



Influence of carbon sources on the physicochemical properties and yield of bacterial cellulose production for drug delivery

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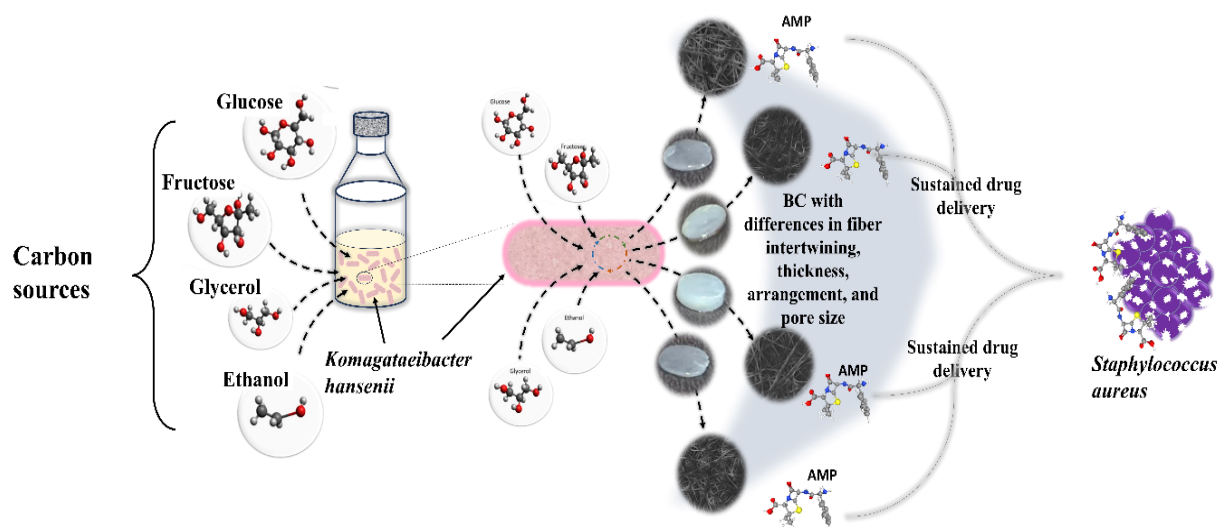
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Abstract: Bacterial cellulose (BC) is a biopolymer of great interest for application in various industrial and medical areas, due to its peculiar characteristics of biocompatibility, tensile strength, high water retention capacity and crystallinity. BC can be produced using culture media with different nutrient sources, under static or agitated cultivation conditions. The objectives of this work were to produce BC membranes, using the bacterial strain *Komagataebacter hansenii* ATCC 23769, in static culture, using media containing different sources of carbon (glucose, fructose, glycerol and ethanol) and nitrogen (yeast extract and peptone) and evaluate comparatively dry mass yield, swelling capacity. The BC obtained processed and characterized by Fourier transform spectroscopy (FTIR) and field emission scanning electron microscopy (FEG-SEM) to evaluate the influence of cultivation media in relation to parameters fiber density, thickness and entanglement of the BC, characteristics of extreme importance for future biomedical applications. The BC produced were impregnated with antibiotic amoxicillin (AMX) and tested for the sustained release capacity of this drug. Membranes with significant swelling capacity were obtained. FTIR spectra demonstrated the maintenance of vibrational modes for the main functional groups of bacterial cellulose, therefore, the different compositions of carbon and nitrogen sources would not influence the chemical properties of the obtained BC membranes. The result of the SEM analyzes allowed the measurement of fiber thicknesses, analysis of the degrees of intertwining and porosity, demonstrating differences in the BC produced in the different cultivation media, in relation to these parameters. All BC membranes produced were capable of releasing, in a sustained manner, for 48 h, the antibiotic AMP and the best results for all tests were produced in M2, M3 M4 and FRU media. The results demonstrate the potential of the SBC to be able to contribute to the design of new drug delivery systems with biomedical applications.

Keywords: Bacterial cellulose. Carbon sources. Physical properties. Sustained release.

Gaphical Abstract

The composition of the culture media can interfere with the biosynthetic metabolism of *K. hansenii*, leading to the production of BC with distinct macro and microscopic physical characteristics that enable its use as a support for sustained AMP release.



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Introduction

First described in 1886 by Brown 1–3 which in turn depend on pore size, pore volume and surface area. In the present study, the effects of structural modifications (in situ and ex situ, bacterial cellulose (BC) constitutes an extracellular biopolymer produced by several bacterial genera such as, *Acetobacter*, *Rhizobium*, *Agrobacterium*, *Aerobacter*, *Achromobacter*, *Azotobacter*, *Salmonella*, *Escherichia* and *Sarcina* 4–6. with special emphasis being given to bacteria of the *Acetobacteraceae* family mainly of the *Gluconacetobacter* 3 genus, later named *G. xylinus* and *G. hansenii*, and currently classified in the genus *Komagataeibacter* 6,7, a Gram-negative bacterium, using a variety of cultivation media with different carbon sources 6,8 as glucose 9, sugarcane molasses 10,11, syrup and fructose 11.

