing, oxidase testing, substrates

utilization, indole and hydrogen sulfide production, and carbohydrate fermentation. The culture was maintained on Hestrin-Schramm (HS) medium (composition is listed in Table 1 with glucose as main carbon source) [12] at 30°C prior to the evaluation.

2.2 Culture medium and conditions

Komagataeibacter xylinus InaCC B404 (1-2 colonies of bacteria grown for 7-10 days in HS plate medium with 1.5% agar w/v) was cultured on 150 ml of modified HS medium with various carbon sources (Table 1) in 500 ml conical flasks for 15 days. The cultures were incubated at 30°C on a rotary shaker at 80 rpm in dark conditions. Uninoculated media were also maintained. Three replicates were maintained for each carbon source.

Table 1. Modified *K. xylinus* InaCC B404 medium with different combinations of carbon sources

Ingredients	g/l
Main carbon source (Mannitol/Sucrose/Xylose/Glucose/Lactose)	20.0
Peptone	5.0
Yeast Extract	5.0
Disodium Hydrogen Phosphate (Na_2HPO_4)	2.7
Citric Acid	1.5

2.3 Harvesting and weighing of bacterial cellulose

After 15 days of cultivation, the bacterial cellulose (BC) formed was separated from the liquid part of the fermentation medium. The fresh BC was then put in an oven at 70°C for 24 h. After that, the weight of dried BC was measured. Both dried bacterial cellulose and the remaining liquid part were kept for further analysis. In order to get the dried BC, the fresh BC was put in an oven at 70°C for 24 h.

2.4 Analysis of fermented broth

2.4.1 The acidity level of liquid medium

The level of acidity of the remaining liquid medium after cellulose production was determined using a pH meter (WA-2015 Lutron, China), along with an uncultured medium. The increasing acidity level (decreasing of pH) of fermented broth was calculated by substracting the pH of unioculated medium (Ctrl) from the pH of inoculated fermented broth (Inoc).

2.4.2 The measurement of utilized sugar during cellulose production

The remaining liquid part of the fermented medium was collected and centrifuged at 5000 rpm for 2 min. The supernatant of the sample, along with an uncultured medium, was then transferred to test tubes and the sugar content in the aqueous solution was measured using Hand-Held Refractometer Atago® Manual (Brix 0-33%). The utilization of sugar was defined using equation 1, where % SU (w/w) is the percentage of sugar utilization by *K. xylinus* InaCC B404 in broth medium, and B_i and B_{BC} are broth medium degree Brix (°Bx) of the initial and after bacterial cellulose production, respectively.

$$\% \text{ SU} = \frac{(B_i - B_{BC})}{B_i} \times 100\% \quad (1)$$

2.5 Analysis of dried bacterial cellulose

2.5.1 Swelling capacity of dried bacterial cellulose

To measure the reversibility of the swelling capacity of the sample, an amount of the dried BC was immersed in sterile distilled water for 72 h at room temperature. Then, the swollen sample was separated from the rest of the water until no free water dripped down and weighed carefully. The swelling capacity of the sample after drying was calculated using equation 2, where SC is the swelling capacity per gram of dried sample, and M_{DBC} and M_{SBC} are the mass weights of the dry and swollen samples (g), respectively.

$$SC = \frac{(M_{SCB} - M_{DBC})}{M_{DBC}} \quad (2)$$

2.5.2 Scanning electron microscope (SEM) observation

The dehydrated bacterial cellulose sample was dissolved using sterile distilled water at the ratios of 1:20 and 1:30 (w/v). The hydrated sample was then dried in an oven at 50°C for 24 h. The dried samples were then laid out in sample stubs for gold (Au) coating using MC1000 Ion Sputter Coater (Hitachi High-Technologies, Japan) and rotary pump. SEM observation was carried out using Hitachi TM3030 low vacuum tabletop microscope (Hitachi High-Technologies, Japan) with appropriate magnification.

2.5.3 Fourier-transform infrared spectroscopy (FTIR) observation

Characterization of functional groups in the dried bacterial cellulose was analyzed by Fourier-Transform Infrared Spectroscopy (FTIR) in a Perkin Elmer Spectrum Two couple (USA) with Universal Attenuated Total Reflectance (UATR). The spectra were generated with 16 scannings per sample in the range of 500-4000 cm^{-1}.

2.6 Statistical analysis

All the assay procedures were repeated in triplicate. Statistical analysis of the data for dry biomass, swelling capacity, as well as sugar utilized percentage measurement was done through Tukey's multiple comparisons test (ordinary one-way ANOVA) by comparing each column. For the analysis of shifting of pH of the liquid part after the fermentation period, an unpaired t-test between inoculated medium (Inoc) and uninoculated control (Ctrl) for each main carbon source was performed. Asterisk signs indicated the P value of the comparison test, * (P value 0.05 - 0.01), ** (P value 0.01- 0.001), *** (P value < 0.001). The relationship between increasing acidity level of environmental liquid broth and dry biomass produced was plotted in a Pearson's correlation plot, with R squared and 1/slope indicating goodness of fit and best-fit value parameter, respectively.

3. Results and Discussion

3.1 Bacterial characteristics

A visual observation showed that *Komagataeibacter xylinus* InaCC B404 was found to be gram-negative, rods shape, occurring singly or in chains, and of size approximately 0.6-0.8 x 2-3 µm under 1000 x magnification. The colonies on HS agar were pale white-creamy, circular, undulate, convex, of dry and crumbly consistency, difficult to disperse using an inoculation loop, and with surface quality that was opaque and shiny (Figure 1).

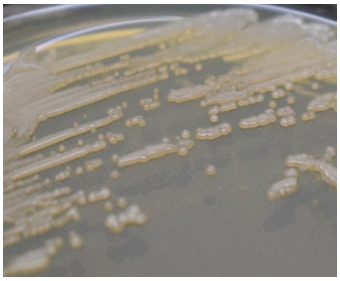

Figure 1. Morphological characteristics of *K. xylinus* InaCC B404

The biochemical properties of *K. xylinus* InaCC B404 are shown in Table 2. The isolate tested positively for catalase; however, it was negative for oxidase tests. The isolate has the ability to produce cellulose and was able to ferment several carbohydrate substrates. An acid condition was produced from substrates such as D-glucose, D-galactose, D-maltose, raffinose, ribose, melibiose, and sucrose.

