Egypt. J. Chem. **Vol. 64,** No. 4 pp. 2213 - 2221 (2021)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Production enhancement of bacterial cellulose nanofiber using local *Komagataeibacter xylinus* SB3.1 under static conditions

Ahmed M. Alemam[a]*, Tharwat I. Shaheen[b], Saad El Din Hassan[a], Said E. Desouky[a] ,Mamdouh S. El-Gamal[a].

[a]*Botany and Microbiology Departmennt, Faculty of Science, Al-Azhar University, Nasr City, Cairo, 11884, Egypt*

[b]*National Research Centre, Textile Research Division,.ElBuhouth St., (former El-Tahrir str.), Dokki, P.O. 12622, Giza, Egypt*

Abstract

Bacterial cellulose (BC) is a nanostructured material mainly produced by genus *Gluconacetobacter* displays unique physicochemical and mechanical properties therefore it has many potential applications in biomedical, biosensor, food and other industries. However, application of bacterial cellulose faced one of the main big problems in industry, viz., low productivity. Herein, this work was undertaken with a view to enhance the BC production using *Komagataeibacter xylinus* SB3.1 under static condition through study the effective culture parameters that played a vital role in nanofiber production. The results revealed the successful production of BC nanofibers using *K. xylinus* SB3.1 under static conditions as obviously indicated from the SEM image. FTIR and XRD affirmed both the chemical structure and crystallographic nature of cellulose I of the produced nanofibers. In the improvement effort, maximum yield of BC reached 6.54 g/L under optimal conditions including the use of mannitol and yeast extract as the sole source of carbon and nitrogen during an 8 day incubation period at 30 ◦C with 8% inoculum size which reveals to the increase in the nanofiber production 3.3 folded times than others with unoptimized condition under the same stationary conditions of growth. This also opens up the window for utilizing a new locally isolated K. xylinus SB3 in industrial manufacturing with potential features.

Keywords: Bacterial Cellulose; Komagataeibacter xylinus; Nanofiber, Acetobacter; FTIR; XRD

Introduction

Cellulose is the most abundant biopolymer, renewable and biodegradable produced in the earth with 180 billion tons per year in nature [1]. BC is a bacterial-based homopolymer of β(1→4) D-glucopyranose units intertwined by intermolecular hydrogen bonds with the formula [$(C_6H_{10}O_5)$n][2], Bacteria of the family *Acetobacteraceae* are most commonly used for BC production, mainly bacteria from the genus *Komagataeibacter* and usually strains of the species *Komagataeibacter xylinus* (previously known as *Gluconacetobacter xylinus*). Gram negative bacteria in *Komagataeibacter* (former *Gluconacetobacter* and *Acetobacter*) genus are mainly cellulose producer these bacteria are isolated from different sources, strictly aerobic and produce BC as an extracellular product at the air liquid interface of the growth media at pH with in 3 and 7 and temperatures ranged from 28 to 30◦C [3, 4]. Plant cellulose (PC) and BC have a much the same chemical structure[5]. However, bacterial cellulose is contrasting from PC in some physicochemical and mechanical properties, including fibrils where, bacterial cellulose are 100 times thinner than that of PC, making it more porous, finer structure (nanoscale microfibrils < 10 nm in width), higher purity (free from hemicellulose and lignin), longer fiber length (polymerization degree between 2000 and 6000), higher crystallinity, higher water absorbing and holding capacity, higher tensile strength, strong biological adaptability, nontoxic and

*Corresponding author e-mail: ahmed.alemam@azhar.edu.eg
Receive Date: 09 December 2020, Revise Date: 10 January 2021, Accept Date: 17 January 2021
DOI: 10.21608/EJCHEM.2021.52972.3096

non-allergenic [6-9]. Therefore, BC represents a potential alternative to plant-derived cellulose and a promising material for many applications[6]. These include a thickening agent and food stabilizer[10], food packaging [11], biomaterial for manufacturing cosmetics [12], artificial skin [13], artificial blood vessels or tissue engineering [14], preparation of optically transparent films [15] and electric conductors [16]. These potential applications of BC largely depend on its price and accessibility. Therefore, strains and production medium must be optimized. Since commercial exploitation of BC is limited by its yield, many researchers have tried to increase the productivity using different carbon and nitrogen sources from *Gluconacetobacter xylinus* [17-19]. Cellulose-producing species belonging to the *Gluconacetobacter* genus have been reported, where BC synthesis has been strictly linked to the cell metabolism. In fact, the culture conditions have a crucial influence on BC production, in particular regarding factors such as carbon and nitrogen sources, temperature, pH and inoculum size and its age as well as incubation time [8, 20, 21]. One of the bacterial cellulose application problems in industry is its low productivity. So, one variable at time method was applied to obtain the optimal culture conditions for highest production of bacterial cellulose. In this work, we aimed to optimize the production of BC nanofibers in order to maximize the production yield through investigate and study the influence of the various culture methods on the BC production by virtue of a new domestic strain, namely, *Komagataeibacter xylinus* SB3.1, which previously isolated from the fermented apple wastes supplied by local markets in Cairo, Egypt [22]. For further optimization, the effect of carbon sources enriched with glucose, fructose, raffinose, sucrose, lactose, maltose, mannitol, and starch were also investigated prolonging with a wide range of nitrogen sources variety such as ammonium sulphate, ammonium nitrate, ammonium chloride, tryptone, peptone, beef extract, yeast extract and urea. Eventually, different incubation times, inoculum sizes, incubation temperatures, and pH values were also investigated.