The BC production can be performed using different methods as static, dynamic, or bioreactor fermentation method. In the static cultivation method are obtained BC films at the air-liquid interface, with fine three-dimensional network structures with excellent mechanical properties 6,12.

In addition to different BC production methods, it is the use of different carbon sources for BC production are also reported in the literature as Hestrin-Schramm (HS) 13, Zhou (Z) 14 and Yamanaka (Y) 15, including sucrose and mannose, dextran and starch, and nitrogen source as peptone, polypeptone, $\text{NH}_4\text{H}_2\text{PO}_4$ and casein hydrolysate 16. The BC production in this different culture media showed different dry mass yield and physicochemical characteristics 6,17.

Although bacterial cellulose (BC) can be produced by several species and has a structure identical to that of vegetable cellulose (VC), consisting of β -(1→4) glycosidic bonds, it is devoid of lignin, pectin and hemicellulose 18, presenting advantages, about VC, such as high purity, high crystallinity, liquid retention capacity, high mechanical resistance, non-toxic, biocompatible and biodegradable 19,20, and due to these characteristics, it can be applied in various fields such as food packaging 21,22 attention towards antimicrobial film is recently on the rise from food scientists for developing eco-friendly, biodegradable, and edible food packaging products over to plastic materials. Bacterial cellulose (BC, coatings or films, adsorbents, cosmetics, electronics 23, and biomedical such as controlled drug delivery systems 6,10,11,24–26 Springer Science+Business Media Dordrecht. This work describes the synthesis of bilayer bacterial cellulose membranes (BCs and filter for protection against respiratory pathogens 27 we present novel, sustainable filters based on bacterial cellulose (BC).

In the field of biomedicine, BC stands out in its application as a physical barrier, acting in wound healing 3,24,28,29} the multilayer fermentation method{,} bacterial cellulose (BC, blood vessels 30, scaffolds for tissue engineering 31, support for controlled drug release 8,25,32

and the application to the wound dressing 33–36"ISSN": "2452199X", "abstract": "The treatment and healing of infected skin lesions is one of the major challenges in surgery. To solve this problem, collagen I (Col-I. Herein, we report the production of BC by *K. hansenii* ATCC 23769 into culture media with different compositions of carbon and nitrogen sources concerning that found for HS and FRU production methods 6. As follows, we explored the influence of these changes on the physicochemical properties of BC, as well as their possible biomedical applications. The obtained BC were comparatively evaluated for dry mass yield and swelling capacity, also characterized by Field emission gun-scanning electron microscopy (FEG-SEM) and Fourier transform spectroscopy (FTIR).

Materials and methods

Materials

The bacteria for BC production *Komagataeibacter hansenii* ATCC 23769 was purchased from André Tosello Foundation. Anhydrous glucose, fructose, sucrose, lactose, and ethanol were purchased from Synth. Yeast extract, peptone, were purchased from Kasvi. Citric Acid, Na_2HPO_4 , NaOH , and glycerol were purchased from Merck.

Methods

BC production and processing

K. hansenii ATCC 23769 was reactivated from stock in glycerol stored at -80°C , in a medium FRU (fructose 60g/L, yeast extract – 5.6 g/L and ethanol – 50mL/L) being kept under constant agitation at 130 rpm at 25°C using Shaker Kasvi Biological Oxygen Demand (B.O.D.) oven at 28°C for 24 hours, and subsequently maintained in static culture at BOD 28°C for 7 days until the production of a BC membrane. Next, the culture was vigorously shaken to remove the bacteria from the blanket, which was used as a pre-inoculum. From the pre-inoculum, was produced a bacterial suspension in the different culture media (Table 1) at an optical density determined on a Cole Parmer 2800 UV/Vis Spectrophotometer at 600nm (OD600), corresponding to McFarland nephelometric scale 1 (3.0×10^8 CFU/mL) for a final volume of 15mL. The production of BC membranes in different culture media was carried out in a 24-well plate. In each well, 3.0 mL of each of the different culture media was placed (in triplicate). The cultures were incubated in B.O.D. at 28°C for 7 days, under static conditions, with daily observations.

The BC produced were washed in distilled water and immersed in NaOH 0,5M in a water bath at 65°C for 20 min. After this procedure, they were washed in distilled water, with constant water change until reaching neutral pH. After pH neutralization, the BC membranes were dehydrated, initially, between absorbent filter papers and, subsequently, dried in a ventilated oven – 400/1ND – Ethik Technology at 60°C until complete dehydration.

Table 1 – Composition of culture media.

Constituents (g/L)	M1	M2	M3	M4	M5	HS	FRU
Glucose (g/L)	–	–	40	50	35	25	–
Fructose (g/L)	40	50	–	–	35	–	60
Glycerol (g/L)	–	–	–	–	15	–	–
Ethanol (mL/L)	50	50	50	50	50	–	50
Yeast Extract (g/L)	5.6	5.6	5.6	5.6	5.6	5	5.6
Peptone (g/L)	–	–	–	–	–	5	–
Citric acid (g/L)	–	–	–	–	–	1.15	–
Na ₂ HPO ₄ (g/L)	–	–	–	–	–	2.5	–

Analysis of dry mass yield of BC membranes

To determine the dry mass yield (Dmy), dry membranes were used. The dry mass yield was calculated using the equation (1):

$$Dmy = \frac{Dm}{v} \quad (1)$$

Where Dm corresponds to the dry mass of the BC membrane (g) and v to the volume of medium consumed (mL).