The characteristics of *K. xylinus* InaCC B404 were aerobic, rod shape, gram-negative, catalase-positive, oxidase-negative, and able to produce cellulose. The isolate was able to ferment some of carbon sources. According to the morphological characteristics and descriptions of *Komagataeibacter* genus [11] and Manual of Systematic Bacteriology [13], the isolate belonged to the family Acetobacteraceae, order Rhodospirillales, and class Alphaproteobacteria. Yamada *et al*. [11] described that *Komagataeibacter* genus produced acid from D-glucose, D-galactose, D-xylose, L-arabinose, or ethanol, but not from D-fructose, L-sorbose, D-sorbitol, maltose, or lactose. However, the biochemical properties of the isolate in our work showed that acid was produced from maltose. These biochemical results are not conclusive enough for the identification of isolate. Contrary to the study of Sarkono and Si [14], based on 16S rDNA molecular approach, the isolate was identified as *Gluconacetobacter xylinus.* A few years ago, *K. xylinus* was known as *Acetobacter xylinum*. Taxonomically it was changed into *Gluconacetobacter xylinus*, and formerly reclassified into *K. xylinus* [15].

3.2 Effect of carbon source on bacterial cellulose production

The appearance of fresh BC produced by *K. xylinus* InaCC B404 with various carbon sources after 15 days of liquid fermentation is shown in Figure 2. Varying the main carbon sources used in the liquid broth affected the product during fermentation, not only influencing the color of the remaining broth, but also the production of BC. Color ranged from dark brown (xylose), light brown (lactose), and white (glucose, mannitol, and sucrose). The yield production of BC by *K. xylinus* InaCC B404 was influenced by the selected main carbon sources (Figure 3). The presence

Table 2. The physiological characteristics of *K. xylinus* InaCC B404

Characterization test	*K. xylinus* InaCC B404
Catalase	+
Oxidase	-
Indole production	-
H_2S formation	-
Citrate utilization	-
Urea utilization	-
Gelatin liquefaction	-
Cellulose production	+
Acid production from	
D-Galactose	+
D-Glucose	+
D-Lactose	-
D-Maltose	+
D-Mannose	-
Melibiose	+
D-Raffinose	+
L-Rhamnose	-
D-Ribose	+
L-Sorbose	-
Sucrose	-

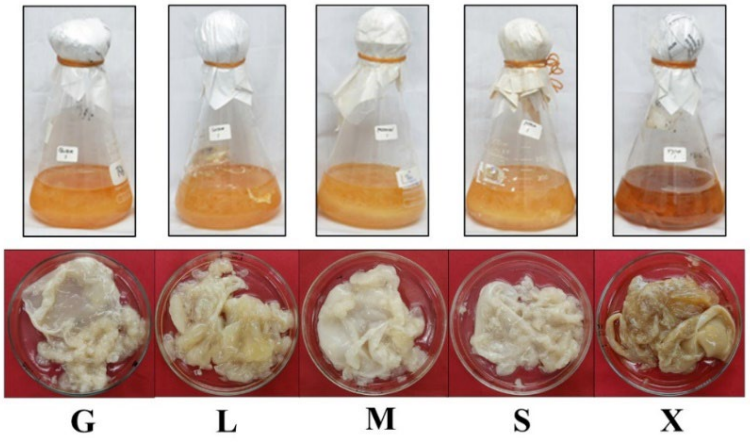

Figure 2. Above, the appearance of 15-day submerged fermentation condition (30°C, 80 rpm, dark condition) of *K. xylinus* InaCC B404 in 150 ml of media with various main carbon sources (G = glucose; L = lactose; M = mannitol; S = sucrose; X = xylose). Below, fresh BC formed after 15 days of submerged fermentation

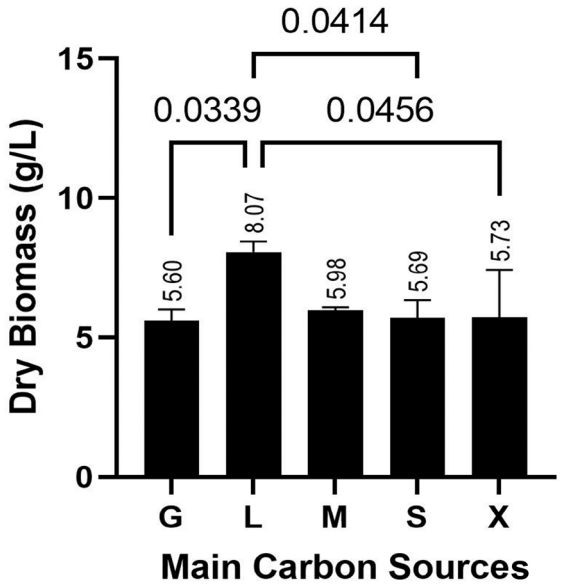

Figure 3. The capability of *K. xylinus* InaCC B404 to produce BC after 15 days of submerged fermentation (30°C, 80 rpm, dark condition) in 150 ml of media with various main carbon sources (G = glucose; L = lactose; M = mannitol; S = sucrose; X = xylose) is defined by weighing the dry biomass. In order to get the dried BC, the fresh BC was put in an oven at 70°C for 24 h. Statistical analysis of the data was done through Tukey's multiple comparisons test (ordinary one-way ANOVA) by comparing each column. P values more than 0.05 are not shown (indicating no significantly differences).

of glucose, mannitol, sucrose, as well as xylose in the media as the main carbon source gave a relatively similar mass of BC. The highest yield was obtained when lactose was used as the main carbon source, and was approximately 44% higher than from glucose (P value > 0.005).

