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Optimization and Characterization of Biocellulose Production from Bacteria Isolated from Passion Fruits

Muhammad Haikal Hizani, Nadiawati Alias*, Syamil Choo Shaiful Bahri, Khairul Anuar Apendi

School of Agriculture Science and Biotechnology, Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin, Besut Campus, 22200 Besut, Terengganu, MALAYSIA.

***Corresponding author: nadiawati@unisza.edu.my**

ABSTRACT

Biocellulose is a strong polymer consisting of nanofibrillar structures that produce a large surface area and a microporous structure. This organic polymer is greatly in demand in various industries, such as the paper industry, biomedical industry and cosmetics industry. In this study, biocellulose production from two bacteria known as endophytic bacterium SV845 (M02) and *Pantoea ananatis* IADCAMBID (M03) isolated from passion fruit was selected. Three different media formulations (Media 1, Media 2, and Media 3) were used in order to optimize the biocellulose production where each media contained a different percentage of carbon sources (glucose and fructose). The highest biocellulose production (1.544 mg/mL) was demonstrated by M02 bacteria strain in Media 1 containing glucose alone which fermented at 30 ºC while at 37 ºC, the highest biocellulose (BC) production was demonstrated by M03 bacteria strain at 2.078 mg/mL in media containing glucose alone (Media 1). Data on pH changes during biocellulose fermentation in all the media were set at an initial pH of 6. The final pH values were observed in the range of 5.34 to 6.08 for M02 strain and 5.95 to 8.53 for M03 strain, respectively. Characterizations of biocellulose were compared to starch using Fourier Transform Infrared (FTIR) spectroscopy. FTIR analysis indicated that the absorption peaks at 3200 cm-1 and 1630 cm-1 were derived from the association of intermolecular and intramolecular hydrogen bonds and H–O–H bending vibration of the absorbed water molecules in cellulose.

Keywords: Biocellulose, Media optimization, Temperature, Characterization, FTIR

INTRODUCTION

Cellulose, classified as the main component of plant biomass as hemicellulose and lignin, is one of the most abundant resources on earth (Ahmed et al., 2019). Biocellulose (BC) is a natural polymer originally produced by bacteria called acetic acid (Dirisu et al., 2017). It is a polysaccharide consisting of a long linear chain of β (1-4) bound D-glucose units with the formula $(C_6H_{10}O_5)_n$ (Mona et al., 2019). BC is an excellent biomaterial for medicinal or cosmetic applications due to its high purity which is free from hemicellulose, lignin, pectin, araban and other impurities (Voon et al., 2016). BC also exhibits unusual properties, including a high level of

crystallinity, water retention capability, tensile strength and moldability (Lin et al., 2013). The genera *Aerobacter*, *Gluconacetobacter*, *Pseudomonas*, *Agrobacterium*, *Salmonella*, *Azotobacter*, *Rhodococcus*, *Rhizobium*, *Achromobacter* and *Sarcina* are the bacteria that can produce cellulose. However, *Gluconacetobacter xylinum* is the only species that can synthesize enough cellulose for commercial purposes (Zakaria & Nazeri, 2012).

Biocellulose is significantly in demand in a broad range of industries, such as the paper industry, biomedical and cosmetics industries. Some examples of biocellulose application in the food industry are powder as a GRAS additive or food ingredient (Niyazbekova et al., 2018). More research needs to be done to substitute synthetic cellulose and plant cellulose with more eco-friendly material like biocellulose in order to control and reduce deforestation and the problem associated with non-biodegradable polymers. Thus, this research aims to optimize biocellulose production from two bacteria strains isolated from Malaysian local fruits and also to characterize the biocellulose produced by FTIR approach.

MATERIALS AND METHODS

Bacteria strains

Potential biocellulose producing bacteria strains (M02 and M03) have been previously isolated from passion fruit by Salman et al., (2020). All the bacteria strains were maintained in 25% glycerol stocks in -80°C. For working bacteria stock, the bacteria cultures were maintained in Hestrin and Schramm medium (HS) agar slants and stored in a 4 °C refrigerator prior to use.