Analysis of Swelling mass percentage

The swelling mass percentage (% Sm) behavior was evaluated by immersing the processed and dried membranes in distilled water at room temperature (25°C) for 4 h until swelling equilibrium was reached. After reaching equilibrium, the BC membranes were carefully removed, and the excess free water on the surface was removed with filter paper, for subsequent determination of the swollen mass on a Shimadzu AUJ 220 analytical balance.

The swelling percentage was calculated using equation (2):

$$\%Sm = \frac{M_{int} - Dm}{M_{int}} \times 100 \quad (2)$$

Where % Sm corresponds to the percentage of swelling of the BC membranes, M_s corresponds to the dry mass of the BC membranes and M_m corresponds to the swollen mass of BC.

Characterization of BC

The characterizations of the BC obtained were

performed by attenuated total reflectance (ATR) using the Agilent Cary 630 FTIR spectrometer, in transmittance mode in the region from 4000 to 600 cm^{-1} , and FEG-SEM, for analysis of the morphological characteristics in relation to the differences between the entanglements, fiber thicknesses and porosity, using the JEOL JSM-6360 LV microscope, after coating with carbon. Fiber thicknesses were measured using the ImageJ program.

Analysis of the capacity for sustained release of antibiotics by BC membranes produced in different culture media

Discs with 10 mm in diameter, produced with BC membranes obtained in different media and cultivation were impregnated with 5.0mg of the antibiotic ampicillin (AMP). The impregnated discs were subjected to thermal treatment in a ventilated oven at 35°C for 8 hours until total removal of the H₂O used as solvent. After drying, the discs were used for diffusion analysis using an adapted methodology described by CLSI [60]. The discs containing the antibiotic were placed on surface of Mueller Hinton agar (MH), inoculated with *Staphylococcus aureus* ATCC 25923 suspension on 0.5 MacFarland nephelometric scale (1.5×10^8 colony forming units per mL – CFU/mL). The plates were incubated in a bacteriological oven at 37°C for 24 hours. After this period, the inhibition zone was measured, and the BC discs were transferred to a new MH agar plate with *S. aureus* ATCC 25923. This process was repeated until there was no more formation of inhibition zone. All experiments were performed in triplicate.

Results and discussion

Production of BC in different culture media

Figure 1 shows the BC membranes obtained in triplicate in the culture media with different compositions before processing.

The Figure 2 shows the BC membranes obtained in triplicate in the culture media with different compositions after processing. As observed, there are differences in the

macroscopic characteristics. These results demonstrate that the difference in BC production is not related to the *K. hansenii* ATCC 23769 strain used, as all cultures were produced from a single pre-inoculum, and under the same culture conditions, indicating that the differences in morphology are related to the different compositions of the culture media.

Figure 1 – Macroscopic characteristics of the BC membranes produced in different culture media, before processing.

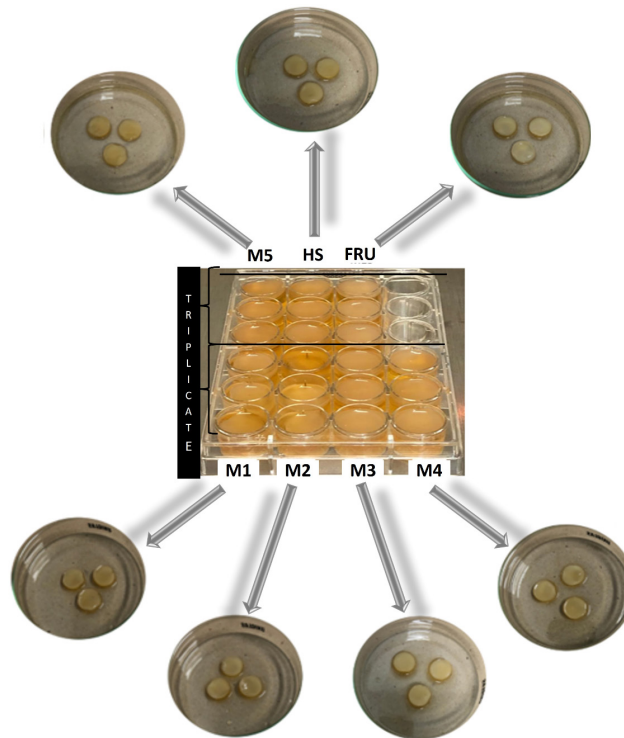
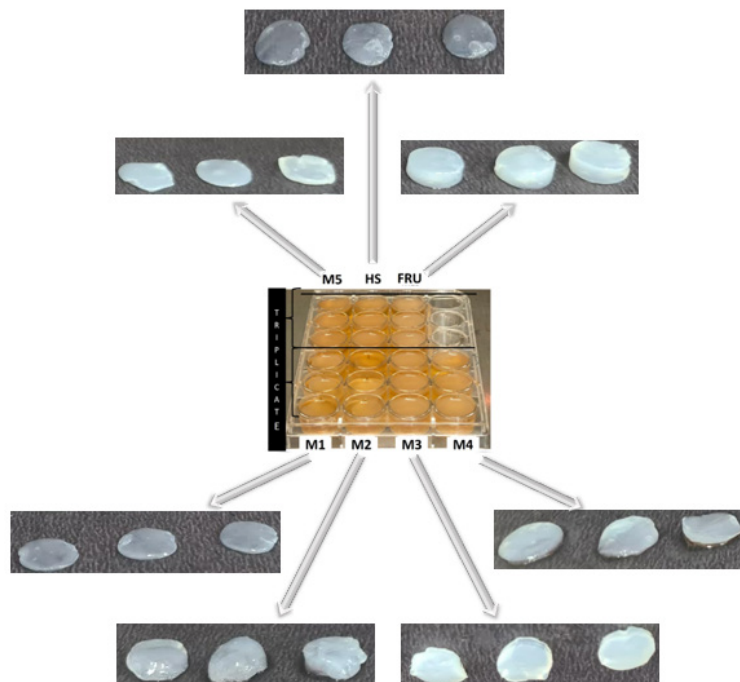