Various factors influence the production of BC by *K. xylinus* InaCC B404 or other cellulose producing bacteria. The optimization of in vitro fermentation conditions is necessary for mass production and the establishment of desirable characteristics of BC. The most crucial factor that has been intensively studied is nutrient variability, including carbon sources, nitrogen sources, phosphorus sources, and trace elements such as mineral and vitamins. The BC synthesis pathway has been elucidated, in which glucose (as prime carbon source) is metabolized to glucose-6-phosphate, and converted to glucose-1-phosphate, then to UDP-glucose that finally gets polymerized to cellulose [8]. However, it is obvious based on our results that even without glucose as the main carbon source, the bacterium is still able to synthesize cellulose. We found that *K. xylinus* InaCC B404 was able to utilize a wide range of main carbon sources, ranging from simple sugars such as xylose ($C_5H_{10}O_5$) and glucose ($C_6H_{12}O_6$), reducing disaccharide (lactose/$C_{12}H_{22}O_{11}$, composed of galactose and glucose), to non-reducing disaccharide (sucrose/$C_{12}H_{22}O_{11}$, composed of fructose and glucose), and even sugar alcohol (mannitol, $C_6H_{14}O_6$).

Lactose as the main carbon source gave the highest yield of bacterial cellulose production by *K. xylinus InaCC* B404, followed by mannitol, xylose, sucrose, and glucose. Even though *K. xylinus* BC pathway utilizes glucose as a primer building block, we found that in submerged fermentation conditions, glucose as the main carbon source produced the least mass of BC. Similarly, under liquid fermentation conditions with organic nitrogen sources such as peptone and

casein hydrolysate, BC production by *K. xylinus* was not optimum when glucose was used as the main carbon source [16]. Lactose itself for many *K. xylinus* strains gave a low yield of BC. However, a study conducted by Singhsa *et al*. [17] showed that some strains of *K. xylinus* produced high BC biomass when utilizing lactose as a carbon source and under agitation, in comparison with glucose, fructose, and sucrose. However, under static conditions, the same strains preferred glucose as a carbon source to produce BC rather than lactose and other carbon sources. This implies that the ability of *K. xylinus* to utilize particular a carbon sources in order to produce BC is strain-dependent, and also dependent on fermentation conditions such as agitation-static conditions.

Our isolate was able to utilize sugar alcohols such as mannitol to produce BC. As a comparison, an isolate of *K. xylinus* was shown to prefer sugar alcohol mannitol, but not xylitol, instead of glucose as the main carbon source to produce BC [18]. In another study, *G. xylinus* was reported to produce BC from C5 sugar alcohol arabitol at more than six times the amount when glucose was used as the carbon source [19]. Hence, sugar alcohol is a carbon source alternative for BC production. Furthermore, sugar alcohols, particularly mannitol and sorbitol, are abundant in plants as well as fungi and play roles as carbohydrate reservoirs in some higher plants [20].

The change of medium acidity was also affected by the selection of carbon sources during fermentation by *K. xylinus* InaCC B404 (Figure 4). Glucose, as the main carbon source, gave the highest increase in acidity, followed by sucrose, mannitol, and xylose. On the other hand, the presence of lactose as the main carbon source resulted in small change of pH in the medium. The level of acidity during the fermentation process affected BC production by *K. xylinus* InaCC B404 (Figure 5). The decrease of pH during fermentation period is linear and negatively correlated with BC yield (R squared 0.8248, 1/slope -2.077).

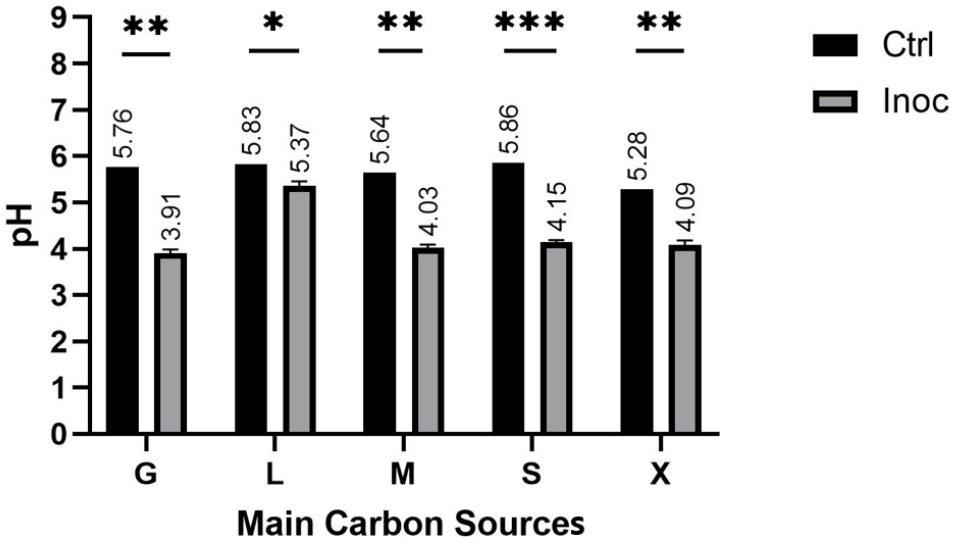

Figure 4. The shifting of pH in liquid part of medium in various main carbon sources (G = glucose; L = lactose; M = mannitol; S = sucrose; X = xylose) after 15 days of submerged fermentation by *K. xylinus* InaCC B404 (Inoc) in comparison with unioculated medium (Ctrl). Statistical analysis of the data was done through Tukey's multiple comparison test (ordinary one-way ANOVA). Asterisk sign indicates the P value of the comparison test, * (P value 0.05 - 0.01), ** (P value 0.01 – 0.001), *** (P value < 0.001). P value more than 0.05 is not shown (indicating no significantly differences).