Materials and Methods

Incubation and Bacterial Cellulose production

The *Komagataeibacter xylinus* SB3.1 strain from apple waste culture was purified and classified as a new strain of *Komagataeibacter* genus [22]. The strain was cultivated in a standard Hestrin–Schramm medium (HS), i.e., aqueous solution of 2% (w/v) glucose, 0.5% (w/v) peptone, 0.5% (w/v) yeast extract, 0.27% (w/v) Na_2HPO_4 and 1.15 g/L citric acid [23]. Standard inoculum was prepared by inoculation of one test tube containing 5 ml of HS medium with 1 ml of tested culture, then incubated at 28- 30°C for 3 days. The contained of this tube was used as a standard inoculum ($O.D_{620}$ nm from 0.21 to 0.44) under static culture.

Factors affecting bacterial cellulose production

These experiments were carried out to obtain maximum BC production by *K. xylinus* SB3.1; various nutritional and physiological parameters were studied such as different media composition, inoculum size, pH, temperature and incubation time, carbon and nitrogen sources were optimized using one variable at a time approach. The yield of BC was determined gravimetrically. Specifically, after rinsed extensively with water, wet BC sheets were dried at 108 °C until constant weight was reached. BC sheets was then weighted to obtain dry weight (W dry, g). BC production was recorded as dry weight of BC per liter of medium (g L^{-1}). The yield of BC was also calculated as follows eq1:

$$\textbf{Yield}\ (\%) = \left(\frac{\text{W dry}}{\Delta C}\right) \times 100 \quad (1)$$

Where W dry is the dry weight of BC (g) and ΔC is the consumption (g) of reducing sugar by the bacteria during cultivation. The reducing sugar concentration of the culture medium was determined using DNS colorimetric method [24] from the standard curve (r^2 = 0.9858) as shown in (Fig. 1). All measurements were performed in triplicate.

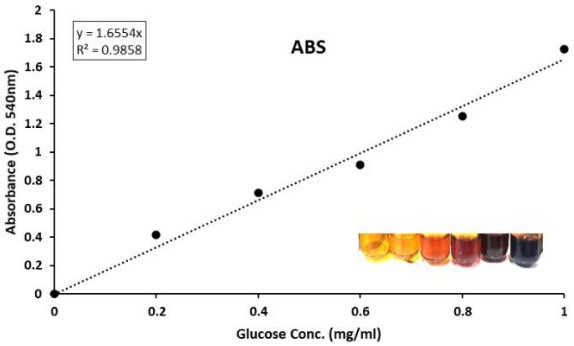


Fig. 1. Calibration curve for the determination of reducing sugars concentration of the culture medium by UV- Visible spectrophotometry.

Effect of different incubation time

The effect of various incubation periods, from 1 to 10 days on the BC production by *K. xylinus*

SB3.1 strain was studied under recommended conditions of previous experiments.

Effect of different inoculum size

Different inoculum size (1,2, 4, 6, 8, 10 and 12%) was tested to investigate their effect for maximal production of cellulose. The production process was performed in 250 ml Erlenmeyer flasks containing 45 ml sterile selected medium for *K. xylinus* SB3.1 strain, then inoculated and incubated at 30°C for 7 days at pH 6 under static condition.

Effect of different inoculum ages

These flasks were then incubated under a shaking condition of 200 rpm and 30°C for different incubation periods at 1, 4, 8, 16 and 21 days.

Effect of different pH values

The influence of various pHs on cellulose production by *K. xylinus* SB3.1 strain was studied by marinating the culture media at pH 4, 5, 6, 7,8 and 9. The production process was conducted in 250 ml Erlenmeyer flasks containing 45 ml sterile medium, each under preferred medium and inoculum, then incubated at 30°C for 7 days at different pH values under static condition.

Effect of different temperatures

To monitor the temperature impacts on cellulose production by studied isolate and reference strains, different temperatures 15,20, 25, 30, 35 and 40°C were tested under preferred conditions for *K. xylinus* SB3.1.

Effect of different carbon source

To study the impacts of different carbon sources on the bacterial cellulose production, carbon source like, glucose, fructose, raffinose, sucrose, lactose, maltose, mannitol, and starch were added at 2% concentration by *K. xylinus* SB3.1.

Effect of different nitrogen source

Ammonium sulphate, ammonium nitrate, ammonium chloride, tryptone, peptone, beef extract, yeast extract and urea which all were screened as a source of nitrogen for the highest production of bacterial cellulose by *K. xylinus* SB3.1 strain at 0.5% concentration.

BC production under optimum condition

The pure culture of *K. xylinus* SB3.1 activated on HS broth medium under a shaking condition of 200 rpm and 30°C for 8 days, subsequently 8% of it was inoculated into optimized HS medium is composed of (2.0% of d-mannitol, 0.5% of tryptone, 0.5% of yeast extract, 0.27 % of Na_2HPO_4, and 0.115 % of citric, 0.2 % (pH 7.0) in 250 ml Erlenmeyer flasks. Flasks were incubated at (30 °C) under static conditions for 8 days. After incubation, the BC yield calculated as mention in equation **(1)**.