Media Preparation

HS medium (Media 1) containing 20 (g/L) glucose, 5 (g/L) yeast extract, 5 (g/L) peptone, 1.15 (g/L) citric acid, and 2.7 (g/L) disodium hydrogen phosphate were prepared as the control media. While for the modified media, compositions of Media 2 were 2 g/L glucose, 18 g/L fructose, 5 (g/L) yeast extract, 5 (g/L) peptone, 1.15 (g/L) citric acid, and 2.7 (g/L) disodium hydrogen phosphate and Media 3 were 20 g/L of fructose, 5 (g/L) yeast extract, 5 (g/L) peptone, 1.15 (g/L) citric acid, and 2.7 (g/L) disodium hydrogen phosphate. pH of the media was adjusted to 6.0 using 5 M NaOH or 5 M HCl. All media were sterilized by autoclaving at 121 °C, 15 psi for 15 - 20 minutes.

Biocellulose fermentation

About 5 mL of the overnight bacteria culture (M02 and M03) were transferred to 250 mL flask containing 50 mL culture medium (Media 1, Media 2 and Media 3). The cultures were then incubated statically at 30 °C and 37 °C for 7 days to induce BC production. pH of the media was recorded pre-fermentation and post-fermentation processes.

Biocellulose dry weight

After 7 days of fermentation, the pellicles were filtered through a 3M membrane filter. The wet weight was taken and recorded by using a weighing scale. Then, the BC pellicles were washed with water and soaked in 0.1 M NaOH at 80°C for 2 hours to remove unwanted bacterial cells possibly attached to the BC pellicles. Then, the pellicles were washed through deionized water several times to ensure the complete removal of the alkali. Finally, the purified biocellulose was dried at 60°C for 12 hours and weighed (Costa et al., 2017). Results were presented as mean of triplicates value with standard deviation (Mean ± SD).

Characterization of Biocellulose by Fourier Transform Infrared (FTIR) spectroscopy

Biocellulose (BC) produced by M02 and M03 bacteria strains were analysed by FTIR spectroscopy. BC samples of 0.5–2 mg were used and the IR spectra were recorded at wavenumbers ranging from 4000 to 400 cm^{-1} using a Shimadzu IR Prestige 21 spectrometer with a resolution of 4 cm^{-1} and 64 scans per sample (Jia et al., 2017).

RESULTS AND DISCUSSION

Optimization of Biocellulose Production

Optimal biocellulose (BC) production was investigated by measuring its dry weight from 3 different media formulations (Media 1, Media 2 and Media 3) for both bacteria strains (M02 and M03). The fermentation process was carried out for 7 days at two different temperatures (30 °C and 37 °C).

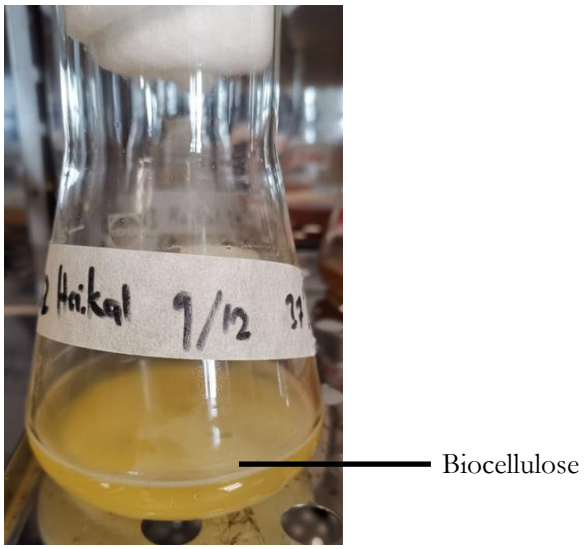

Fig. 1. Example of BC produced

For endophytic bacterium SV845 (M02), the highest BC production (Fig. 1) was achieved at 30 °C when using Media 1 containing glucose alone at about 1.544 mg/mL (dry weight). This was followed by Media 3 (100% fructose) and Media 2 (90% fructose + 10% glucose) with BC dry weight at 1.342 mg/mL and 1.322 mg/mL respectively (Fig. 2). The findings were consistent with the findings by Hodel et al. (2020) and Molina-Ramírez et al. (2017), which suggested that BC production is influenced by the type and concentration of carbon source used. From this study, glucose was found as the best carbon source for BC production for M02 bacteria strain. Effective biocellulose production for M02 bacteria strain is attributed to its ability to utilize glucose as carbon source during the fermentation, followed by BC polymerization (Mikkelsen et al., 2009). Meanwhile, for the fermentation performed at 37 °C, the highest BC production by M02 strain was achieved when using Media 3 containing fructose alone at about 1.440 mg/mL (dry weight). This was followed by Media 1 (100% glucose) and Media 2 (90% fructose + 10% glucose), with BC dry weight of 1.208 mg/mL and 1.208 mg/ml respectively (Fig. 2).