Figure 2 – Macroscopic characteristics of the BC membranes produced in different culture media, after processing.



Dry mass yield (*Dmy*) and percentage of swelling (%*Sm*) of BC membranes produced in different culture media

It was observed that the results obtained in the *Dmy* analysis shown in Table 2 demonstrate that the membranes produced in media M2, M3, M4 and FRU obtained higher *Dmy*. However, there were no significant differences in relation to %*Sm*. These results reinforce the fact that, although the membranes were produced in different culture media, they still maintained similar swelling capabilities.

The results obtained demonstrate that the BC produced has a great possibility of being applied as a support for drug release, since BC is a biomaterial known for its hypoallergenic, biocompatible, and nanostructured characteristics. In this way, the BC will be able to contribute to the design of new drug delivery systems with biomedical applications.

FEG-SEM analysis of the BC produced in different culture media

The FEG-SEM comparative analysis of the dried BC produced in different culture media showed a significant difference in fiber interlacing, thickness, arrangement, and pore size, Figure 3. It is possible to verify that the variation in the carbon source triggered surface morphological differences both in relation to the thickness (Panels C) and the interlacing of the cellulose fibers, and porosity (Panels A, B, and C). The Figure 4 show comparative analysis of fiber measure of BC produced in different culture media.

The results obtained suggest that the composition of the culture medium can interfere with the biosynthetic metabolism of *K. hansenii*, leading to the production of BC with distinct macro and microscopic physical characteristics since all samples presented heterogeneous degrees of intertwining and porosity. Similar results were obtained by Machado et al.³⁷ using culture media composed of different concentrations of Sugarcane molasses supplemented with different concentrations of glucose.

Table 2 – Dry mass yield of BC membranes produced in different cultivation and swelling media.

BC	Dry mass yield (g/L)	
M1	0.340 (0.030*)	88.196 (2.429*)
M2	0.373 (0.074*)	85.531 (3.546*)
M3	0.403 (0.081*)	83.779 (5.673*)
M4	0.377 (0,046*)	84.008 (5.637*)
M5	0.347 (0.055*)	84.181 (1.606*)
HS	0.310 (0.061*)	86.872 (3.254*)
FRU	0.400 (0.056*)	80.255 (3.922*)

* Standard deviation

Figure 3 – SEM of the CB membrane produced by *K. hansenii* in the different culture media (magnifications of A: 25,000; B: 50,000 and C:100,000x).