Characterization of BC obtained after optimization

The properties and characteristics of the overripe banana medium derived BNC samples produced at different scales were subsequently determined using the following techniques. All the samples were dried in oven at 50 °C until reach constant weight, except for SEM analysis.

Scanning electron microscopy (SEM)

SEM analysis was performed using a scanning electron microscopy (FEG 250, Quanta, Japan). The samples cut from the freeze-dried BC was coated with gold film before analysis.

X-ray diffraction (XRD)

The X-ray diffraction (XRD,) technique was employed to determine the crystallinity index and crystallite size of the bacterial cellulose sample. XRD patterns of the lyophilized films were recorded using a Bruker D8 Advance diffractometer (Bruker, Carlsruhe, Germany) at the CuKα radiation wavelength (λ = 1.54 Å), generated at a voltage of 40 kV and a filament emission of 40 mA. Samples were scanned in a 2θ range between 5° and 90°. The scan speed was 0.5°·min−1 with a step size of 0.02°, and the scans were collected from 2θ = 5° to 50°. The crystallinity index (CrI) was calculated using the peak intensity method:

$$\boldsymbol{CrI}(\%) = \left(\frac{I_{(200)} - I_{(am)}}{I_{(200)}}\right) \times 100$$

, where $I_{(200)}$ is the intensity at (200) peak and $I_{(am)}$ is the minimum intensity between (110) and (200) peaks. The mean crystallite (*CrS*) size was calculated from the X-ray line broadening of the (002) diffraction peak according to Scherrer's Equation:

$$\boldsymbol{CrS} = \frac{k\lambda}{\beta cos\theta}$$

where, k is the shape factor (0.89), β is the wavelength of X-ray radiation (1.54 Å), β is the full width at half maximum height calculated in radians and θ is the Bragg's angle.

Fourier transform infrared (FTIR) spectroscopy

The series of BC derivatives were freeze dried. And the IR spectra of sample was obtained using a Bruker FTIR Equinox 55 spectrometer with an ATR attachment in the range of 500–4,000 cm^{-1} with a resolution of 4 cm^{-1}

Results and discussion

Effect of different incubation period on cellulose production.

To obtain maximum cellulose production from *K. xylinus* SB3.1, different incubation time was studied range from 1 to 10 days. Data represented in **Fig. 2** showed that the cellulose production was observed in all incubation time, but, 8 days cultivation time is considered the optimal time and achieved 3.12 g/l yield. The cellulose production approximately stable after the optimum incubation time for *K. xylinus* SB3.1. The obtained results are achieved in short term than those use the same media under static condition [25] who proved that the optimum bacterial cellulose production achieved at 10 days.

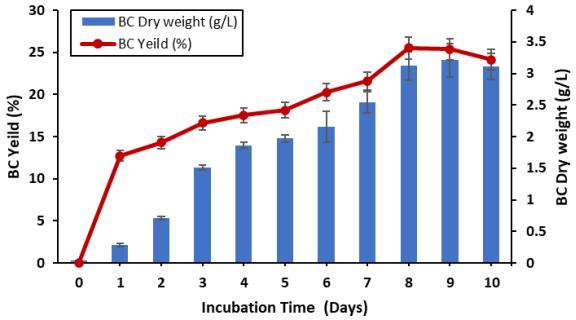
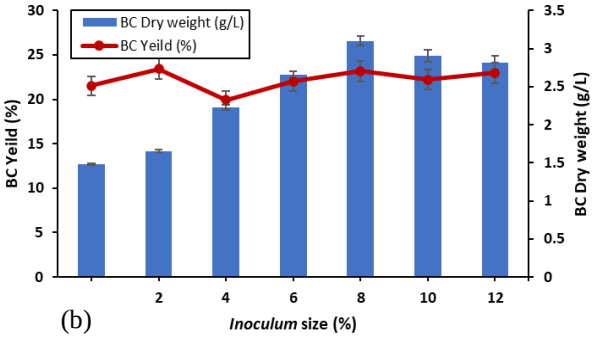


Fig. 2. Effect of different static incubation period on bacterial cellulose production.

Effect of various inoculum sizes on BC production.

The inoculum volume plays an important role in cellulose production. To study the effect of inoculum size of *K. xylinus* SB3.1 inoculum size ranging from 1% to 12% (v/v) was examined for cellulose production. The results are presented in **Fig. (3.a)** clearly showed that the cellulose production was observed in all inoculum size tested but lower and higher values than 8% inoculum showed there is a decrease in cellulose production. By this experiment, we can conclude that 8% inoculum size is optimum for cellulose production and achieved 3.1g/L yield compared to other inoculum sizes.

(a)



(b)

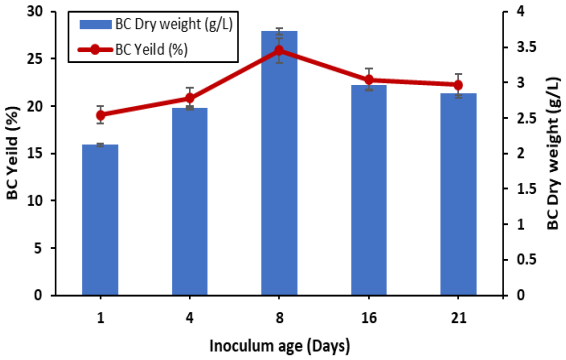


Fig. 3. (a)Effect of different inoculum size on the production of BC, (b) Effect of different inoculum size on the production of BC.