After 15 days of submerged fermentation by *K. xylinus* InaCC B404, the fermented broth that contained lactose as main carbon source had the lowest pH drop (< 1), but resulted in the highest BC biomass. On the other hand, when other carbon sources, particularly glucose, were used as primary carbon sources, the level of acidity of the broth plummeted by more than 1 unit, with glucose giving the largest pH drop among other carbon sources, and the lowest yield of BC. *Komagataeibacter xylinus* InaCC B404 belongs to the acetic acid bacteria (AAB) group which produce acid, particularly carboxylic acid, during fermentation. However, BC formation is limited by environmental acidity. It has been reported that the production is sub-optimal below 4 [21], making it a challenge to keep the level of acidity high during fermentation in order to achieve high yield. During fermentation with glucose as the main carbon source, the bacteria oxidize glucose to the acidic product, gluconic acid, which readily increases the acidity of the broth, even in the early stage of fermentation [22], thus limited bacterial cellulose production.

During the fermentation period, the efficiency of *K. xylinus* InaCC B404 using available sugar was dependent on the main carbon source in the medium (Figure 6). The Brix degree of all initial fermentation broths was set up at 4.00. In the course of the fermentation period, the bacterial isolate used the least available sugar when lactose was the main carbon source in the medium. The highest sugar utilization was obtained in the presence of mannitol as the main carbon source.

The sugar consumption ability and efficiency of *K. xylinus* InaCC B404 during fermentation varied depending on the carbon sources. In our findings, the isolate utilized less sugar but yielded higher BC when lactose was used as the main carbon source (compared to Figure 3). Such a desirable result shows the value of careful selection of carbon source or substrate for BC production.

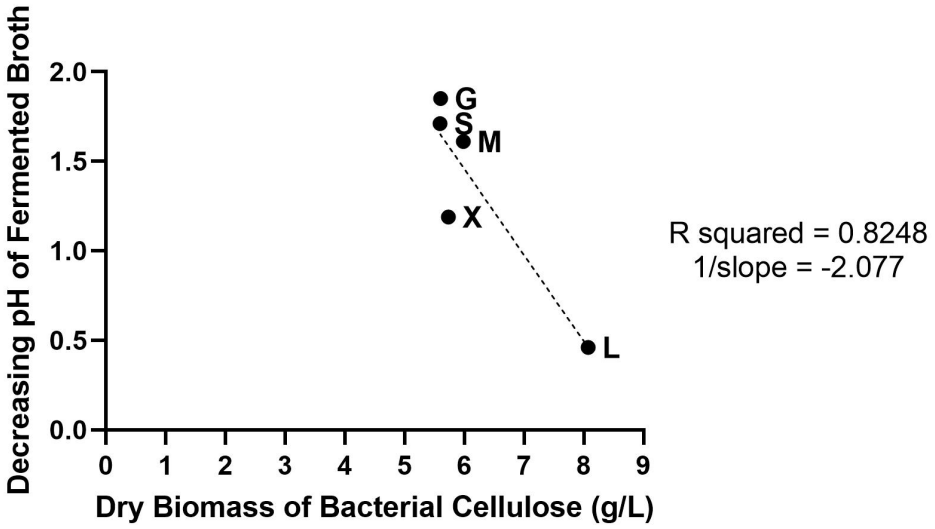

Figure 5. Pearson's correlation plot between the increasing acidity level of environmental liquid broth during 15 days of fermentation by *K. xylinus* InaCC B404 in various main carbon sources (G = glucose; L = lactose; M = mannitol; S = sucrose; X = xylose) with the yield of bacterial cellulose (BC) produced. R squared and 1/slope indicate goodness of fit and best-fit value parameters, respectively.

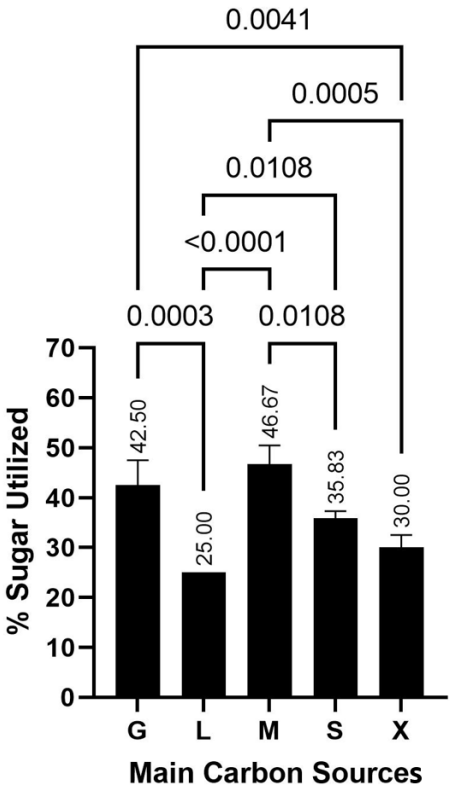

Figure 6. The efficiency of *K. xylinus* InaCC B404 at utilizing sugars as various main carbon sources (G = glucose; L = lactose; M = mannitol; S = sucrose; X = xylose) in order to biosynthesize BC was calculated based on the degree of Brix value of liquid part of the medium after 15 days of submerged fermentation. Statistical analysis of the data was done through Tukey's multiple comparison test (ordinary one-way ANOVA) by comparing each column. P values of more than 0.05 are not shown (indicating no significant differences).

3.3 Physicochemical properties of bacterial cellulose produced

The ability of dried natural BC to absorb water was relatively the same for all carbon sources (Figure 7), with lactose giving a slightly higher result than the other carbon sources, followed by sucrose, mannitol, glucose, and xylose. The swelling capacity of unmodified BC produced by *K. xylinus* InaCC B404 was relatively the same and low for various carbon sources used. A slightly high swelling capacity (> 3) was obtained when lactose was utilized as the main carbon source compare to swelling capacities from other carbon sources (< 3). Even though the dried natural BC gave low swelling capacity values, it had some beneficial properties when set side by side with other sources of cellulose. For example, compared to plant-based cellulose, BC has high purity with no hemicellulose and lignin, as well as high biocompatibility with other materials [23]. Due to its low water-absorbing ability, the natural BC needs to be modified in order to obtain desirable characteristics of hydrogel to be used for various applications. Such modifications, either in-situ [24, 25] as well as ex-situ [26, 27] have been the subjects of thoroughly study recently.