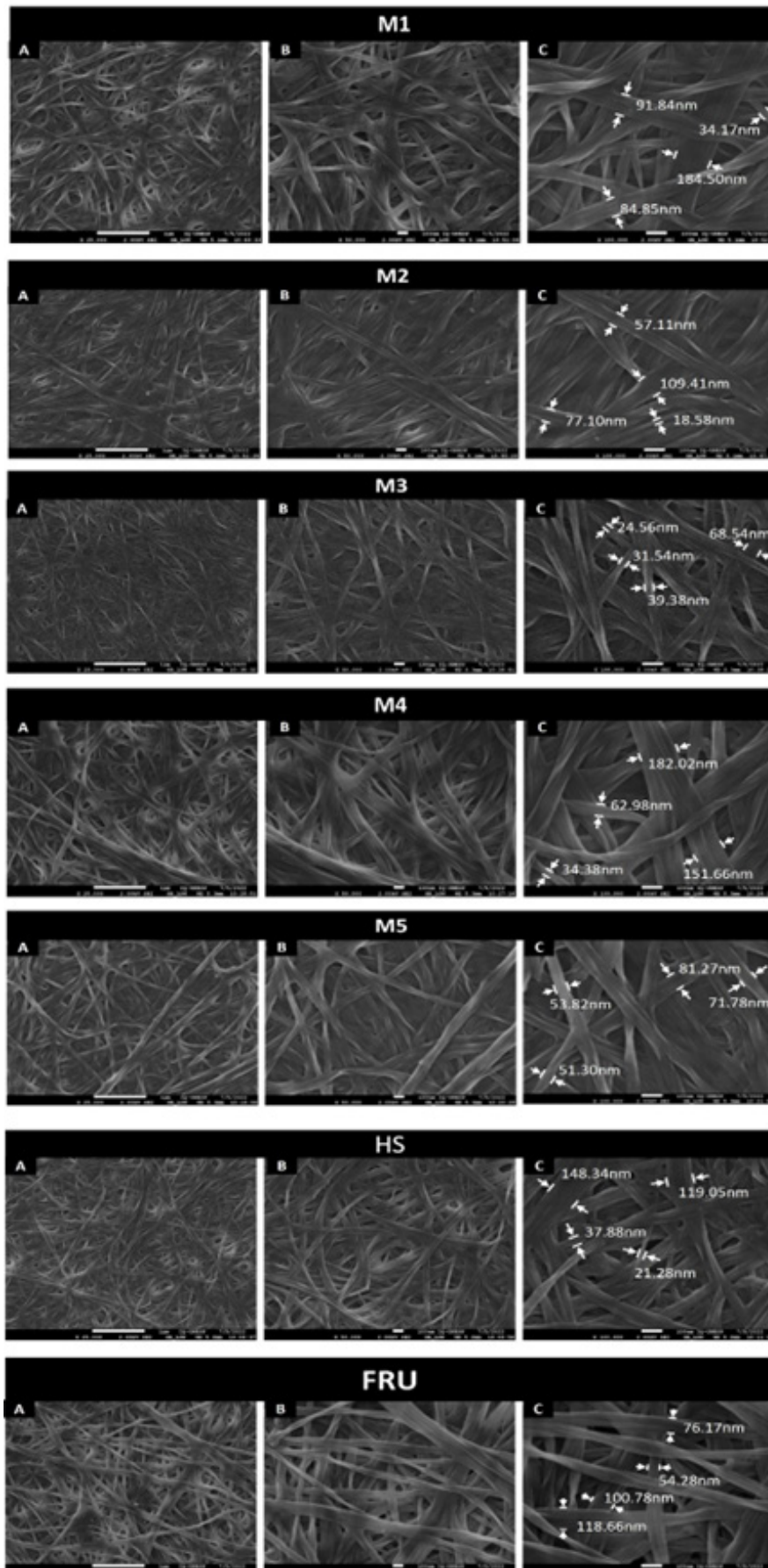
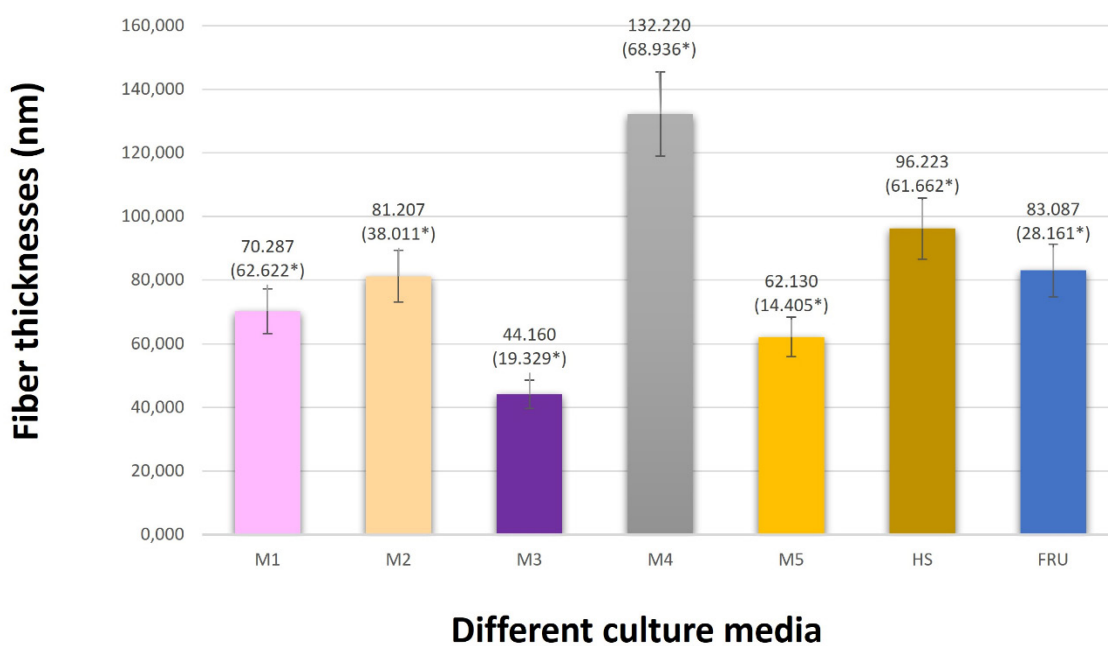


Figure 4 – Comparative analysis of fiber measure of BC produced in different culture media.