Effect of various inoculum age on BC production.

The results presented in **Fig. (3.b)** show that, the inoculum age display the positive effect on bacterial cellulose production. However, the inoculum age after 8 days provided the highest yield and achieved 3.72g/L yield other than inoculation, due to the increasing the number of inoculation cells. The obtained results are in accordance with those of several investigators [21] who proved that the inoculum age had impact effect on bacterial cellulose production.

Impact of various pH values on BC production.

The pH plays a significant role in cell growth and BC production. The cellulose production was observed at pH ranged from 4 to 9 for *K. xylinus* SB3.1 stain as can be seen in **Fig. (4.a).** On the other hand, cellulose and cell growth cannot be achieved at pH 2 and 10. By this investigation, we can conclude that pH 7 is optimum for cellulose production and achieved 4.4 g/l which is maximum yield compared to other pH values for *K. xylinus* SB3.1.

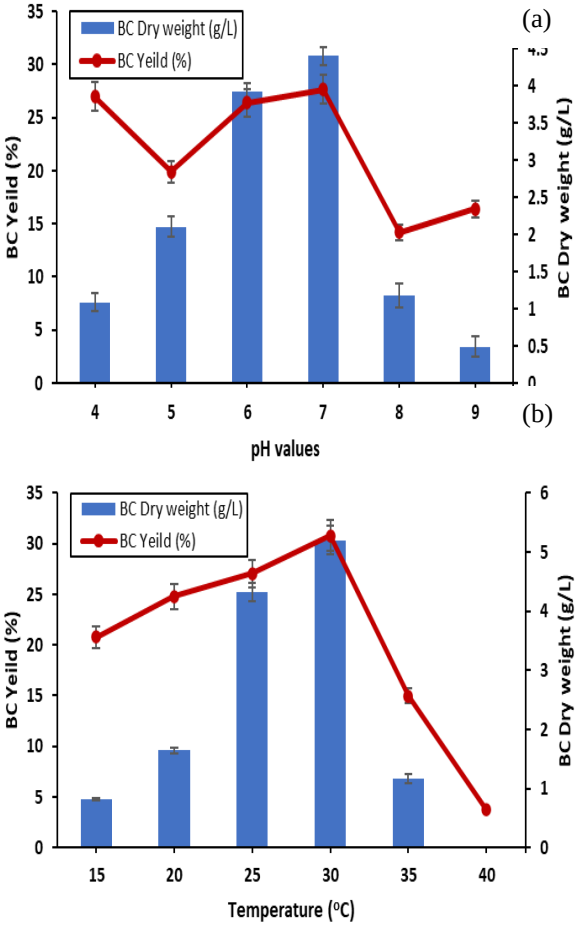


Fig. 4. (a) Effect of different pH values on production of BC, (b) Effect of different incubation temperature on production of BC .

Several studies showed that the pH value range for cellulose production was about 4–9[3, 6], and the optimum pH for cellulose production varies with the bacterial strains, but was usually attributed to a neutral to slightly acidic pH range [20].Lin *et al*., [3] reported that the *G. xylinus* 23769 produces cellulose ranged from 0.64 to 1.4 g/L at pH between 4 to 9 under incubation from 4 to 9 days using HS medium, while the present study achieved the maximum production of cellulose ranged from 1.08 to 4.4g/l at pH from 4 to 8 after 8 days using HS medium by *K. xylinus* SB3.1.When the culture pH falls below 4 as a consequence of gluconate accumulation, cellulose synthesis declines. Once all of the glucose in the media has been oxidized, the bacteria begin to metabolize the gluconate and a gradual increase in culture pH is observed as the bacteria consume the gluconate. This could be attributed to the facts that the glucose was transformed into other product than BC such as gluconic acid via direct oxidation during production process which finally led to significantly reduced in the pH of production medium. The pH value decreases during production because of the accumulation of by-products like gluconic, acetic or lactic acids [26, 27]

Effect of different incubation temperatures on BC production.

Cellulose production and cell growth were directly affected by temperature. To study the effect of various temperature on cellulose production by *K. xylinus* SB3.1, temperature vary from 15 to 40°C (with unit increase of 5°C) was examined as shown in **Fig. (4.b)**. The results indicated that the *K. xylinus* SB3.1 exhibits cellulose production (0.82 to 5.2 g/L) at temperatures ranged from 15 to 35°C, but no cellulose production and cell growth when temperature reached to 40°C. The optimum temperature for high cellulose production (5.2 g/L) is 30°C. The optimum temperature supplies the bacterium with an enough energy which improve the cellulose biosynthetic pathway to transform glucose into cellulose. Zahan *et al*., [28] reported that, at incubation temperature of 40°C, there is no significant growth appeared of *A. xylinum* 0416 and cellulose production as well. This is probably due to the harsh and inappropriate environment created by the incubation at this temperature, these data are similar with data obtained from this study. Son *et al.* [29] reported that at incubation temperature of 35°C and / or above, the bacteria do not multiply even in an optimal medium due to denaturation of cell components such as nucleic acids and proteins, this directed to the growth of cells and BC production were not observed under incubation temperature of 35°C and above, this may be matching to the results obtained from this work.

Effect of various carbon sources on cellulose production.

