PREPARATION AND CHARACTERIZATION OF BACTERIAL CELLULOSE PRODUCED IN MODIFIED HESTERIN-SCHRAMM MEDIUM BY *GLUCONACETOBACTER XYLINUS*

MOHAMMADMAHDI ALIGHANBARI,[*] FIROOZEH DANAFAR,[*] FERESHTEH BAKHTIARI[*] and MAZIAR JAJARMI[**]

[*]*Department of Chemical Engineering, Faculty of Engineering, Shahid Bahonar University of Kerman, Iran*
[**]*Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran*
✉ *Corresponding author: F. Danafar, Danafar@uk.ac.ir*

Received October 11, 2022

This work investigated the suitability of using date syrup for producing bacterial cellulose (BC) by *Gluconacetobacter xylinus* (PTCC 1734). In the preliminary study, BC was produced in Hesterin-Schramm (HS) medium. The highest production yield was achieved after 12 days of cultivation at the initial pH of 8. Considering this result, BC was produced in modified HS media containing date syrup. In the modified HS1 (MHS1) medium, glucose was omitted, and in the modified HS2 (MHS2) medium, neither glucose nor peptone was present. Interestingly, the production yield in the modified media was about 75% higher than the production using HS medium. The amounts of BC produced using MHS1 and MHS2 media were obtained as 5.03 gL^{-1} and 5.17 gL^{-1}, respectively. Structure analysis of these specimens indicated long fibrils, with diameters less than 70 nm. However, the crystallinity index of BC obtained from MHS1 media, containing peptone, was higher than that of BS produced in MHS2.

Keywords: bacterial cellulose, date syrup, Hesterin-Schramm media, *Gluconacetobacter xylinus*

INTRODUCTION

Due to its abundance and eco-friendliness, cellulose is well known not only as a renewable source of energy, but also for the fabrication of different types of commodities.[1,2] Cellulose is a water-insoluble exopolysaccharide made of β (1 → 4) linked D-glucose units.[2] Plants are the main source of cellulose; about 40-50% of wood and 90% of cotton consists of cellulose.[3] The other sources of cellulose are algae and fungi, marine animals, invertebrates, and bacterial species.[2,4] Plant cellulose also has impurities, such as lignin, hemicelluloses, pectin and other non-cellulosic polymers, which need to be removed using a chemical or enzymatic purification method. The purification step may have unwilling effects on the quality of the final product. Besides, the purification methods are generally expensive, which increases the final price of cellulose. In the case of using chemical agents for purification, the undesired substance enters the environment and causes pollution.[3,5]

Bacterial species, such as *Komagataeibacter* (known as *Gluconacetobacter* and *Acetobacter*), *Rhizobium*, *Agrobacterium*, *Aerobacter*, *Achromobacter*, *Zeobacter*, *Salmonella*, *Azotobacter*, *Alcaligenes*, *Pseudomonas*, *Sarcina*, *Enterobacter*, *Rhodococcus* and *Escherchia* produce extracellular cellulose as part of their natural metabolic pathway.[1,6–8] Bacterial cellulose (BC) is the purest form of cellulose that has no impurities, and thus no need for a purification step.[4] The nanoscale structure of bacterial cellulose, accompanied by the purity, leads to outstanding characteristics, such as high water holding capacity, mechanical strength, elasticity, moldability, high degree of porosity, high

crystallinity, high degree of polymerization, low density, non-toxicity, high resilience, retention ability, biological adaptability, and good thermal stability.[9–12] Accordingly, bacterial cellulose has a vast range of applications in various fields, for instance, in medical and biomedical engineering and pharmaceuticals (in wound healing, burn treatment, medical device, tissue repair, surgical implants, regenerative medicine, drug delivery, biomaterials, artificial blood vessels, scaffolds for tissue engineering, dental grafting bone tissue *etc*.);[5,12,13] in electrical engineering (for producing paper-based devices, acoustic diagrams, electrical conductors, magnetic materials, sensors and biosensors, fuel cells, batteries, *etc*.);[1,3,6,9] in food engineering (composites for food packaging, transparent coatings, ingredients, carrier, stabilizer, *etc*.)[1,9,14] and in the field of water treatment as adsorbent.[12]

Commercial production of BC has been limited by the use of traditional cultural media (such as Hesterin-Schramm, Yamanaka, and Zhou), which are expensive and have a low production yield.[11,15,16] Researchers have focused investigations and efforts on finding suitable carbon and nitrogen sources to decrease the expenses of BC production and pave the route to commercial production of BC.[17–19]

Among the bacterial species, Gram-negative, aerobic, non-pathogenic, and non-photosynthetic bacteria, *Gluconacetobacter xylinus* can convert and metabolize a wide range of different carbon and nitrogen sources to produce extracellular cellulose and has the highest productivity.[1,3,12,20–22] This bacterial type can be suitable for dissimilar substrates and can tolerate medium acidity easily.[23–26] According to Figure 1, *Gluconacetobacter xylinus* converts glucose to cellulose as part of its natural metabolism. In the first step, glucose converts to glucose-6-phosphate; then, glucose-6-phosphate isomerizes to glucose-1-phosphate; after that, glucose-1-phosphate is altered to uridine diphosphate glucose (UDP-glucose) and finally, cellulose synthase turns UDP-glucose to cellulose.[22,27]