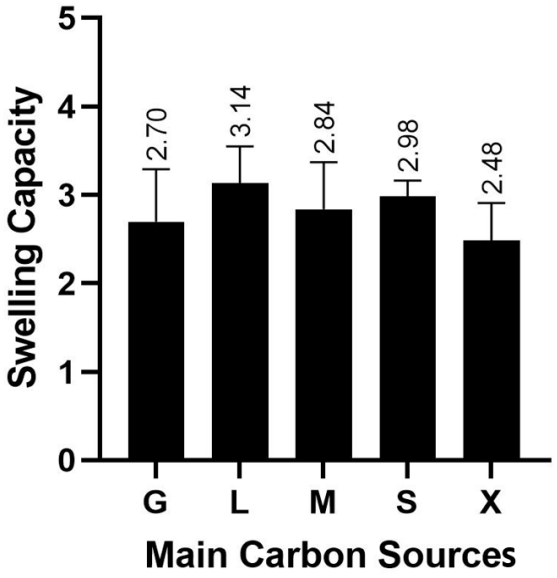

Figure 7. The reversibility of water-absorbing capacity or swelling capacity of dried BC produced by *K. xylinus* InaCC B404BC in various main carbon sources (G = glucose; L = lactose; M = mannitol; S = sucrose; X = xylose). The dried BC was submerged in sterile distilled water for 72 h at room temperature. Statistical analysis of the data was done through Tukey's multiple comparison test (ordinary one-way ANOVA) by comparing each column. P values of more than 0.05 are not shown (indicating no significant differences).

The morphology characteristic of BC produced by *K. xylinus* InaCC B404 from culture media with various main carbon sources was evaluated by SEM, and the results are shown in Figure 8. At such magnification, the BC appeared to form a network, but differences of ruggedness were observed.

Based on SEM micrographs, the appearance of BC derived from lactose and glucose were more rugged than other BCs, and also appeared more porous. On the other hand, the BC derived from sucrose and mannitol were appeared to be smoother. Similarly, the characterization of BC produced by an acid-resistant strain of *Komagataeibacter medellinensis* in various carbon sources showed that the BC produced from glucose had the highest porosity, followed by sucrose and fructose [28]. The porosity of BC produced regarding utilized carbon sources is related to the formation of the nanoribbon network and branching rates of BC [29]. Furthermore, a study showed that carbon sources influence the variability of bacterial cellulose porosity produced by *Gluconacetobacter hansenii* [30]. In this study, the bacterium was fed with two main carbon sources, fructose and glycerol, among other carbon sources, and was reported to produce BC with the highest pore surface area. The porosity of BC is a critical feature that influences other fundamental properties such as stability, permeability, and even compatibility with other materials used for further processing. Therefore, the manipulation of fermentation conditions, including selection of suitable carbon source or other nutrients, is an effective and simple strategy to obtain desirable porosity of BC.

The FTIR spectra of BC samples produced by *K. xylinus* InaCC B404 in different carbon sources are presented in Figure 9. The spectra showed a similar pattern of the vibrational bands,

suggesting that the cellulose produced from various media had identical basic chemical structures. The details of pointed bands are presented in Table 3.

It is obvious that the chemical composition of BC was relatively similar for all carbon sources (Figure 9, Table 3). However, the production yield as well as physical properties such as swelling capacity and porosity, were influenced by the choice of carbon sources. The spectral bands that characterize natural unmodified bacterial cellulose are clearly presented in the spectra of cellulose produced from various main carbon sources. In particular, the existence of peaks at 1402-1410 cm^{-1} and at 1054 cm^{-1} for all BC, which can be assigned to CH_2 bending and C–O–H bond of carbohydrates, respectively, are observed [31].

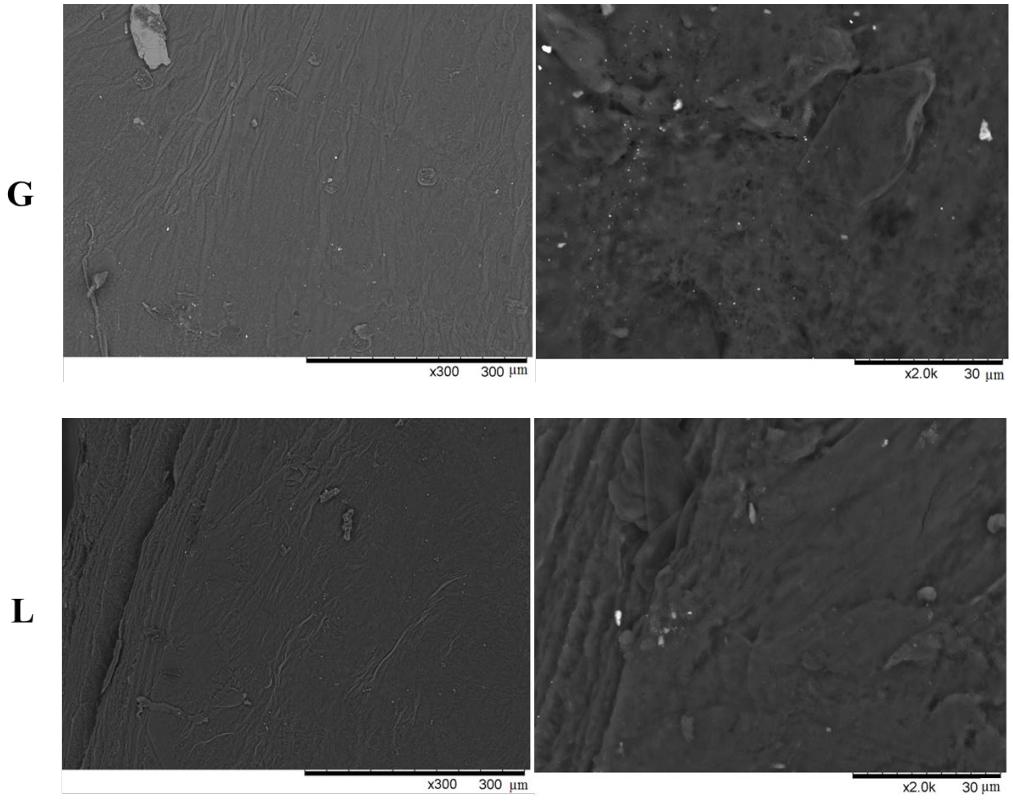

Figure 8. Scanning electron microscope (SEM) micrograph of dried BC network synthesized by *K. xylinus* InaCC B404 in various main carbon sources (G = glucose; L = lactose; M = mannitol; S = sucrose; X = xylose) after15 days of submerged fermentation. The left side shows the 3D cellulose network at magnification 300x, and the right panel shows micrograph at magnification of 2000x.