To investigate the effect of carbon source on BC production, carbon sources like mono, di and polysaccharide such as glucose, fructose, raffinose, sucrose, lactose, maltose, mannitol, and starch were supplemented at 2% (w/v) in a suitable medium. The results are described and presented in **Fig. (5.a).** The maximum BC production (6.12g/L) was observed in glucose followed for *K. xylinus SB3.1*. In the present study, *K. xylinus* SB3.1 shows the ability to use a wide variety of carbon sources for cellulose production and D-mannitol seems to be the most suitable carbon source [30]. Most of the researchers show that the productivity of cellulose production by *Gluconacetobacter* is influenced by carbon source availability and the aggregation of metabolic by-products that cause adverse growth conditions [8].

Molina-Ramírez *et al.*, [31] expressed that the most efficient carbon source is glucose which achieves 2.80 g/l cellulose at concentration 2% after 8 days by *Komagataeibacter medellinensis*, however the cellulose reached to 3.3 g/l after 15 days when mixed glucose/sucrose used as a carbon source. The present work achieves 6.12 g/L cellulose after 8 days by *K. xylinus* SB3.1 at 2% mannitol. Other study describes the addition of polysaccharide such as starch on the production media that affect the physical properties of cellulose membrane obtained from *Gluconacetobacter xylinus* BTCC B796 [32].

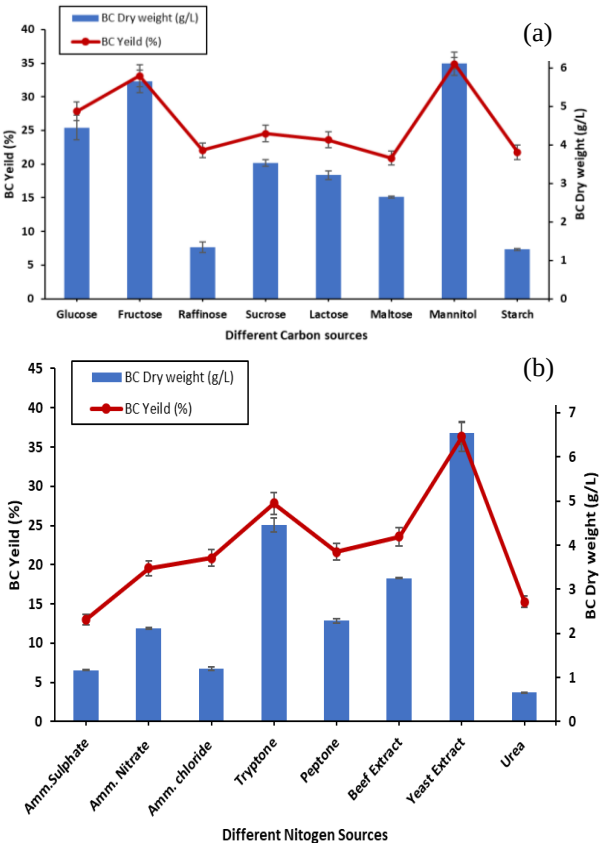


Fig. 5. (a) Effect of different carbon sources on BC production, (b) Effect of different nitrogen sources on BC production

Impact of different nitrogen sources on cellulose production.

The highest production efficiency of cellulose depends not only the carbon source but also on nitrogen source. In this experiment different organic and inorganic nitrogen source was applied. The results represented in **Fig. (5.b)** clearly showed that the maximum cellulose production was observed when used organic nitrogen source than inorganic nitrogen source. As well as yeast extract exhibits high cellulose production for *K. xylinus* SB3.1 than other nitrogen source and these data are finding agreement with the previous report [33]. Maximum cellulose production was achieved with yeast extract to obtained cellulose yield of 6.54 g/L. Çoban and Biyik [34] reported that a higher cellulose production was achieved in a medium composed of glucose and supplemented with yeast extract as the finest nitrogen source, He *et al* [35] achieved higher BC production mannitol with yeast extract, this data agreement with the present study.

Bacterial nanocellulose production under optimized condition

The yield of BC production is influenced by pH, temperature, inoculum size, and incubation condition [3, 6, 33]. Therefore, BC production by *K. xylinus* SB3.1 was studies by providing those critical factors at the optimum level. The results showed that increasing the BC production from 18.13% at starting experiment to reach 36.29 % under the optimum condition within 8 days as shown in **Fig. 6**.

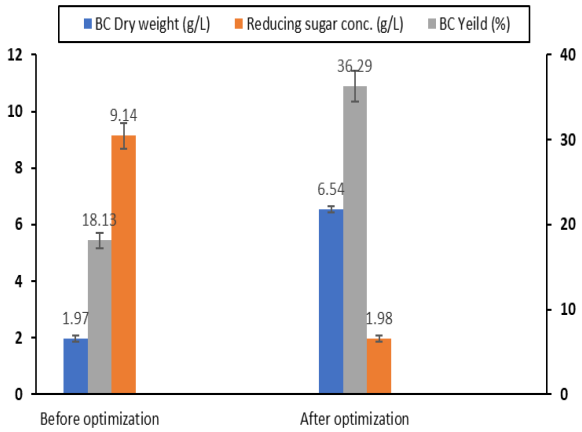


Fig. 6. BC production under optimum condition by *K. xylinus* SB3.1

Morphology of BC

The interior morphology of the purified BC membranes was analyzed by SEM and images were shown in **Fig. (7.a)**. BC sheet displayed three-dimensional networks consisting of ultrafine cellulose nanofibrils exhibited a more regular networks homogeneously distributed and connected. The histogram based on the SEM images illustrated their average size and size distribution. As seen in the **Fig. (7.b)** the average diameter of BC film microfibrils was 39.5 nm, the smallest and the largest diameters being 20 nm and 75 nm, respectively. This property was stated by earlier researcher [22].