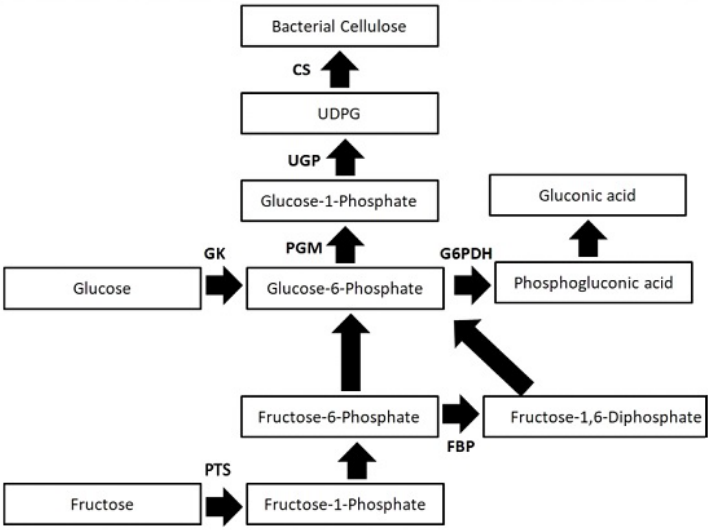

Figure 1: Metabolic pathway of cellulose synthesis by *G. xylinus* (GK – glucokinase, PTS – phosphotransferases system, FBP – fructose-1,6-biphosphate phosphatase, G6PDH – glucose-6-phosphate dehydrogenase, PGM – phosphoglucomutase, UGP – pyrephosphorylase uridine diphosphoglucose, CS – cellulose synthase)[22,27–29]

Several parameters, such as the type and the amount of carbon, nitrogen, and microelements sources, surface-to-volume ratio, temperature, pH, inoculum ratio, dissolved oxygen, and by-products, influence the bacterial growth and the production yield of BC.[12] The combination of culture medium and bacterial strain are important factors as the metabolic pathway can be defined by the culture medium composition, and every bacterial type can have its specific metabolic process.[30,31] It is worth mentioning that not all the carbon sources are converted to bacterial cellulose

by the bacteria, and it depends on their type, as well as the carbon sources. Bacteria produce some undesirable by-products, such as gluconic acid and acetic acid, during their growth pathway, as indicated in Figure 1. These by-products hurt bacterial growth and BC production, by decreasing the pH of the culture medium.[32–34] Disposal foods, agricultural and industrial wastes often include valuable components that can be substituted for expensive culture medium items.[2,6,12,19,23,34–43]

To increase BC productivity and decrease the BC production cost, many non-conventional ingredients, like industrial and agricultural wastes, have been examined as carbon and nitrogen sources.[30] Date syrup is one of these items, which, due to its adequate levels of glucose, fructose, sucrose and protein, is a potential candidate for low-cost carbon and nitrogen sources. Another culture medium containing date syrup was called 'operational culture medium' and was used to cultivate the bacteria for the extracellular production of cellulose.

In this study, the effect of the initial pH of the culture medium on the yields of BC production was studied, and the optimum initial pH was determined. Then, the ability of date syrup for producing bacterial cellulose was investigated. In this respect, the Hesterin-Schramm (HS) medium was modified by substituting the expensive components with the date syrup. In MHS1, the date syrup was used as a carbon source instead of glucose. In the second medium (MHS2), glucose and peptone did not exist and date syrup was used as both carbon and nitrogen source. The yield of cellulose, as a mass of BC produced per initial carbon mass in culture media, was determined for each medium. The structure and morphology of the products with the highest yield obtained from each medium were then characterized and analyzed using XRD, DLS, and FE-SEM.

EXPERIMENTAL

Microorganisms and materials

Gluconacetobacter xylinus PTCC 1734 (Persian Type Culture Collection) was purchased from Iranian Research Organization for Science and Technology.

Glucose, peptone, sodium hydrogen phosphate, hydrogen chloride, sodium hydroxide, and citric acid monohydrate were purchased from Merck (Germany). The yeast extract was Que-Lab brand (USA). Date syrup was purchased from Dombaz, Kian Kaveh Azma, Iranian Companies.

Culture media

In this study, two different culture media were prepared. HS is a traditional complex medium that is used in most research as a main or standard medium. Hesterin-Schramm (HS) medium, which contained pure chemicals, was used as seed culture. The composition of this culture medium is shown in Table 1. At first, all chemicals were dissolved in distilled water and then the solution pH was adjusted to 7 by using 1 molar sodium hydroxide solution. The medium was sterilized at 121 °C for 21 minutes. After cooling the culture medium to the ambient temperature, inoculation was done by adding the provided seed bacterial sample. Inoculated solutions were kept at 30 °C till a layer of cellulose can be observed, then it was stored at 4 °C and used as the seed culture.

Bacterial cellulose (BC) was produced using two different modified HS media. Table 1 shows the compositions of these two media. In the MHS1 medium, date syrup was used instead of glucose, while keeping all other constituents of the HS medium. In MHS2, not only glucose, but also peptone was replaced by date syrup. Based on the date syrup chemical composition analysis, 31.05 g glucose is present in 100 g of date syrup. Accordingly, 32.79 g date syrup can substitute for 20 g glucose in the HS medium. The quantity of date syrup used in each culture medium is shown in Table 1.

Table 1
Composition of operational culture media

Composition	HS ($g.L^{-1}$)	MHS1 ($g.L^{-1}$)	MHS2 ($g.L^{-1}$)
Glucose	20.0	–	–
Date syrup	–	32.79	32.79
Peptone	5.00	5.00	–
Yeast extract	5.00	5.00	5.00
Sodium hydrogen phosphate	2.70	2.70	2.70
Citric acid monohydrate	1.15	1.15	1.15

To examine the effects of the initial pH of the medium on BC production, six HS media with different pH (4-9) were prepared. The amount of BC produced and the final pH of the culture media were

recorded every 4 days for 20 days as the total period time of BC production.