For *Pantoea ananatis* strain IADCAMB10 (M03), the highest BC production was achieved also at 30 °C when using Media 2 which contains 90% fructose + 10% glucose at about 1.478 mg/mL of dry weight. This was followed by Media 3 (100% fructose) and Media 1 (100% glucose) with the dry weight of 1.280 mg/mL and

0.434 mg/mL respectively. This shows that the M03 bacteria strain prefers media containing mixed carbon sources (glucose and fructose) compare to the single carbon source in Media 1. Zakaria & Nazri (2012) reported that media containing mixed carbon sources with 70% glucose and above would produce lower BC yield than media containing fructose alone. This has supported our findings as showed in Fig. 2 and Fig. 3.

The variability in BC production when using different carbon sources could be due to the fact that certain bacteria would have a different level of glucose metabolic pathways which convert glucose to energy for general activity via oxidative metabolism (Ross et al., 1991). According to Rani & Appaiah (2011), BC production can increase with the increment of glucose concentrations of up to 40 g/L but initially decreased BC production above that concentration, along with the decrease in pH of the medium. This is due to the initial high glucose concentration which leads to the accumulation of gluconic acid, which reduces the pH of the medium below the optimal level and hence inhibits the bacteria growth and production of BC (Son et al., 2001). The formation of gluconic acid can be diminished in the presence of lignosulfonate as stated by Naritomi et al. (1998). Besides that, the accumulation of acetic or lactic acid in static cultures decreases the pH far lower than the optimal range required for good BC yield (Aswini et al., 2020).

Many previous studies suggested that there is no specific pattern for a given bacterial species to utilize any desired carbon and nitrogen sources for BC production (Mikkelsen et al., 2009; Aswini et al., 2020). In this present investigation, we use yeast extract for all the media tested. Besides carbon sources, corn steep liquor (CSL) was stated to be the most suitable nitrogen source for BC production. Increasing CSL concentration up to 8 percent can increase the BC yield due to the presence of lactate and methionine in CSL, which are effective components for improved cellulose production (Matsuoka et al., 1996). Lactate stimulates cell growth by facilitating the tricarboxylic acid (TCA) cycle and also by generating oxidation energy as lactate is converted to pyruvate would results in increased BC production as stated by Naritomi et al. (1998). Meanwhile, methionine increases the growth rate by reducing the lag time leading to an increase in the BC production rate (El-Saied et al., 2008). Inhibition of glucose oxidation by adding ethanol and acetic acid as an additional source of electrons has been reported to increase cellulose yield (Velasco-Bedrán & López-Isunza, 2007). The potential reason for the increase in yield is that ethanol stimulates cell growth and serves as an energy source for the production of ATP. Increased levels of ATP contribute to an abundant flow of glucose 6-phosphate into the BC biosynthetic pathway via inhibition of glucose 6-phosphate dehydrogenase. The increase in glucose 6-phosphate would increase the BC yield. While acetic acid is used as a monomeric raw material resulting in a polymerization reaction and a glucose mass flow resulting in the conversion of glucose to cellulose.