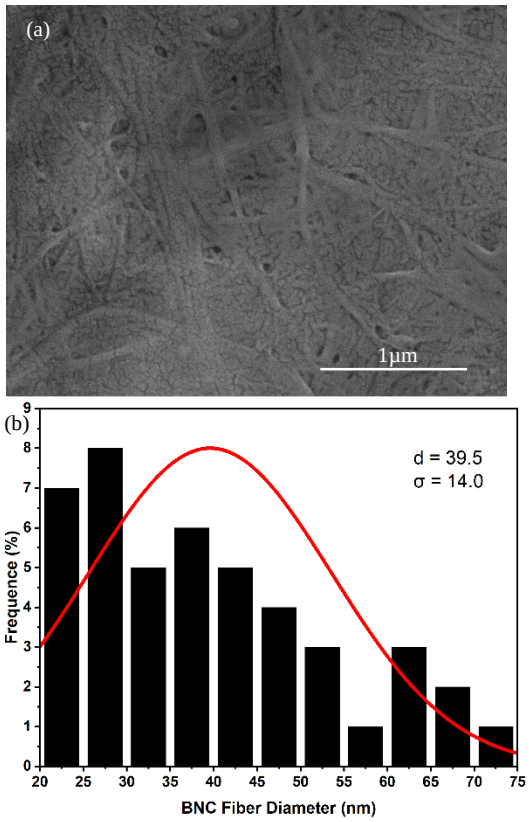


Fig. 7. Scanning electron microscopic morphology of (a) BNC fiber, (b) BNC fiber size distribution histograms

FITR spectra of BC was shown in Fig. 8a. The spectra showed essentially the same absorption peaks to those in previous reports [36, 37], indicating that the fermentation product of *K. xylinus* SB3.1is cellulose. Specifically, the characteristic peaks at 3345 cm^{-1}, 1425 cm^{-1} and 1160 cm^{-1} were assigned to O–H stretching, CH_2 symmetric bending and C–O–C asymmetric stretching vibration peak of cellulose type I, respectively. The peaks at 2893 $cm^{-1}$and 1650 cm^{-1} were from C–H stretching of CH_2 groups and water absorbed, respectively [37-39]. The crystalline structure, crystallinity and crystallite size of the BC were investigated by XRD. The diffraction diagrams were shown in **Fig. 8b**.

The BC showed three distinct peaks appeared at 2θ = 14.5°, 16.7° and 22.8°, which were assigned to the cellulose $100I_\alpha$,$010I_\beta$ and $110I_\alpha$ phases, respectively [40-43]. It suggested that BC exhibited a typical crystalline form of cellulose I. Crystallinity and crystallite size of BC were 82.29%, 4.77 nm. These results indicated that BC sheet have quite similar crystalline structures.

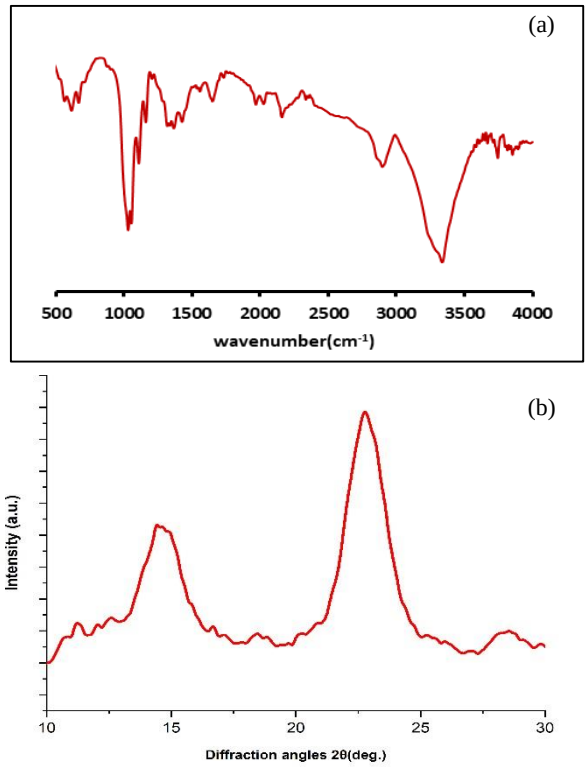


Fig. 8. (a) FTIR spectra of the BC, (b) X-Ray diffraction patterns of BC.

Conclusion

The study concluded that the optimum conditions for bacterial cellulose production by an

Egyptian local strain *Komagataeibacter xylinus SB3.1*. Different, inoculum size, pH, temperature, carbon, and nitrogen parameters were studied for optimal BC production. At the end of optimization, the HS media composed of mannitol as a carbon and yeast extract as a nitrogen source at 30◦C, pH 7, with the inoculum size 8% for 8 days achieved 6.45 g/L BC

Acknowledgments

The author expresses their sincere thanks to the Botany and Microbiology Department, Faculty of Science (Boys), Al-Azhar University, Egypt and the National Research Centre, Egypt for providing the necessary research facilities.

References

[1] J. Engelhardt, Sources, industrial derivatives and commercial application of cellulose, Carbohydr Eur 12 (1995) 5-14.