BC production and yield

Inoculation was prepared by adding 3 mL of seed culture to 30 mL of operational culture under the biological hood. The inoculated cultures were kept in an incubator at 30 °C. Every 4 days until twelve days, three flasks were taken out and the pH of the suspension and the amount of BC produced were determined. Each set of experiments was repeated three times under static conditions and averaged.

To measure the amount of BC produced, the surface layer was taken out from the culture media and washed by adding 1% (V/V) sodium hydroxide solution and stirred for 30 minutes at 80 °C to eliminate the bacteria trapped in BC tissues. Then, the BC layers were put in distilled water at 80 °C for 30 minutes to reach a neutral pH. Washed BC layers were placed in an oven at 55 °C for 4 days to eliminate moisture and reach constant weight. Dry BC layers were weighed and the yield of BC production was calculated based on Equation 1. Yield is defined as the ratio of the dry mass of cellulose produced to the initial mass of carbon mass:

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

where m_0 – dry weight of produced BC, and C is the initial carbon mass in culture media.

Characterization of BC

To determine the characteristics of BC, some analysis tests were conducted as described further.

Fourier transform infrared spectroscopy was performed in the range of 400-4000 cm^{-1}. For FTIR spectroscopy analysis, the sample in the form of powder was mixed with dried potassium bromide in a ratio of 1 to 100, and then pressed under the pressure of 5-8 $tons.cm^{-1}$ to obtain tablets. This tablet was introduced into the Brucker Tensor 27 IR system for analysis.[43]

XRD analysis indicates the crystallinity and structure type of the material. This analysis was carried out using an Asenware XDM-300. Samples were scanned between 0°-50° in 2Θ and the crystallinity index was calculated based on Equation 2:[44]

$$CI\ (\%) = \frac{(I_{002} - I_{am})}{I_{002}} \quad (2)$$

where I_{002} is the maximum diffraction intensity, and I_{am} is the diffraction intensity in the amorphous region and at the peak 2Θ=18°.

The structure of BC can be investigated by high resolution FE-SEM. Before taking the images, the sample was subjected to platinum sputtering, which is necessary to make the sample conductive.[45] FE-SEM images were taken using a TESCAN MIRA3-XMU, for BC samples obtained from MHS1 and MHS2 with an initial pH of 7. These two culture media were selected as they showed the highest yield in BC production. These samples were also analyzed by Dynamic Light Scattering (DLS) to measure the size of the BC structure produced. Before the analysis, 0.1 g of the sample was dissolved in 1000 mL of distilled water and then placed in an ultrasonic bath for 5 minutes. After a few minutes, the sample was introduced into the dynamic light scattering instrument (Zen 3600, Marvern).[46]

RESULTS AND DISCUSSION

Production of bacterial cellulose in HS medium

For each test, the amount of bacterial cellulose produced per liter of HS medium was determined every four days, up to twelve days. The yield of production, as presented in Table 2, was calculated using Equation 1. Referring to these results, the culture media with an initial pH of 4-5 presented no BC; while the one with an initial pH of 6 did not have a measurable amount of BC until day four.

According to the results presented in Table 2, BC production can be categorized into three groups considering the initial pH of culture media. In acidic culture media (initial pH of 4 or 5, there was no measurable BC, even after 12 days. By increasing the initial pH to 6, the BC production started and it could be measured after 8 days. By increasing the initial pH of the culture media to 7, the BC production enhanced to 1.70 ± 0.30 ($g.L^{-1}$) after twelve days. Adjusting the initial pH of the culture media to 8 led to an increase in the BC biosynthesis to 2.97 ± 0.13 $g.L^{-1}$ after 12 days of cultivation. However, further increasing the pH to 9 significantly lowered the BC production.

The results obtained in the current study were compared with those presented in other studies for BC production. This comparison revealed slight differences attributed to the variety of the microorganisms and/or the different production conditions used. For instance, Mikkelsen[47] *et al.* reported the BC production of 3.10 g. L^{-1} by using *Gluconacetobacter xylinus* strain ATCC 53524, at 30 °C, after four days of cultivation with the initial pH 5 of the HS culture. Similarly, Çakar F.[15] *et al.* reported BC production by using a culture of *Gluconacetobacter xylinus* FC01 in the HS culture medium with an initial pH of 5, at 30 °C was 0.43 g. L^{-1} after 6 days of cultivation.

Souza[22] *et al.* reported 4.3 $g.L^{-1}$ BC was produced by *Gluconacetobacter xylinus* strain ATCC53582 after 7 days using HS medium, at 30 °C and initial pH of 5. Ye *et al*. reached 3 $g.L^{-1}$ BC after 10 days of cultivation of *Gluconacetobacter xylinus* strain NRRL B- 759 in HS medium, at 30 °C and an initial pH of 5.[48] Khan[19] *et al.* obtained 2.57 g. L^{-1} of BC production after 16 days of cultivation of *K. xylinus* IITRDKH20 in HS medium at 30 °C with an initial pH of 6.

In the current research, it was indicated that *Gluconacetobacter xylinus* PTCC 1734 did not produce any BC using HS medium when the initial pH was lower than 6 even after 12 days.