BC characterization by FTIR

Figure 5 shows the FTIR spectra for BC membranes, suggesting the keeping of vibrational modes for the principal functional groups of bacterial cellulose. Therefore, the different compositions of carbon and nitrogen sources would not influence the chemical properties of the obtained BC membranes. As can be seen from the characteristic stretches of BC, the range of 3350–3500 cm^{-1} attributed to the O–H stretch, the 2800–2900 cm^{-1} range attributed to the C–H stretches, the 1160 cm^{-1} range attributed to the C–O–C stretch, while the range 1035–1060 cm^{-1} is due to C–O stretching were maintained.

Analysis of the capacity for sustained release of antibiotics by BC membranes produced in different culture media by disc diffusion methods.

The results obtained demonstrate that BC membranes

produced in different culture media, impregnated with AMP (BC–AMP) were able to release antibiotic uninter- ruptedly for 48h, demonstrated by the zones of inhibition of bacterial growth around of the discs.

The Figure 6 shows Sustained/controlled release using BC–AMP by disc diffusion assay of the triplicates after 24 and 48 h.

During diffusion for 24 hours, it was not possible to measure inhibition halos, as there was a burst of release that led to the diffusion of the antibiotic throughout the plaque, completely inhibiting bacterial growth. After 48 h, it was possible to observe the formation of inhibition zones around all BC discs, however, with differences in the measurement of the inhibition zones. This fact is due to the different AMP retention capacities of the different BVmembranes, since all discs were impregnated with the same concentration of AMP.

Figure 6 – Sustained/controlled release using BC–AMP by disc diffusion assay of the triplicates after 48 h. The shows the averages of the measures of the zones of inhibition by time of diffusion after 24 and 48h.

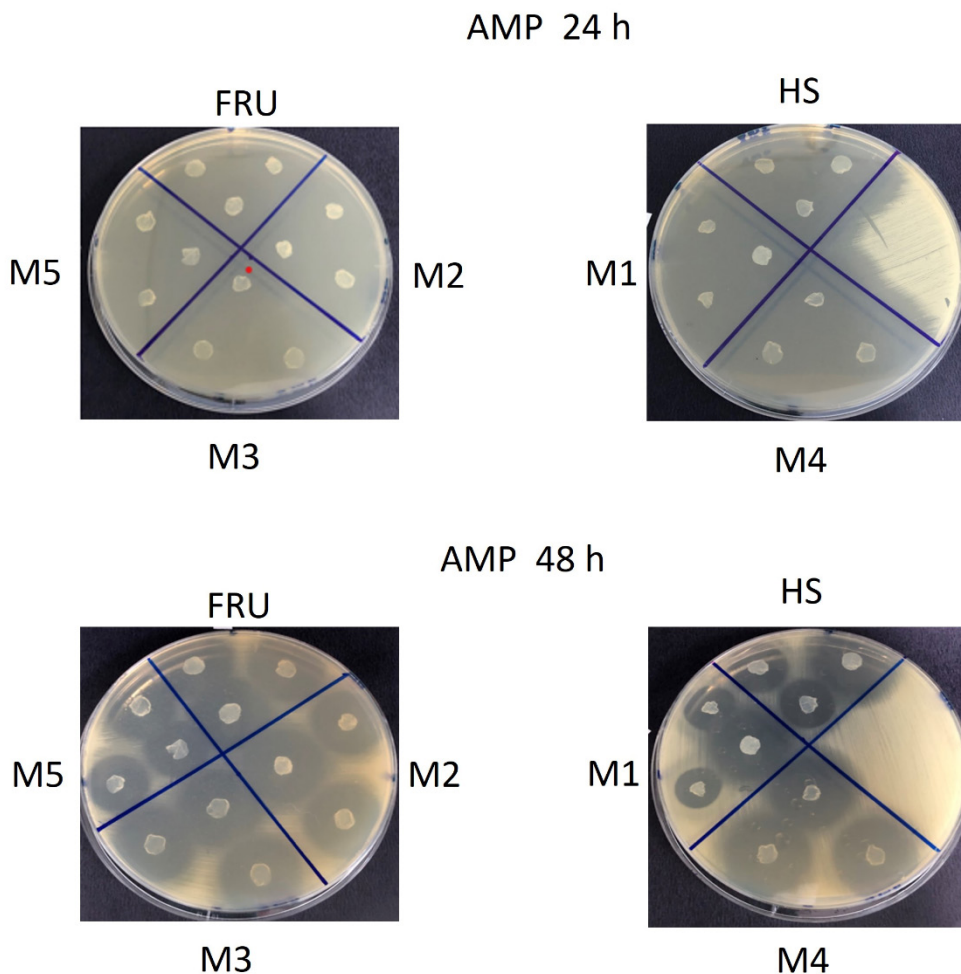
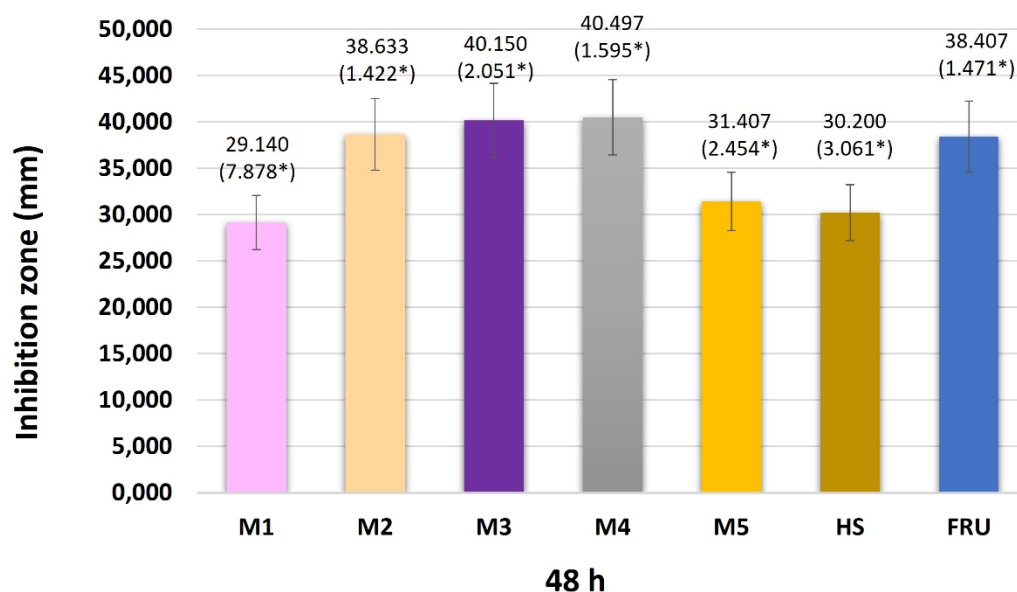