[2] Y. Huang, C. Zhu, J. Yang, Y. Nie, C. Chen, D. Sun, Recent advances in bacterial cellulose, Cellulose 21(1) (2014) 1-30.

[3] S.-P. Lin, Y.-H. Huang, K.-D. Hsu, Y.-J. Lai, Y.-K. Chen, K.-C. Cheng, Isolation and identification of cellulose-producing strain Komagataeibacter intermedius from fermented fruit juice, Carbohydrate polymers 151 (2016) 827-833.

[4] R.T. Machado, J. Gutierrez, A. Tercjak, E. Trovatti, F.G. Uahib, G. de Padua Moreno, A.P. Nascimento, A.A. Berreta, S.J. Ribeiro, H.S. Barud, Komagataeibacter rhaeticus as an alternative bacteria for cellulose production, Carbohydrate polymers 152 (2016) 841-849.

[5] Y. Wan, L. Hong, S. Jia, Y. Huang, Y. Zhu, Y. Wang, H. Jiang, Synthesis and characterization of hydroxyapatite–bacterial cellulose nanocomposites, Composites Science and Technology 66(11-12) (2006) 1825-1832.

[6] M. Iguchi, S. Yamanaka, A. Budhiono, Bacterial cellulose—a masterpiece of nature's arts, Journal of materials science 35(2) (2000) 261-270.

[7] H. Bäckdahl, G. Helenius, A. Bodin, U. Nannmark, B.R. Johansson, B. Risberg, P. Gatenholm, Mechanical properties of bacterial cellulose and interactions with smooth muscle cells, Biomaterials 27(9) (2006) 2141-2149.

[8] P.R. Chawla, I.B. Bajaj, S.A. Survase, R.S. Singhal, Microbial cellulose: fermentative production and applications, Food Technology and Biotechnology 47(2) (2009) 107-124.

[9] M. Ul-Islam, T. Khan, J.K. Park, Water holding and release properties of bacterial cellulose obtained by in situ and ex situ modification, Carbohydrate Polymers 88(2) (2012) 596-603.

[10] Z. Shi, Y. Zhang, G.O. Phillips, G. Yang, Utilization of bacterial cellulose in food, Food hydrocolloids 35 (2014) 539-545.

[11] K.L. Spence, R.A. Venditti, O.J. Rojas, Y. Habibi, J.J. Pawlak, The effect of chemical composition on microfibrillar cellulose films from wood pulps: water interactions and physical properties for packaging applications, Cellulose 17(4) (2010) 835-848.

[12] I. Kawaguchi, K. Nakamura, Make-up tissue paper for removing cosmetics, comprises glycerin impregnated into a tissue paper which consists of pulp fiber, and bacterial cellulose entangled in the pulp interfiber forming a network structure, JP2009077752-A, 2007.

[13] J. Kingkaew, S. Kirdponpattara, N. Sanchavanakit, P. Pavasant, M. Phisalaphong, Effect of molecular weight of chitosan on antimicrobial properties and tissue compatibility of chitosan-impregnated bacterial cellulose films, Biotechnology and Bioprocess Engineering 19(3) (2014) 534-544.

[14] M. Scherner, S. Reutter, D. Klemm, A. Sterner-Kock, M. Guschlbauer, T. Richter, G. Langebartels, N. Madershahian, T. Wahlers, J. Wippermann, In vivo application of tissue-engineered blood vessels of bacterial cellulose as small arterial substitutes: proof of concept?, journal of surgical research 189(2) (2014) 340-347.

[15] V. Palaninathan, N. Chauhan, A.C. Poulose, S. Raveendran, T. Mizuki, T. Hasumura, T. Fukuda, H. Morimoto, Y. Yoshida, T. Maekawa, Acetosulfation of bacterial cellulose: An unexplored promising incipient candidate for highly transparent thin film, Materials Express 4(5) (2014) 415-421.

[16] D. Müller, J. Mandelli, J. Marins, B. Soares, L. Porto, C. Rambo, G. Barra, Electrically conducting nanocomposites: preparation and properties of polyaniline (PAni)-coated bacterial cellulose nanofibers (BC), Cellulose 19(5) (2012) 1645-1654.

[17] S. Masaoka, T. Ohe, N. Sakota, Production of cellulose from glucose by Acetobacter xylinum, Journal of fermentation and bioengineering 75(1) (1993) 18-22.

[18] M.E. Embuscado, J.S. Marks, J.N. BeMiller, Bacterial cellulose. I. Factors affecting the production of cellulose by Acetobacter xylinum, Food Hydrocolloids 8(5) (1994) 407-418.

[19] K. Ramana, A. Tomar, L. Singh, Effect of various carbon and nitrogen sources on cellulose synthesis by Acetobacter xylinum, World Journal of Microbiology and Biotechnology 16(3) (2000) 245-248.

[20] A. Krystynowicz, M. Koziołkiewicz, A. Wiktorowska-Jezierska, S. Bielecki, E. Klemenska, A. Masny, A. Płucienniczak, Molecular basis of cellulose biosynthesis disappearance in submerged culture of Acetobacter xylinum, Acta Biochimica Polonica 52(3) (2005) 691-698.

[21] N.A. Atwa, A.I. El-Diwany, H. El-Saied, A.H. Basta, Improvement in bacterial cellulose production using Gluconacetobacter xylinus ATCC 10245 and characterization of the cellulose pellicles produced, Egyptian Pharmaceutical Journal 14(2) (2015) 123.