By increasing the initial pH of cultivation, the amounts of BC produced increased. The yield of BC production after twelve days of cultivation was obtained as 5.33%, 8.50%, and 14.83% at an initial pH of 6, 7, and 8, respectively. In the literature, according to the culture medium composition, microorganism strain, and incubation conditions, such as temperature, incubation time, static or agitated solution, *etc*., an initial pH was suggested in a different range, namely, 5.4 to 6.3,[1] or 4 to 6.[12] On the other hand, on the metabolic pathway of BC production, gluconic acid is produced as a by-product, which lowers the pH of the medium.[28,31] The conversion of glucose to gluconic acid and the accumulation of gluconic acid in the culture medium impedes bacterial growth and cellulose production.[2]

In this study, the maximum BC production by *Gluconacetobacter xylinus* (PTCC 1734) using an HS medium was obtained at the initial pH of cultivation in the range of 7-8 (shown in Table 2). The pH changes in the media were recorded during the twelve days of cultivation, as presented in Table 2. It was observed that the amount of BC production was reduced after 12 days of cultivation. The data in Table 3 revealed the acidification of the culture media with cellulose production. Referring to Figure 1, gluconic acid is a by-product in the pathway of cellulose formation. According to the results presented in Table 2, increasing the initial pH delays the acidification of the culture medium and can help to produce more BC. However, there is a limitation in increasing the pH. When the initial pH was set at 9, no BC was produced as this pH is far from the optimum pH (6.5-7) determined for bacterial cultivation. Based on the data in Table 2, the maximum BC production belongs to the culture media with an initial pH of 6 to 8.

By measuring the amount of BC produced, it was found that the amount of BC produced did not change considerably after the twelfth day. Two probabilities can justify this observation; acidification of the media or lack of nutrition in the culture medium. To examine whether acidic media or lack of nutrients stop BC formation, it was supposed there are sufficient nutrients in the media and the low pH ceases the BC formation, as bacteria cannot survive in this condition. Accordingly, on day 12^{th}, 5 mL of sodium hydroxide (1 molar) was added to the culture to increase the pH of the culture medium from 4.23 to 6.3. The amount of BC produced was measured on the days 16^{th} and 20^{th} of cultivation, as presented in Table 3. It was observed that the BC production continued until the 16^{th} day of cultivation as the amount of BC increased from 2.07 to 4.30 $g.L^{-1}$. However, there is no significant difference in the amounts of BC produced after 16 and 20 days of cultivation (Table 3). Consequently, increasing the medium pH can be helpful until the 16^{th} day of cultivation and the amount of BC produced decreased to 3.83 on the 20^{th} day. Such observation can be attributed to the lack of required nutrition, especially carbon and nitrogen sources for bacterial activities. It seems BC was consumed by the bacteria as the mass of BC decreased. Accordingly, the reason for stopping BC production after the 12^{th} day is acidification of the media, not lack of nutrients. However, it is true until the 16^{th} day (in this research), and after that, the medium does not contain enough nutrients for bacteria to activate.

BC production in modified HS cultures

In this work, based on the HS medium, the carbon source was modified using date syrup and also date syrup protein was evaluated by elimination of peptone from the HS medium. Based on the results obtained for BC production in the HS culture medium, the pH of the MHS1 and MHS2 media was adjusted to 7 and 8. Table 4 shows the amounts of BC produced in MHS1 and MHS2 and the pH of the culture media recorded during the 12 days of cultivation. Based on the results in Table 4, more BC was produced using modified culture media accompanied by fewer pH changes during cultivation. The differences between the two cultures were only in existing peptone in the media, as MHS2 had no peptone in it.

Table 2
Yields of bacterial cellulose produced in HS medium and final pH of culture media every four days to twelve days

Initial pH	Fourth day			Eighth day			Twelfth day		
	Amount ($g.L^{-1}$)	Percent yield	Final pH	Amount ($g.L^{-1}$)	Percent yield	Final pH	Amount ($g.L^{-1}$)	Percent yield	Final pH
4	–	–		–	–		–	–	
5	–	–		–	–		–	–	
6	–	–	5.38±0.05	0.57±0.33	2.83	4.43±0.03	1.07±0.13	5.33	3.70±0.45
7	0.30±0.03	1.50	4.76±0.02	0.96±0.29	4.80	4.22±0.13	1.70±0.30	8.50	4.19±0.22
8	0.30±0.12	1.50	4.94±0.07	1.60±0.40	8.00	4.93±0.28	2.97±0.13	14.83	4.96±0.44
9			5.06±0.21	–		5.33±0.07	–		5.36±0.07

Table 3
Amounts of BC produced (gL^{-1}) and pH changes after adding 5 mL of sodium hydroxide (1 molar) to the HS medium at initial pH 7

Day	12^{th} day	16^{th} day	20^{th} day
BC production amount ($g.L^{-1}$)	2.07±0.09	4.30±0.03	3.83±0.21
Final pH	6.3±0.49	5.90±0.48	5.92±0.68

Table 4
Amounts of BC produced ($g.L^{-1}$), percent yield, and final pH after 12 days in MHS1 and MHS2 with initial pH of 7

Medium	Fourth day			Eighth day			Twelfth day		
	Amount ($g.L^{-1}$)	Percent yield	Final pH	Amount ($g.L^{-1}$)	Percent yield	Final pH	Amount ($g.L^{-1}$)	Percent yield	Final pH
MMHS1	1.30±0.33	6.5	4.96±0.14	3.83±0.17	19.17	5.46±0.12	5.03±0.03	25.17	5.83±0.03
MMHS2	2.57±0.13	12.83	4.91±0.18	4.50±0.30	22.50	5.54±0.15	5.17±0.03	25.83	5.59±0.09

Table 5
Chemical composition of date syrup (100 g)

Component	Glucose	Fructose	Protein	Magnesium	Calcium	Sodium	Potassium
Amount (g)	31.05	33.10	1.58	0.0852	0.1573	0.1573	1.3109

Table 6
Amounts of BC produced ($g.L^{-1}$), pH changes, and yield percentage calculated for BC production after 12 days in MHS2 with initial pH 8

Day	Fourth day	Eighth day	Twelfth day
BC production ($g.L^{-1}$)	1.1±0.05	3.50±0.24	4.43±0.29
Final pH	5.07±0.20	5.43±0.08	5.57±0.32
Yield percentage	5.50	17.50	22.15

The amounts of BC produced using MHS1 in the early days of cultivation were lower than when using MHS2. However, the difference got smaller as the cultivation proceeded. On the 12th day of cultivation, the BC production in MHS1 and MHS2 reached 5.03 ± 0.03 and 5.17 ± 0.03 g. L^{-1}, respectively.