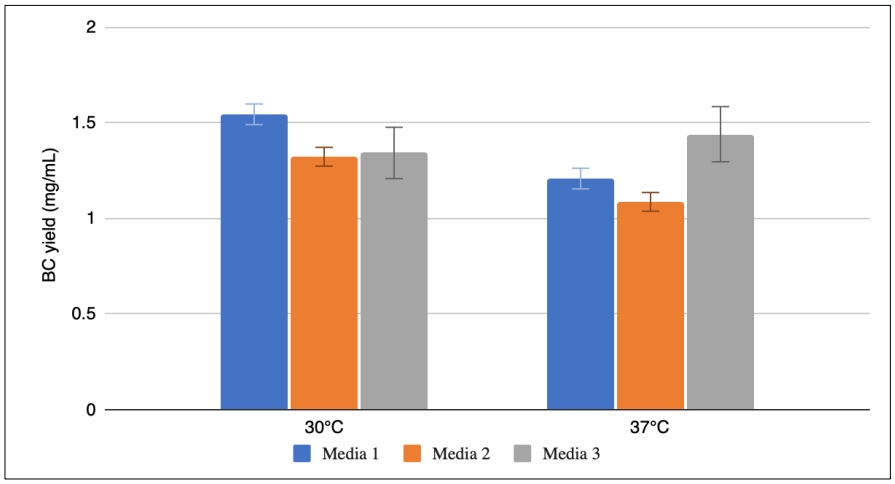

Fig. 2. BC produced by *endophytic bacterium* SV845 (M02) at 30 °C and 37 °C

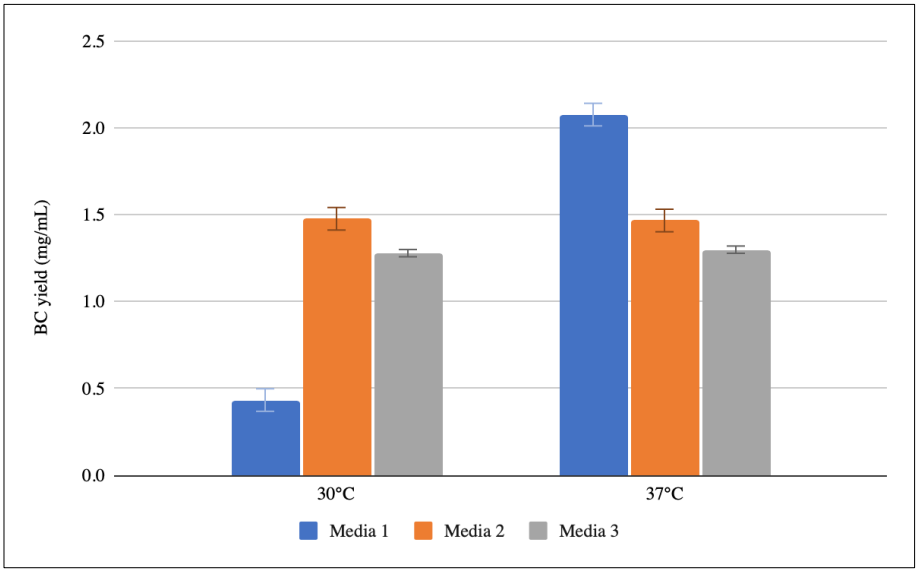

Fig. 3. BC produced by *Pantoea ananatis* strain IADCAMB10 (M03) at 30 °C and 37 °C

Changes of pH

pH is an essential factor for microbial growth and BC production. Although bacteria can regulate their internal hydrogen ion concentration very efficiently, maintaining pH control is required when the bacteria are exposed to pH levels beyond their optimum range. In addition, the pH of the media has an important influence on the structure and permeability of the cell membrane, which may have a significant impact on the biochemical activities of the bacteria.

According to Fig. 4, the pH value for most of the media cultures was generally found to decrease throughout the fermentation process for M02 bacteria strain. At 30 °C of fermentation, the lowest pH was showed on Media 3 (with fructose alone) at 5.34 and 6.30 for M02 and M03 respectively. The changes of pH were not too obvious for the other media (Media 1 and Media 2) for bacteria strain M02 which were around pH 5.43 and 5.38 respectively. This is due to the production and accumulation of glucosidic acid from glucose by M02 bacteria strain which decreases the pH of the media. Previous research by Dirisu et al. (2017) stated that the optimum pH for growth and activity of *Acetobacter xylinum* is between 4 and 7 while pH 6.5 contributes to the highest biocellulose yield. However, the risk of contamination will increase as pH approaches 7 as many other microorganism species can live in this pH state. As a result, lower pH (acidic pH) would reduce the risk of contamination by other bacteria species due to the acidic environment. In the meantime, the pH of the culture medium cannot be lower than pH 4 which would lead to the inactivation of the bacterium itself and inhibits biocellulose production (Zakaria & Nazeri, 2012). In contrast, bacteria strain M03 has shown higher pH (Fig 5) in most of the media used which is expected could be due to the contamination that occurred during the fermentation process.