[22] A. Alemam, ISOLATION AND CHARACTERIZATION OF CELLULOSE NANO FIBER PRODUCING BACTERIAL STRAIN FROM FERMENTED FRUITS, Al-Azhar Journal of Pharmaceutical Sciences 62(2) (2020) 152-163.

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

[24] M. Abdelraof, M.S. Hasanin, H. El-Saied, Ecofriendly green conversion of potato peel

wastes to high productivity bacterial cellulose, Carbohydrate polymers 211 (2019) 75-83.
[25] N. Tyagi, S. Suresh, Isolation and characterization of cellulose producing bacterial strain from orange pulp, Advanced Materials Research, Trans Tech Publ, 2013, pp. 475-479.
[26] B. Rangaswamy, K. Vanitha, B.S. Hungund, Microbial cellulose production from bacteria isolated from rotten fruit, International journal of polymer science 2015 (2015).
[27] K.A. Zahan, N. Pa'e, I.I. Muhamad, Process parameters for fermentation in a rotary disc reactor for optimum microbial cellulose production using response surface methodology, BioResources 9(2) (2014) 1858-1872.
[28] K.A. Zahan, K. Nordin, M. Mustapha, M.N. Mohd Zairi, Effect of incubation temperature on growth of Acetobacter xylinum 0416 and bacterial cellulose production, Applied Mechanics and Materials, Trans Tech Publ, 2015, pp. 3-8.
[29] H.J. Son, M.S. Heo, Y.G. Kim, S.J. Lee, Optimization of fermentation conditions for the production of bacterial cellulose by a newly isolated Acetobacter, Biotechnology and applied biochemistry 33(1) (2001) 1-5.
[30] M. Rana, M.U. Dahot, Optimization of culture conditions to produce secondary metabolites by Pleurotus ostreatus, Pak. J. Biotechnol 14(2) (2017) 251-256.
[31] C. Molina-Ramírez, M. Castro, M. Osorio, M. Torres-Taborda, B. Gómez, R. Zuluaga, C. Gómez, P. Gañán, O.J. Rojas, C. Castro, Effect of different carbon sources on bacterial nanocellulose production and structure using the low pH resistant strain Komagataeibacter medellinensis, Materials 10(6) (2017) 639.
[32] Y.K. Sya'Di, E.T. Wahyuni, E. Rahayu, C. MN, The effect of polysaccharide in the fermentation medium on physical properties of bacterila cellulose from gluconacetobacter xylinus BTCC B796, Pak. J. Biotechnol 14 (2017) 323-327.
[33] H. Bıyık, E.P. Çoban, Evaluation of different carbon, nitrogen sources and industrial wastes for bacterial cellulose production, Evaluation 5(1) (2017).
[34] E.P. Çoban, H. Biyik, Effect of various carbon and nitrogen sources on cellulose synthesis by Acetobacter lovaniensis HBB5, African Journal of Biotechnology 10(27) (2011) 5346-5354.
[35] X. He, H. Meng, H. Song, S. Deng, T. He, S. Wang, D. Wei, Z. Zhang, Novel bacterial cellulose membrane biosynthesized by a new and highly efficient producer Komagataeibacter rhaeticus TJPU03, Carbohydrate Research 493 (2020) 108030.
[36] M.N. Thorat, S.G. Dastager, High yield production of cellulose by a Komagataeibacter rhaeticus PG2 strain isolated from pomegranate as a new host, RSC advances 8(52) (2018) 29797-29805.
[37] H.E. Emam, T.I. Shaheen, Investigation into the role of surface modification of cellulose nanocrystals with succinic anhydride in dye removal, Journal of Polymers and the Environment 27(11) (2019) 2419-2427.
[38] K.V. Ramana, H.V. Batra, Occurrence of Cellulose-Producing Gluconacetobacter spp. in fruit samples and Kombucha tea, and production of the biopolymer, Applied biochemistry and biotechnology 176(4) (2015) 1162-1173.
[39] M. Abdelraof, S. Ibrahim, M. Selim, M. Hasanin, Immobilization of L-methionine γ-lyase on different cellulosic materials and its potential application in green-selective synthesis of volatile sulfur compounds, Journal of Environmental Chemical Engineering 8(4) (2020) 103870.
[40] S. Gea, C.T. Reynolds, N. Roohpour, B. Wirjosentono, N. Soykeabkaew, E. Bilotti, T. Peijs, Investigation into the structural, morphological, mechanical and thermal behaviour of bacterial cellulose after a two-step purification process, Bioresource technology 102(19) (2011) 9105-9110.
[41] A.D. French, Idealized powder diffraction patterns for cellulose polymorphs, Cellulose 21(2) (2014) 885-896.
[42] T.I. Shaheen, A. Fouda, Green approach for one-pot synthesis of silver nanorod using cellulose nanocrystal and their cytotoxicity and antibacterial assessment, International journal of biological macromolecules 106 (2018) 784-792.
[43] M. Abdelraof, M.S. Hasanin, M.M. Farag, H.Y. Ahmed, Green synthesis of bacterial cellulose/bioactive glass nanocomposites: Effect of glass nanoparticles on cellulose yield, biocompatibility and antimicrobial activity, International journal of biological macromolecules 138 (2019) 975-985.