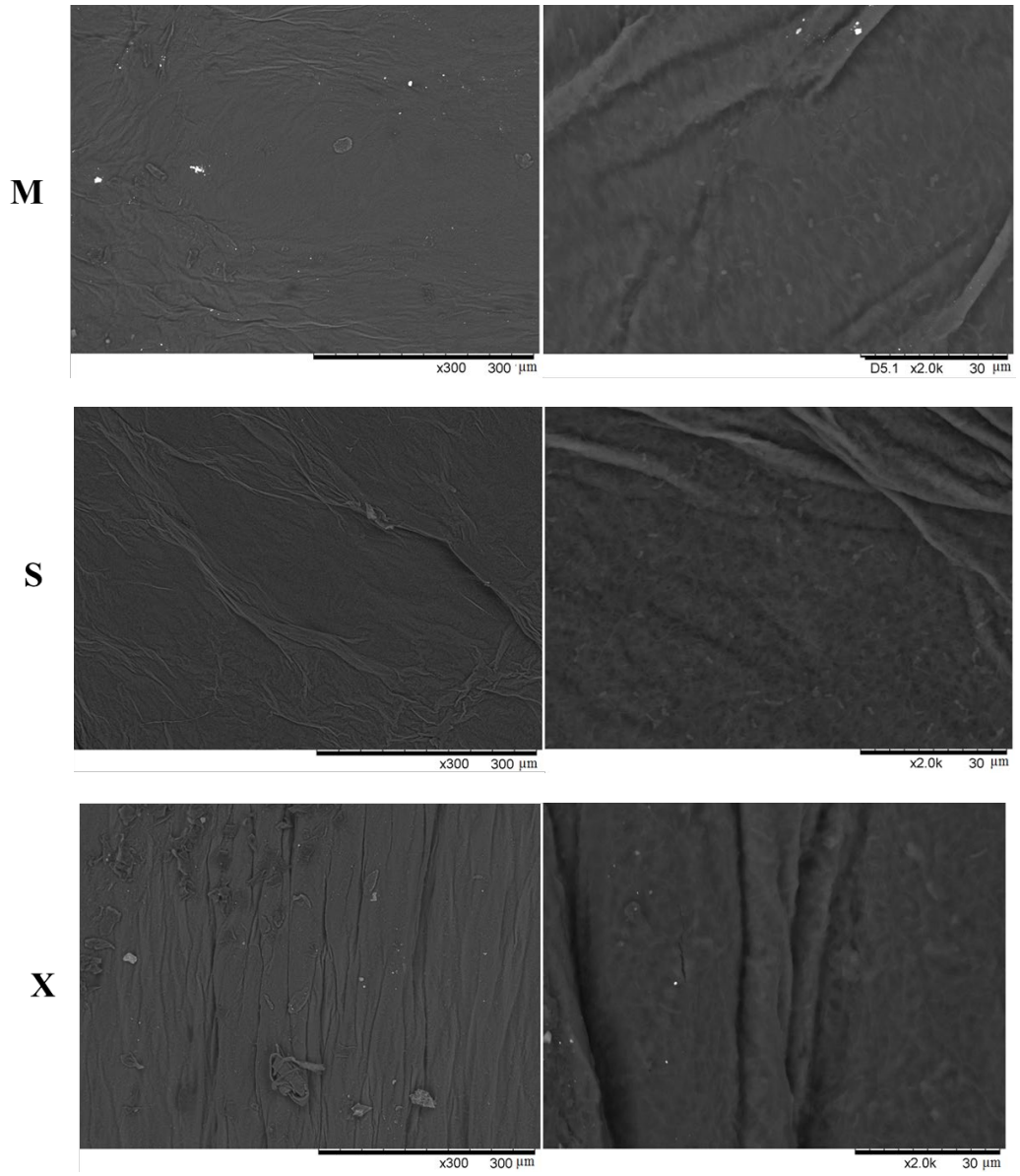

Figure 8. (cont.) Scanning electron microscope (SEM) micrograph of dried BC network synthesized by *K. xylinus* InaCC B404 in various main carbon sources (G = glucose; L = lactose; M = mannitol; S = sucrose; X = xylose) after15 days of submerged fermentation. The left side shows the 3D cellulose network at magnification 300x, and the right panel shows micrograph at magnification of 2000x.