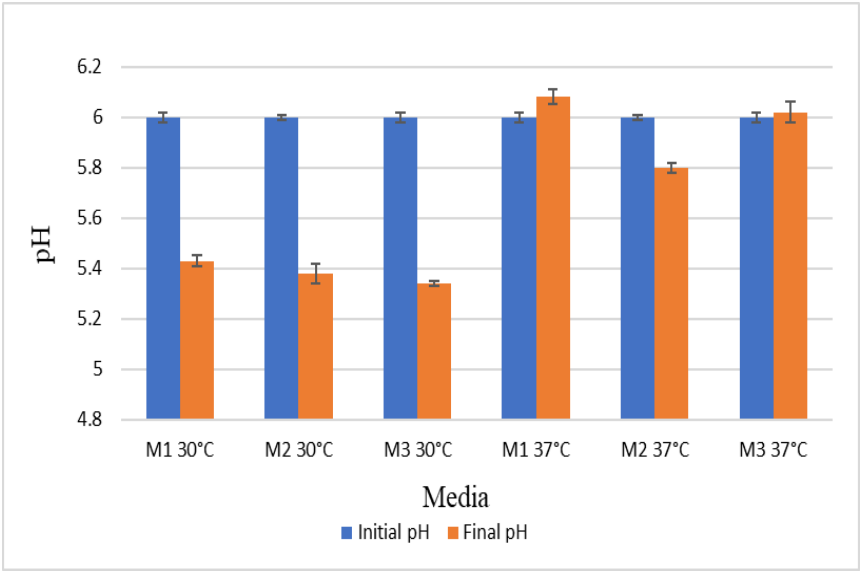

Fig. 4. Comparison of pH changes in 3 media (Media 1, Media 2 and Media 3) for *Endophytic bacterium* SV845 (M02) at 30 °C and 37 °C.

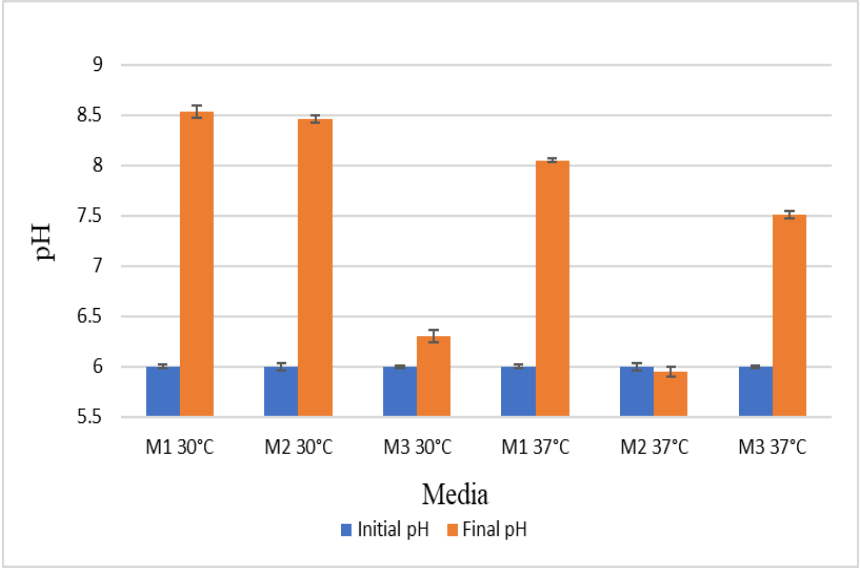

Fig. 5. Comparison of pH changes in 3 media (Media 1, Media 2 and Media 3) for *Pantoea ananatis* strain IADCAMB10 (M03) at 30 °C and 37 °C.