The factory the date syrup was bought from provided the datasheet for the important components of the product, as presented in Table 5. According to the data, date syrup contains glucose, fructose, protein, magnesium, calcium, sodium, and potassium. These elements can have positive effects on bacterial growth and improve BC production. The highest yield of BC production was 25.83%, produced in MHS2. To examine whether the yield of BC increased with the initial pH, an experiment was conducted: the initial pH of MHS2 was increased to 8, and its results are presented in Table 6. Considering the results presented in Table 2, the maximum amount of BC produced using HS culture was about 2.97 gL^{-1} (yield = 14.8) at pH 8. When the MHS2 medium was used, about 4.43 gL^{-1} (22.15%) BC was formed at the same pH.

Characterization of produced BC

Fourier-transform infrared spectroscopy (FTIR)

The FTIR spectra for the samples were identical. As an example, the FTIR spectrum for the BC sample produced in MHS1 at the initial pH=7 is shown in Figure 2. Several peaks, such as those at 3375.09 cm^{-1}, 2920.73 cm^{-1}, and 1163.67 cm^{-1}, demonstrate the O-H stretching vibration bond, C-H bond, and C-O-C stretching bond, respectively.[19,43,48] Other peaks, like that at 1060.93 cm^{-1}, are related to C-O and those at the wavelength of 1330.08 cm^{-1} and 1432.92 cm^{-1} are connected to O-H rotational bond and CH_2 stretching bond.[19,22] There are slight differences between the FTIR spectrum in Figure 1 and those typical of pure cellulose. The differences can be due to some impurities present in the sample.

X-ray diffraction (XRD)

The crystalline structure of BC was examined by XRD analysis.[2] The diffractograms of the BC produced in MHS1 and MHS2 media (Figs. 3 and 4) present three peaks related to cellulose. Diffraction angles of 14.80º, a weak peak at 17.55º, and at 23.00º can be identified in Figure 3 for BC from MHS1, and those at 14.90º, 17.05º and 22.50º – in Figure 4 for BC from MHS2. These three peaks are characteristic of cellulose type I.[2] Cellulose type I is abundant in nature, and cellulose type II has a complex hydrogen bonding in comparison with cellulose type I. Based on this, cellulose type I has a parallel structure, unlike cellulose type II, which has an impact on its chemical and physical properties. The crystallinity index of the BC produced in MHS1 and MHS2 media, calculated by using Equation 2, has been found of 42% and 53%, respectively. The only difference between these two media consisted in the presence of an extra nitrogen source, *i.e.* peptone, existing in the MHS1 culture medium. Based on the CrI calculation and Figures 3 and 4, the amount and the crystallinity of the BC produced in MHS2 were higher than in MHS1 during the early eight days of cultivation.