Figure 7 – Averages of the measures of the zones of inhibition by time of diffusion for 48 h.

* Standard deviation

All BC membranes tested were capable of maintaining the release of the AMP for 48h, however, the amount of AMP released was not the same, as can be seen by the difference in measurements of the inhibition zones (Figure 6). This fact may be related to the physical characteristics of the different membranes since all BC discs were impregnated with the same amount of AMP (5.0mg). Although there were no significant differences between BC membranes produced in the different culture media in relation to %Sm, the M2, M3 M4 and FRU showing best sustained release capacity of AMP in relation to the other BC, a fact that may be related to the degree porosity and fiber thickness.

Conclusion

The results obtained suggest that the composition of the culture medium can interfere with the biosynthetic metabolism of *K. hansenii*, leading to the production of BC with distinct macro and microscopic physical characteristics. FTIR spectra demonstrated the maintenance of vibrational modes for the main functional groups of BC, therefore, the different compositions of carbon and nitrogen sources would not influence the chemical properties of the obtained membranes. The result of the SEM analyzes allowed the measurement of fiber thicknesses, analysis of the degrees of intertwining and porosity, demonstrating differences in the BC produced in the different cultivation media, in relation to these parameters. There was a significant difference in relation to the thickness and intertwining of the fibers and degrees of porosity of the BC produced in the different media. Although there were no significant differences between BC membranes produced in the different culture media in relation to %Sm, all were capable of releasing AMP for 48h. However, the M2, M3, M4, and FRU showing best sustained release capacity of AMP in relation to the other BC which may be related to

the thickness and intertwining rates of the fibers. Although, the results of this work demonstrate the influence of culture media on the physical characteristics of membranes, the products obtained with great possibility of being used as a support for drug release, since BC is a biomaterial known for its hypoallergenic, biocompatible, and nanostructured characteristics. In this way, the BC will be able to contribute to the design of new drug delivery systems with biomedical applications.

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Declarations

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical approval: All ethical rules were obeyed; no human or animal experiments were performed.

References

[1].UI–Islam, M., Khan, T. and Park, JK. Water holding and release properties of bacterial cellulose obtained