Characterization of biocellulose by FTIR analysis

Fourier Transform Infrared (FTIR) spectrocopy is used to analyse the chemical composition of the BC pellicle based on the absorption of the radioactive material by the chemical bonds present in the compound (Zakaria & Nazeri, 2012). Typical BC molecular structure $(C_6H_{10}O_5)n$ has the chemical bonding of OH stretching, C–H stretching, CH_2 bending, C–O–C stretching, and C–O stretching (Gomes et al., 2013). The following Fig. 6, Fig. 7, Fig. 8, and Fig. 9 were the results from the FTIR analysis from starch (control) and biocellulose samples produced from this study.

The FTIR analysis of the BC produced revealed typical BC IR spectra which were very similar to each other, indicating no change in the functional source structure. Cellulose is a biopolymer consisting of β-D-glucopyranose units bound together by β-1,4-glycosidic bonds. Fig 7 and Fig 8 shows biocellulose FTIR spectra produced by the M02 bacteria strain at 30 °C and 37 °C while Fig. 9 shows biocellulose FTIR spectra produced by the M03 bacteria strain at 37 °C. All the data shows a 3271.27 cm^{-1} band that can be associated with intermolecular and intramolecular hydrogen bonds. The spectra also consist of a 1631.78 cm^{-1} band that can be associated with the H–O–H bending vibration of the absorbed water molecules in the cellulose as also reported by Siew et al. (2012).

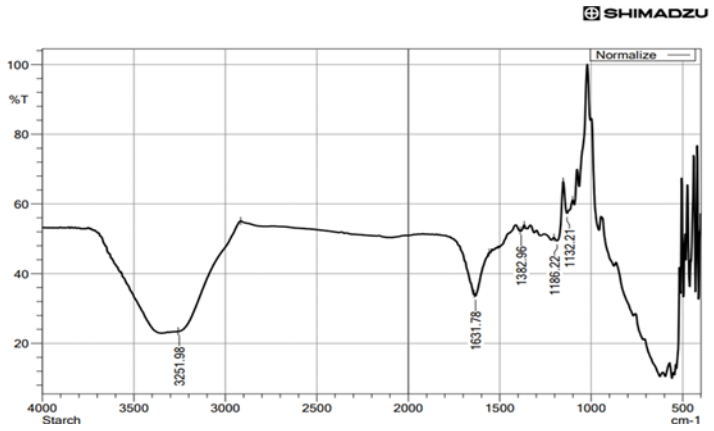

Fig. 6. FTIR image of starch (control)