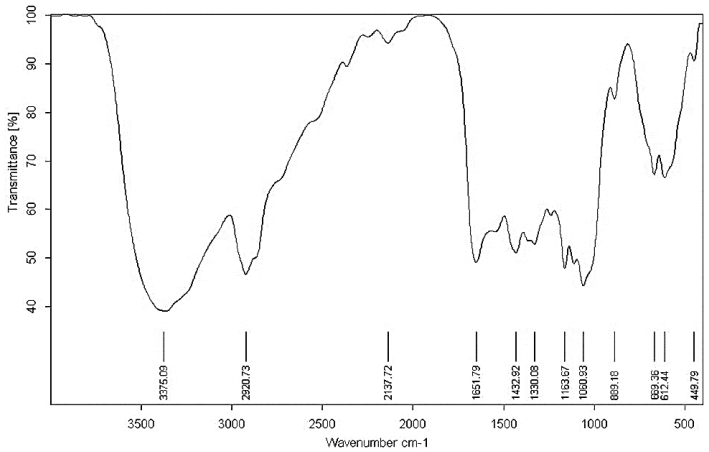

Figure 2: FTIR spectrum of BC from MHS1

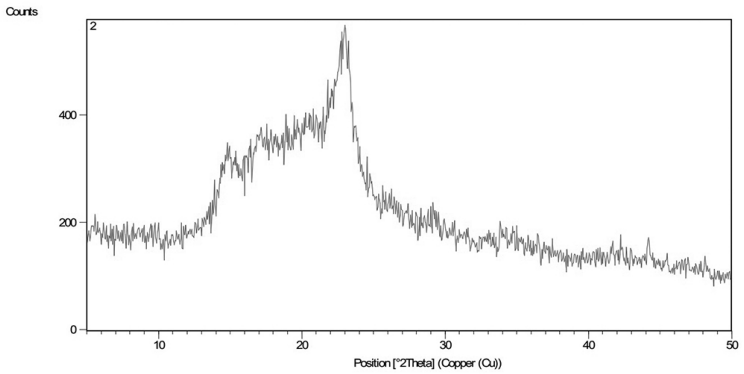

Figure 3: X-ray diffractogram of BC produced in MHS1 with initial pH 7

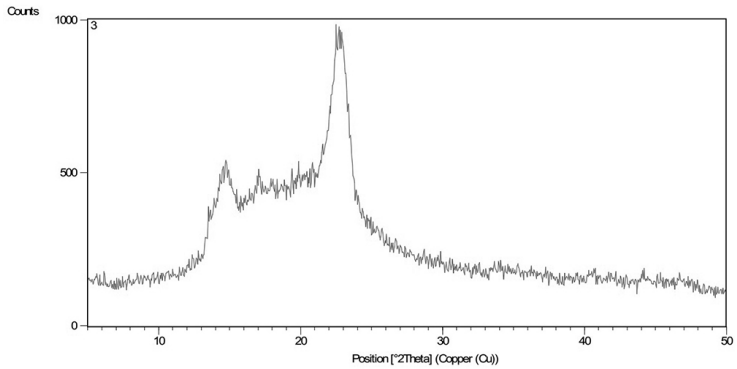

Figure 4: X-ray diffractogram of BC produced in MHS2 with initial pH 7

Other researchers reported a wide range of crystallinity index values for BC. For instance, Khan[19] *et al.* produced BC in pineapple peel extract medium in 7 days, and found 91.82% crystallinity, while that in sweet lime peel extract medium had 79.08% crystallinity. Souza E.[22] *et al.* recorded CrI values of 87%, 80%, and 79% for BC using HS medium, cashew apple juice with yeast extract, and cashew apple juice with soybean molasses media, respectively, in 7 days. The CrI of BC produced in MHS1 and MHS2 media, in comparison with those for BC obtained from other sources reported in the literature, are in the lower range. One of the reasons could be the impurities of date syrup. On the other hand, it must be also taken into consideration the fact that BC crystallinity can be affected by the bacterial strain, and culture conditions, such as

temperature, static or agitated culture, incubation time, drying method, *etc*.[2,49] In addition, agricultural waste used for the production of BC must be submitted to mild pretreatments, so as to have minimum negative influence on the quality of BC.

Field emission scanning electron microscopy (FE-SEM)

Figures 5 and 6 present the FE-SEM micrographs for the BC produced in the MHS1 and MHS2 media, respectively. These images reveal that the produced BC was constituted from fibers of nanometer dimensions. However, in corroboration with XRD analysis, the BC produced using MHS1 makes up a network of long and lean cellulosic fibers.

Dynamic light scattering (DLS)

DLS analysis of the BC sample produced from MHS1 is shown in Figure 7. The size distribution of BC is uniform, within the range of 35-65 nm. The size of BC nanofibrils are close to the ranges reported by other researchers, such as Avcioglu[2] (35-75 nm), Khan[19] (40-50 nm), and Sanabria[43] (50-60 nm).

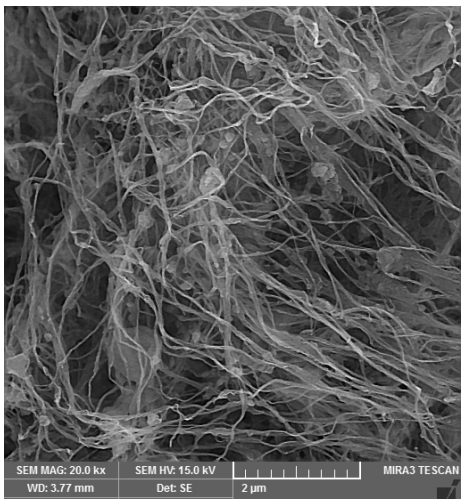

Figure 5: FE-SEM image of BC produced in MHS1 with initial pH of 7

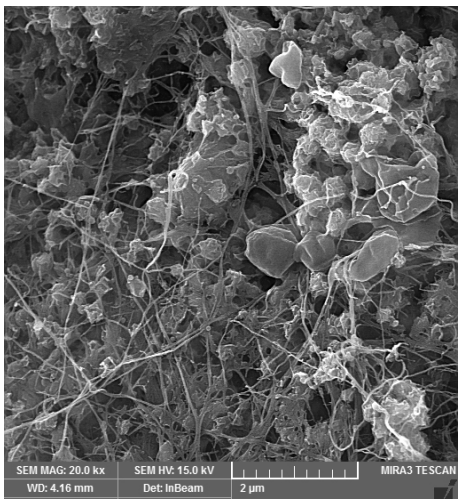

Figure 6: FE-SEM image of BC produced in MHS2 with initial pH of 7

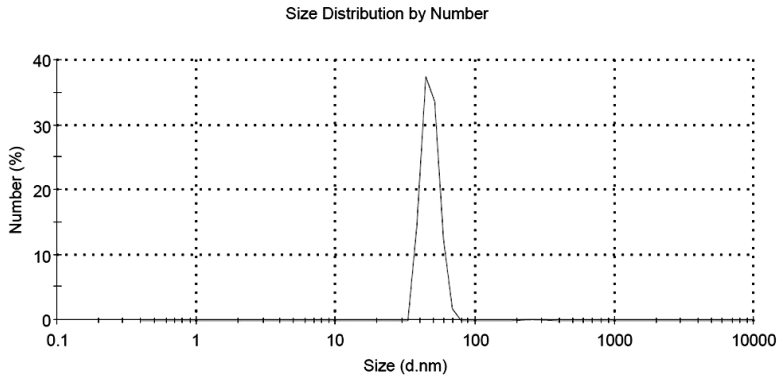

Figure 7: Size distribution of BC fibril diameters (MHS1 with initial pH 7)

CONCLUSION

This research investigated the effects of initial pH on the yield of BC production. Choosing the appropriate initial pH, the potential application of date syrup, as a cheap and sustainable substrate, was evaluated for bacterial cellulose production. BC was produced using *G. xylinus* (PTCC 1734), at first in HS medium in the pH range of 4-9. The highest yield of BC was obtained at the initial pH of 8, when 2.97 gL^{-1} cellulose was produced. After 12 days of cultivation, cellulose production was stopped due to acidification of the medium. Adding 5 mL of sodium hydroxide to the culture continued BC production for the next 4 days.