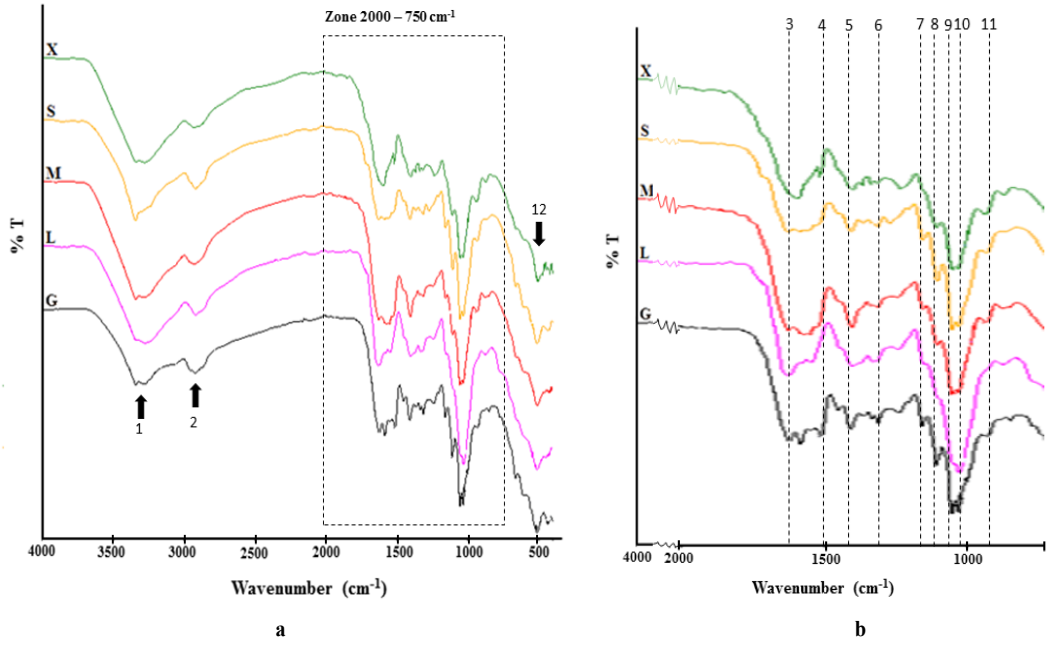

Figure 9. The FTIR spectra of dried BC biosynthesized by *K. xylinus* InaCC B404 in media with various main carbon sources (G = glucose; L = lactose; M = mannitol; S = sucrose; X = xylose) after 15 days of submerged fermentation. (a) comparison of complete spectra; (b) magnification spectra at area range wavenumber between 2000-750 cm^{-1}. The arrows and dotted lines show important peaks related to bacterial cellulose characteristics. Wavenumber and proposed functional group of each peak number are shown in Table 3.

Table 3. Comparative analysis of FTIR spectra of dried BC produced by *K. xylinus* InaCC B404

Peak No.	Wavenumber (cm^{-1})					Functional Groups
	G	L	M	S	X	
1	3343.41	3273.58	3342.76	3345.58	3276.45	O–H stretching
2	2922.08	2922.00	2928.76	2922.88	2930.21	C–H stretching
3	1625.15	1632.07	1627.92	1628.77	-	H–O–H bending from absorbed water
4	1510.97	-	-	-	1519.76	
5	1410.59	1402.50	1404.80	1408.04	1402.20	CH_2 bending
6	1313.29	-	1315.51	-	-	The CH2 group out-of-plane wagging
7	1159.40	-	1156.65	-	-	Antisymmetric bridge stretching C–O–C of 1,4-β- D-glucoside
8	1108.23	-	1106.82	1106.64	1108.09	C–O bending vibration or bonds of C–C from monomer of polysaccharide
9	1054.73	-	1053.98	1053.39	1053.10	C–O–C vibration of pyranose ring skeletal or C–O–H bond from carbohydrates
10	1031.99	1029.49	1033.46	-	-	
11	-	-	935.01	-	938.97	
12	513.75	511.85	509.84	510.98	503.39	

4. Conclusions

The type of carbon sources clearly affected the bacterial cellulose (BC) production yield by our strain *Komagataeibacter xylinus* InaCC B404. This study found that lactose as the main carbon source gave the highest yield compared to other carbon sources. It appeared that the chemical composition of BC was relatively similar for all carbon sources, but the production yield as well as physical properties such as swelling capacity and porosity were influenced by the choice of carbon source. The study highlights the values of the potential use of lactose as a carbon source in the production of BC by *K. xylinus* InaCC B404.

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References

[1] Tomášková, I., Svatoš, M., Macků, J., Vanická, H., Resnerová, K., Čepl, J., Holuša, J., Hosseini, S.M. and Dohrenbusch, A., 2020. Effect of different soil treatments with hydrogel on the performance of drought-sensitive and tolerant tree species in a semi-arid region. *Forests*, 11(2), https://doi.org/10.3390/f11020211.

[2] Mazloom, N., Khorassani, R., Zohury, G.H., Emami, H. and Whalen, J., 2020. Lignin-based hydrogel alleviates drought stress in maize. *Environmental and Experimental Botany*, 175, http://dx.doi.org/10.1016/j.envexpbot.2020.104055.

[3] Jamnická, G., Ditmarová, Ľ., Kurjak, D., Kmeť, J., Pšidová, E., Macková, M., Gömöry, D. and Střelcová, K., 2013. The soil hydrogel improved photosynthetic performance of beech seedlings treated under drought. Plant. *Soil and Environment*, 59(10), 446-451.

[4] Wei, J., Yang, H., Cao, H. and Tan, T., 2016. Using polyaspartic acid hydro-gel as water retaining agent and its effect on plants under drought stress. *Saudi Journal of Biological Sciences*, 23(5), 654-659.

[5] Li, Y., Huang, G., Zhang, X., Li, B., Chen, Y., Lu, T., Lu, T.J. and Xu, F., 2013. Magnetic hydrogels and their potential biomedical applications. *Advanced Functional Materials*, 23(6), 660-672.

[6] Klein, M. and Poverenov, E., 2020. Natural biopolymer-based hydrogels for use in food and agriculture. *Journal of the Science of Food and Agriculture*, 100(6), 2337-2347.

[7] Ross, P., Mayer, R. and Benziman, M., 1991. Cellulose biosynthesis and function in bacteria. *Microbiological Reviews*, 55(1), 35-58.

[8] Lustri, W.R., Barud, H.G.O., Barud, H.S., Peres, M.F.S., Gutierrez, J., Tercjak, A., Junior, O.B.O. and Ribeiro, S.J.L., 2015. Microbial cellulose—biosynthesis mechanisms and medical applications. In: M. Poletto and H.L.O. Junior, eds. *Cellulose-Fundamental Aspects and Current Trends*. London, IntechOpen, pp. 133-157.

[9] Basu, A., Das, D., Bapat, P., Wangikar, P.P. and Phale, P.S., 2009. Sequential utilization of substrates by *Pseudomonas putida* CSV86: signatures of intermediate metabolites and online measurements. *Microbiological Research*, 164(4), 429-437.