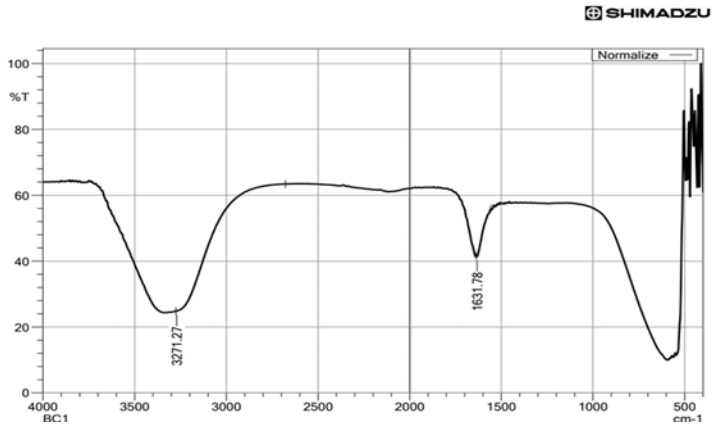

Fig. 7. FTIR image of biocellulose produced by M02 at 30 °C

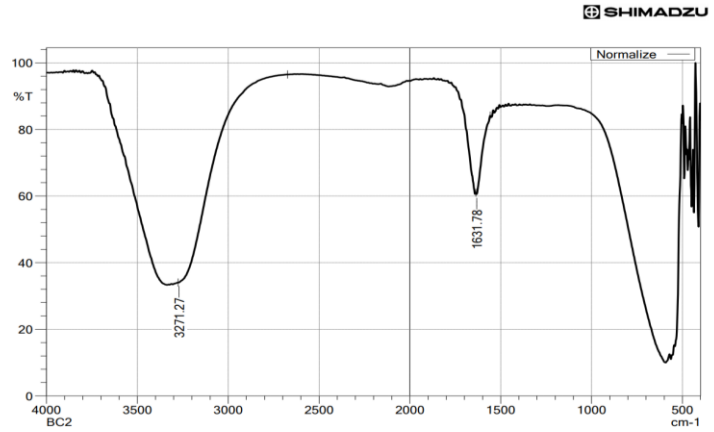

Fig. 8. FTIR image of biocellulose produced by M02 at 37 °C

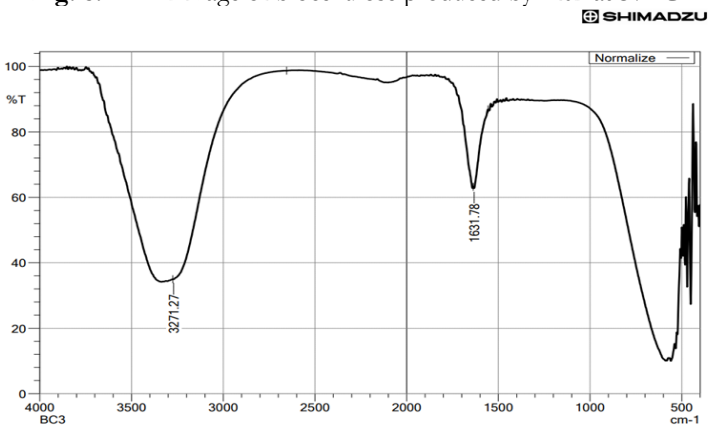

Fig. 9. FTIR image of biocellulose produced by M03 at 37 °C

CONCLUSION

As a conclusion, the production of biocellulose by *endophytic bacterium* SV845 (M02) and *Pantoea ananatis* strain IADCAMB10 (M03) in different media, carbon sources and temperatures were successfully performed. From this study, M02 bacteria strain has produced the highest BC yield compared to M03 strain and the best temperature for BC fermentation was identified at 30 °C. Structural characterization of biocellulose produced was performed by Fourier Transform Infrared (FTIR). From the FTIR spectra, it was confirmed that the components produced by *endophytic bacterium* SV845 (M02) and *Pantoea ananatis* strain IADCAMB10 (M03) were cellulose with existing bands representing strong bonds of hydrogen bonding and H–O–H bending of absorbed water molecules representing cellulose properties. Nevertheless, more studies need to be carried out to improve BC production from these strains in the future since their potential is vast to be explored.

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REFERENCES

Ahmed, A., Saleh, A., Soliman, N., Ibrahim, M., El-Shinnawy, N., & Abdel-Fattah, Y. (2019). Biocellulose production by *Gluconacetobacter hansenii* ATCC 23769: Application of statistical experimental designs and cellulose membrane characterization. *Egyptian Journal of Chemistry,* ***62***(11), 2077-2092.

Aswini, K., Gopal, N. O., & Uthandi, S. (2020). Optimized culture conditions for bacterial cellulose production by *Acetobacter senegalensis* MA1. *BMC Biotechnology,* ***20***(46), 1-16.

Costa, A. F. S., Almeida, F. C. G., Vinhas, G. M., & Sarubbo, L. A. (2017). Production of Bacterial Cellulose by *Gluconacetobacter hansenii* Using Corn Steep Liquor As Nutrient Sources. *Frontiers in Microbiology,* ***8***(2027), 1–12.

Dirisu, C., Rosenzweig, J., Lambert, E., & Oduah, A. (2017). PH effect and pH changes during Biocellulose production by *Gluconacetobacter xylinus* in moringa oleifera tea-sugar medium. *Journal of Advances in Microbiology,* ***7***(2)**,** 1-7.

El-Saied, H., El-Diwany, A. I., Basta, A. H., Atwa, N. A., & El-Ghwas, D. E. (2008). Production and characterization of economical bacterial cellulose. *Bioresources,* ***3***(4), 1196-1217.

Gomes, F. P., Silva, N. H., Trovatti, E., Serafim, L. S., Duarte, M. F., Silvestre, A. J., Neto, C. P., & Freire, C. S. (2013). Production of bacterial cellulose by *Gluconacetobacter sacchari* using dry olive mill residue. *Biomass and Bioenergy,* ***55***, 205-211.