To decrease the BC production cost, date syrup was examined to be used as a carbon and nitrogen source in the HS medium. Based on the experiment results, when the initial pH of the

culture was 7, about 5.03 and 5.17 gL^{-1} cellulose was produced in MHSI and MHS2, respectively. By increasing the initial pH of MHS1 to 8, BC production increased to 5.57 gL^{-1}. Due to the presence of various sugars, proteins, and other elements in date syrup, it has the potential of being a carbon and nitrogen source for BC production. Interestingly, the yield of BC production using modified HS media was about 75% higher than the yield of BC obtained from the common HS medium. However, the crystallinity of the BC produced in the modified medium that contained peptone was higher than that of the BC produced in the absence of this component.

REFERENCES

[1] S. Barshan, M. Rezazadeh-Bari, H. Almasi and S. Amiri, *Int. J. Biol. Macromol.*, **136**, 1188 (2019), https://doi.org/10.1016/j.ijbiomac.2019.06.192

[2] N. H. Avcioglu, M. Birben and I. Seyis Bilkay, *Process Biochem.*, **108**, 60 (2021), https://doi.org/10.1016/j.procbio.2021.06.005

[3] M. Güzel and Ö. Akpınar, *Int. J. Biol. Macromol.*, **162**, 1597 (2020), https://doi.org/10.1016/j.ijbiomac.2020.08.049

[4] B. Behera, D. Laavanya and P. Balasubramanian, *Bioresour. Technol.*, **346**, 126659 (2022), https://doi.org/10.1016/j.biortech.2021.126659

[5] A. Rastogi and R. Banerjee, *Process Biochem.*, **91**, 297 (2020), https://doi.org/10.1016/j.procbio.2019.12.021

[6] K. Naloka, K. Matsushita and G. Theeragool, *Int. J. Biol. Macromol.*, **150**, 1113 (2020), https://doi.org/10.1016/j.ijbiomac.2019.10.117

[7] V. V. Revin, A. V. Dolganov, E. V. Liyaskina, N. B. Nazarova, A. V. Balandina *et al.*, *Polymers* (*Basel*), **13**, 1422 (2021), https://doi.org/10.3390/polym13091422

[8] C. Chen, W. Ding, H. Zhang, L. Zhang, Y. Huang *et al.*, *Carbohyd. Polym.*, **278**, 118995 (2022), https://doi.org/10.1016/j.carbpol.2021.118995

[9] E. A. Skiba, V. V. Budaeva and E. V. Ovchinnikova, *Chem. Eng. J.*, **383**, 123128 (2020), https://doi.org/10.1016/j.cej.2019.123128

[10] G. Chen, G. Wu, L. Chen, W. Wang, F. F. Hong *et al.*, *Carbohyd. Polym.*, **215**, 280 (2019), https://doi.org/10.1016/j.carbpol.2019.03.080

[11] X. Zhai, D. Lin, W. Li and X. Yang, *Int. J. Biol. Macromol.*, **149**, 186 (2020), https://doi.org/10.1016/j.ijbiomac.2020.01.230

[12] S. N. N. Said Azmi, S. N. N. F. Mohd Fabli and F. A. Faisul Aris, *Mater. Today*, **42**, 101 (2019), https://doi.org/10.1016/j.matpr.2020.10.220

[13] A. H. Bhat, I. Khan, M. A. Usmani, R. Umapathi and. S. M. Z. Al-Kindy, *Int. J. Biol. Macromol.*, **129**, 750 (2018), https://doi.org/10.1016/j.ijbiomac.2018.12.190

[14] C. Sharma and N. K. Bhardwaj, *Mater. Sci. Eng. C*, **104**, 109963 (2019), https://doi.org/10.1016/j.msec.2019.109963

[15] F. Çakar, A. Kati, I. Özer, D. D. Demirbağ, F. Şahin *et al.*, *Biochem. Eng. J.*, **92**, 35 (2014), https://doi.org/10.1016/j.bej.2014.07.002

[16] N. M. S. Sá, A. L. A Mattos, L. M. A. Silva, E. S. Brito, M. F. Rosa *et al.*, *Int. J. Biol. Macromol.*, **161**, 1337 (2020), https://doi.org/10.1016/j.ijbiomac.2020.07.269

[17] R. F. Dórame-Miranda, N. Gámez-Meza, L. Medina-Juárez, J. M. Ezquerra-Brauer, M. Ovando-Martínez *et al.*, *Carbohyd. Polym.*, **207**, 91 (2019), https://doi.org/10.1016/j.carbpol.2018.11.067

[18] E. E. Kiziltas, A. Kiziltas and D. J. Gardner, *Carbohyd. Polym.*, **124**, 131 (2015), https://doi.org/10.1016/j.carbpol.2015.01.036

[19] H. Khan, V. Saroha, S. Raghuvanshi, A. K. Bharti and D. Dutt, *Carbohyd. Polym.*, **260**, 117807 (2021), https://doi.org/10.1016/j.carbpol.2021.117807

[20] C. H. Kuo, J. H. Chen, B. K. Liou and C. K. Lee, *Food Hydrocoll.*, **53**, 98 (2016), https://doi.org/10.1016/j.foodhyd.2014.12.034

[21] E. Bilgi, E. Bayir, A. S. Urkmez and E. E. Hames, *Int. J. Biol. Macromol.*, **90**, 2 (2016), https://doi.org/10.1016/j.ijbiomac.2016.02.052

[22] E. F. Souza, M. R. Furtado, C. W. P. Carvalho, O. Freitas-Silva and L. M. F. Gottschalk, *Int. J. Biol. Macromol.*, **146**, 285 (2020), https://doi.org/10.1016/j.ijbiomac.2019.12.180

[23] Y. Liu, F. Wang and Y. Sun, *Bioresources*, **16**, 1042 (2020), https://doi.org/10.15376/biores.16.1.1042-1062