[10] Goetghebuer, L., Servais, P. and George, I.F., 2017. Carbon utilization profiles of river bacterial strains facing sole carbon sources suggest metabolic interactions. *FEMS Microbiology Letters*, 364(10), https://doi.org/10.1093/femsle/fnx098.

[11] Yamada, Y., Yukphan, P., Vu, H.T.L., Muramatsu, Y., Ochaikul, D., Tanasupawat, S. and Nakagawa, Y., 2012. Description of *Komagataeibacter* gen. nov., with proposals of new combinations (Acetobacteraceae). *The Journal of General and Applied Microbiology*, 58(5), 397-404.

[12] Hestrin, S. and Schramm, M.J.B.J., 1954. Synthesis of cellulose by *Acetobacter xylinum*. 2. Preparation of freeze-dried cells capable of polymerizing glucose to cellulose. *Biochemical Journal*, 58(2), 345.

[13] Komagata K., Iino T. and Yamada Y., 2014. The family Acetobacteraceae. In: E. Rosenberg, E.F. DeLong, S. Lory, E. Stackebrandt and F. Thompson, eds. *The Prokaryotes.* Berlin: Springer.

[14] Sarkono, S. and Si, M., 2015. *Kajian Bakteri Asam Asetat Penghasil Selulosa Endogenik Buah Masak dan Eksogenik Inokulum Nata*. Ph.D. Universitas Gadjah Mada, Indonesia.

[15] Parte, A.C., 2018. LPSN–List of prokaryotic names with standing in nomenclature (bacterio.net), 20 years on. *International Journal of Systematic and Evolutionary Microbiology*, 68(6), 1825-1829.

[16] Ramana, K.V., Tomar, A. and Singh, L., 2000. Effect of various carbon and nitrogen sources on cellulose synthesis by *Acetobacter xylinum*. *World Journal of Microbiology and*

Biotechnology, 16(3), 245-248.

[17] Singhsa, P., Narain, R. and Manuspiya, H., 2018. Physical structure variations of bacterial cellulose produced by different *Komagataeibacter xylinus* strains and carbon sources in static and agitated conditions. *Cellulose*, 25(3), 1571-1581.

[18] Gullo, M., China, S.L., Petroni, G., Gregorio, S.D. and Giudici, P., 2019. Exploring K2G30 genome: a high bacterial cellulose producing strain in glucose and mannitol based media. *Frontiers in Microbiology*, 10, https://doi.org/10.3389/fmicb.2019.00058.

[19] Oikawa, T., Morino, T. and Ameyama, M., 1995. Production of cellulose from D-arabitol by *Acetobacter xylinum* KU-1. *Bioscience, Biotechnology, and Biochemistry*, 59(8), 1564-1565.

[20] Moing, A., 2000. Sugar alcohols as carbohydrate reserves in some higher plants. In: A.K. Gupta and N. Kaur, eds. *Developments in Crop Science*. Vol. 26. Amsterdam: Elsevier, pp. 337-358.

[21] Jonas, R. and Farah, L.F., 1998. Production and application of microbial cellulose. *Polymer Degradation and Stability*, 59(1-3), 101-106.

[22] Sun, B., Zi, Q., Chen, C., Zhang, H., Gu, Y., Liang, G. and Sun, D., 2018. Study of specific metabolic pattern of *Acetobacter xylinum* NUST4.2 and bacterial cellulose production improvement. *Cellulose Chemistry and Technology*, 52(9-10), 795-801.

[23] Sukara, E. and Meliawati, R., 2016. Potential values of bacterial cellulose for industrial applications. *Jurnal Selulosa*, 4(01), 7-16.

[24] Huang, H.C., Chen, L.C., Lin, S.B., Hsu, C.P. and Chen, H.H., 2010. In situ modification of bacterial cellulose network structure by adding interfering substances during fermentation. *Bioresource Technology*, 101(15), 6084-6091.

[25] Perotti, G.F., Barud, H.S., Messaddeq, Y., Ribeiro, S.J. and Constantino, V.R., 2011. Bacterial cellulose–laponite clay nanocomposites. *Polymer*, 52(1), 157-163.

[26] Buyanov, A.L., Gofman, I.V., Revel'skaya, L.G., Khripunov, A.K. and Tkachenko, A.A., 2010. Anisotropic swelling and mechanical behavior of composite bacterial cellulose–poly (acrylamide or acrylamide–sodium acrylate) hydrogels. *Journal of the Mechanical Behavior of Biomedical Materials*, 3(1), 102-111.

[27] Sakaguchi, M., Ohura, T., Iwata, T., Takahashi, S., Akai, S., Kan, T., Murai, H., Fujiwara, M., Watanabe, O. and Narita, M., 2010. Diblock copolymer of bacterial cellulose and poly (methyl methacrylate) initiated by chain-end-type radicals produced by mechanical scission of glycosidic linkages of bacterial cellulose. *Biomacromolecules*, 11(11), 3059-3066.

[28] Molina-Ramírez, C., Castro, M., Osorio, M., Torres-Taborda, M., Gómez, B., Zuluaga, R., Gómez, C., Gañán, P., Rojas, O.J. and Castro, C., 2017. Effect of different carbon sources on bacterial nanocellulose production and structure using the low pH resistant strain *Komagataeibacter medellinensis*. *Materials*, 10(6), https://doi.org/10.3390/ma10060639.

[29] Kim, U.J., Eom, S.H. and Wada, M., 2010. Thermal decomposition of native cellulose: influence on crystallite size. *Polymer Degradation and Stability*, 95(5), 778-781.

[30] Ashrafi, Z., Lucia, L. and Krause, W., 2019. Bioengineering tunable porosity in bacterial nanocellulose matrices. *Soft Matter*, 15(45), 9359-9367.

[31] Wang, S.S., Han, Y.H., Ye, Y.X., Shi, X.X., Xiang, P., Chen, D.L. and Li, M., 2017. Physicochemical characterization of high-quality bacterial cellulose produced by Komagataeibacter sp. strain W1 and identification of the associated genes in bacterial cellulose production. *RSC Advances*, 7(71), 45145-45155.