Hodel, K. V., Fonseca, L. M., Santos, I. M., Cerqueira, J. C., Santos-Júnior, R. E., Nunes, S. B., Barbosa, J. D., & Machado, B. A. (2020). Evaluation of different methods for cultivating Gluconacetobacter hansenii for bacterial cellulose and montmorillonite Biocomposite production: Wound-dressing applications. *Polymers*, ***12***(267), 1-17.

Jia, Y., Wang, X., Huo, M., Zhai, X., Li, F., & Zhong, C. (2017). Preparation and characterisation of a novel bacterial cellulose/chitosan bio-hydrogel. *Nanomaterials and Nanotechnology*, ***7***, 1–8.

Lin, S., Loira Calvar, I., Catchmark, J. M., Liu, J., Demirci, A., & Cheng, K. (2013). Biosynthesis, production and applications of bacterial cellulose. *Cellulose*, ***20***(5), 2191-2219.

Matsuoka, M., Tsuchida, T., Matsushita, K., Adachi, O., & Yoshinaga, F. (1996). A Synthetic Medium for Bacterial Cellulose Production by *Acetobacter xylinum* subsp. sucrofermentans. *Bioscience, Biotechnology, and Biochemistry*, ***60***(4), 575-579.

Mikkelsen, D., Flanagan, B., Dykes, G., & Gidley, M. (2009). Influence of different carbon sources on bacterial cellulose production by *Gluconacetobacter xylinus* strain ATCC 53524. *Journal of Applied Microbiology,* ***107***(2), 576-583.

Molina-Ramírez, C., Castro, M., Osorio, M., Torres-Taborda, M., Gómez, B., Zuluaga, R., Gómez, C., Gañán, P., Rojas, O., & Castro, C. (2017). Effect of different carbon sources on bacterial Nanocellulose production and structure using the low pH resistant strain *Komagataeibacter medellinensis*. *Materials*, ***10***(639), 1-13.

Mona, S., Bajar, S., Deepak, B., Kiran, B., & Kaushik, A. (2019). Microbial cellulose: Production and application. *Materials for Biomedical Engineering*, 309-322.

Naritomi, T., Kouda, T., Yano, H., & Yoshinaga, F. (1998). Effect of ethanol on bacterial cellulose production from fructose in continuous culture. *Journal of Fermentation and Bioengineering*, ***85***(6), 598-603. h

Niyazbekova, Z., Nagmetova, G., & Kurmanbayev, A. (2018). An overview of bacterial cellulose applications. *Eurasian Journal of Applied Biotechnology,* ***2****,* 1-14 https://doi.org/10.11134/btp.2.2018.3

Rani, M. U., & Appaiah, A. (2011). Optimization of culture conditions for bacterial cellulose production from *Gluconacetobacter hansen*ii UAC09. *Annals of Microbiology*, ***61***(4), 781-787.

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

Salman, N. A. S., Alias, N., & Widowati, R. (2020). Screening and identification of biocellulose producing bacteria from Malaysian local fruits. *Bioscience Research*, 17(SI-1), 179-188.

Siew, K. W., Junaidi, Z., & Shahril, M. (2012). Enhancement of biocellulose production in mixed medium culture. UMP.

Son, H., Heo, M., Kim, Y., & Lee, S. (2001). Optimization of fermentation conditions for the production of bacterial cellulose by a newly isolated *Acetobacter* sp.A9 in shaking cultures. *Biotechnology and Applied Biochemistry*, ***33***(1), 1-5.

Velasco-Bedrán, H., & López-Isunza, F. (2007). The unified metabolism of *Gluconacetobacter entanii* in continuous and batch processes. *Process Biochemistry*, ***42***(8), 1180-1190.

Voon, W., Rukayadi, Y., & Meor Hussin, A. (2016). Isolation and identification of biocellulose-producing bacterial strains from Malaysian acidic fruits. *Letters in Applied Microbiology*, ***62***(5), 428-433.

Zakaria, J., & Nazeri, M. A. (2012). Optimization of Bacterial Cellulose Production from Pineapple Waste: Effect of Temperature, pH and Concentration: Proceedings of an EnCon 2012, 5th Engineering Conference, 10-12 July 2012, Kuching, Sarawak.

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