[24] V. Tuan, B. Flanagan and D. Mikkelsen, *Carbohyd. Polym.*, **80**, 337 (2010), https://doi.org/10.1016/j.carbpol.2009.11.019

[25] S. S. Kim, S. Y. Lee, K. J. Park, S. M. Park, H. J. An *et al.*, *Saudi J. Biol. Sci.*, **24**, 314 (2015), https://doi.org/10.1016/j.sjbs.2015.09.031

[26] M. J. Tabaii and G. Emtiazi, *Appl. Food Biotechnol.*, **3**, 35 (2016), https://doi.org/10.22037/afb.v3i1.10582

[27] P. Ross, R. Mayer and M. Benziman, *Microbiol. Rev.*, **55**, 35 (1991), https://doi.org/10.1128/mr.55.1.35-58.1991

[28] M. Moniri, A. B. Moghaddam and S. Azizi, *Nanomaterials*, **7**, 1 (2017), https://doi.org/10.3390/nano7090257

[29] K. Ji, W. Wang and B. Zeng, *Sci. Rep.*, **6**, 21863 (2016), https://doi.org/10.1038/srep21863

[30] G. Sperotto, L. G. Stasiak, J. P. M. G. Godoi, N. C. Gabiatti and S. S. De Souza, *Cellulose*, **28**, 2649 (2021), https://doi.org/10.1007/s10570-021-03754-5
[31] R. R. Singhania, A. K. Patel and Y. S. Tseng, *Bioresour. Technol.*, **344**, 126343 (2021), https://doi.org/10.1016/j.biortech.2021.126343
[32] Z. Li, S. Q. Chen, X. Cao, L. Li, J Zhu *et al.*, *J. Microbiol. Biotechnol.*, **31**, 429 (2021), https://doi.org/10.4014/jmb.2010.10054
[33] P. Gomes, N. H. C. S. Silva, E. Trovatti, L. S. Serafim, M. F. Duarte *et al.*, *Biomass Bioenerg.*, **55**, 205 (2013), https://doi.org/10.1016/j.biombioe.2013.02.004
[34] Q. Wang, P. C. Nnanna and F. Shen, *Ind. Crop. Prod.*, **162**, 113256 (2021), https://doi.org/10.1016/j.indcrop.2021.113256
[35] S. M. F. Silva, H. L. Ribeiro, A. L. A. Mattos, M. de F Borges, M. de F Rosa *et al.*, *J. Food Sci. Technol.*, **58**, 1979 (2021), https://doi.org/10.1007/s13197-020-04709-7
[36] K. Filippi, H. Papapostolou and M. Alexandri, *Bioresour. Technol.*, **343**, 125989 (2022), https://doi.org/10.1016/j.biortech.2021.125989
[37] S. Lee, A. Abraham, A. C. S. Lim, O. Choi, J. G. Seo *et al.*, *Bioresour. Technol.*, **342**, 125918 (2021), https://doi.org/10.1016/j.biortech.2021.125918
[38] W. Jaroennonthasit, N. T. Lam and P. Sukyai, *Int. J. Biol. Macromol.*, **191**, 299 (2021), https://doi.org/10.1016/j.ijbiomac.2021.09.028
[39] E. Leonarski, K. Cesca, E. Zanella, B. U. Stambuk, D. de Oliveira *et al.*, *LWT*, **135**, 1 (2021), https://doi.org/10.1016/j.lwt.2020.110075
[40] Y. A. Ramírez Tapias, M. V. Di Monte, M. A. Peltzer and A. G. Salvay, *Food Chem.*, **372**, 131346 (2022), https://doi.org/10.1016/j.foodchem.2021.131346
[41] X. Ma, H. Yuan, H. Wang and H. Yu, *Bioprocess. Biosyst. Eng.*, **44**, 2231 (2021), https://doi.org/10.1007/s00449-021-02599-3
[42] S. A. Villarreal-Soto, J. Bouajila, S. Beaufort, D. Bonneaud, J. P. Souchard *et al.*, *J. Vinyl Addit. Technol.*, **27**, 183 (2021), https://doi.org/10.1002/vnl.21795
[43] O. L. Saavedra-Sanabria, D. Durán, J. Cabezas, I. Hernández, C. Blanco-Tirado *et al.*, *Carbohyd. Polym.*, **274**, 118645 (2021), https://doi.org/10.1016/j.carbpol.2021.118645
[44] P. H. F. Pereira, N. F. Souza, H. L. Ornaghi and M. R. de Freitas, *Ind. Crop. Prod.*, **150**, 112305 (2020), https://doi.org/10.1016/j.indcrop.2020.112305
[45] M. H. Salehi, H. Golbaten-Mofrad and S. H. Jafari, *Int. J. Biol. Macromol.*, **173**, 467 (2021), https://doi.org/10.1016/j.ijbiomac.2021.01.121
[46] Y. Lu, J. Li, L. Ge, W. Xie and D. Wu, *Carbohyd. Polym.*, **255**, 117483 (2021), https://doi.org/10.1016/j.carbpol.2020.117483
[47] D. Mikkelsen, B. M. Flanagan, G. A. Dykes and M. J. Gidley, *J. Appl. Microbiol.*, **107**, 576 (2009), https://doi.org/10.1111/j.1365-2672.2009.04226.x
[48] Y. E. Öz and M. Kalender, *Cellulose Chem. Technol.*, **55**, 1060 (2021), https://doi.org/10.35812/CelluloseChemTechnol.2021.55.90
[49] S. Mohamad, L. C. Abdullah, S. S. Jamari, S. S. O. Al Edrus, M. M. Aung *et al.*, *J. Mater. Sci.*, **57**, 1462 (2022), https://doi.org/10.1007/s10853-021-06685-5