- by in situ and ex situ modification. *Carbohydr. Polym.* 88, 596–603 (2012).
- [2]. Shah, N., Ul-Islam, M., Khattak, WA. and Park, JK. Overview of bacterial cellulose composites: A multi-purpose advanced material. *Carbohydrate Polymers* vol. 98 1585–1598 at <https://doi.org/10.1016/j.carbpol.2013.08.018> (2013).
- [3]. Cacicedo, ML. et al. Bioresource Technology Progress in bacterial cellulose matrices for biotechnological applications. *Bioresour. Technol.* 213, 172–180 (2016).
- [4]. Ullah, H., Wahid, F., Santos, HA. and Khan, T. Advances in biomedical and pharmaceutical applications of functional bacterial cellulose-based nanocomposites. *Carbohydr. Polym.* 150, 330–352 (2016).
- [5]. Islam, MU., Ullah, MW., Khan, S., Shah, N. and Park, JK. Strategies for cost-effective and enhanced production of bacterial cellulose. *Int. J. Biol. Macromol.* 102, 1166–1173 (2017).
- [6]. Lazarini, SC., Yamada, C., da Nóbrega, TR. and Lustrini, W. R. Production of sphere-like bacterial cellulose in cultivation media with different carbon sources: a promising sustained release system of rifampicin. *Cellulose* (2022) doi:10.1007/s10570-022-04644-0.
- [7]. Yamada, Y. Transfer of *Gluconacetobacter kakaiceti*, *Gluconacetobacter medellinensis* and *Gluconacetobacter maltaceti* to the genus *Komagataeibacter* as *Komagataeibacter kakaiceti* comb. nov., *Komagataeibacter medellinensis* comb. nov. and *Komagataeibacter maltaceti* comb. *Int. J. Syst. Evol. Microbiol.* 64, 1670–1672 (2014).
- [8]. Candido, T. Z. et al. Silver Nimesulide Complex in Bacterial Cellulose Membranes as an Innovative Therapeutic Method for Topical Treatment of Skin Squamous Cell Carcinoma. 1–18 (2022).
- [9]. Yang, F. et al. A recombinant strain of *Komagataeibacter xylinus* ATCC 23770 for production of bacterial cellulose from mannose-rich resources. *N. Biotechnol.* 76, 72–81 (2023).
- [10]. Machado, R. T. A. et al. *Komagataeibacter rhaeticus* grown in sugarcane molasses-supplemented culture medium as a strategy for enhancing bacterial cellulose production. *Ind. Crops Prod.* 122, 637–646 (2018).
- [11]. Lazarini, S. C. et al. Characterization of bilayer bacterial cellulose membranes with different fiber densities: a promising system for controlled release of the antibiotic ceftriaxone. *Cellulose* 23, (2016).
- [12]. Wang, J., Tavakoli, J. & Tang, Y. Bacterial cellulose production, properties and applications with different culture methods – A review. *Carbohydrate Polymers* vol. 219 63–76 at <https://doi.org/10.1016/j.carbpol.2019.05.008> (2019).
- [13]. Hestrin, S., Schramm, M. Synthesis of cellulose by *Acetobacter xylinum*. 2. Preparation of freeze-dried cells capable of polymerizing glucose to cellulose*. *Biochem. J.* 58, 345–352 (1954).
- [14]. Zhou, LL., Sun, DP., Hu, LY., Li, YW. and Yang, J. Z. Effect of addition of sodium alginate on bacterial cellulose production by *Acetobacter xylinum*. *J. Ind. Microbiol. Biotechnol.* 34, 483–489 (2007).
- [15]. Yamanaka, S. et al. The structure and mechanical properties of sheets prepared from bacterial cellulose. *J. Mater. Sci.* 24, 3141–3145 (1989).
- [16]. Öz, YE., Kalender, MA. novel static cultivation of bacterial cellulose production from sugar beet molasses: Series static culture (SSC) system. *Int. J. Biol. Macromol.* 225, 1306–1314 (2023).
- [17]. Mohammadkazemi, F., Azin, M. and Ashori, A. Production of bacterial cellulose using different carbon sources and culture media. *Carbohydr. Polym.* 117, 518–523 (2015).
- [18]. Kumar, V. et al. Efficient and economic process for the production of bacterial cellulose from isolated strain of *Acetobacter pasteurianus* of RSV-4 bacterium. *Bioresour. Technol.* 275, 430–433 (2019).
- [19]. Barshan, S., Rezazadeh-Bari, M., Almasi, H. and Amiri, S. Optimization and characterization of bacterial cellulose produced by *Komagataeibacter xylinus* PTCC 1734 using vinasse as a cheap cultivation medium. *Int. J. Biol. Macromol.* 136, 1188–1195 (2019).
- [20]. Ghozali, M., Meliana, Y. & Chalid, M. Synthesis and characterization of bacterial cellulose by *Acetobacter xylinum* using liquid tapioca waste. *Mater. Today Proc.* (2021) doi:10.1016/j.matpr.2020.12.274.
- [21]. Jang, EJ. et al. Antibacterial and biodegradable food packaging film from bacterial cellulose. *Food Control* 153, 109902 (2023).
- [22]. Lahiri, D. et al. Bacterial cellulose: Production, characterization and application as antimicrobial agent. *Int. J. Mol. Sci.* 22, 1–18 (2021).
- [23]. Zhang, L. K. et al. Bacterial cellulose based composites enhanced transdermal drug targeting for breast cancer treatment. *Chem. Eng. J.* 370, 749–759 (2019).
- [24]. Gomes, H. et al. A multipurpose natural and renewable polymer in medical applications : Bacterial

- cellulose. 153, 406–420 (2016).
- [25]. Lazarini, SC. et al. Influence of chemical and physical conditions in selection of *Gluconacetobacter hansenii* ATCC 23769 strains with high capacity to produce bacterial cellulose for application as sustained antimicrobial drug–release supports. *J. Appl. Microbiol.* 125, 777–791 (2018).
- [26]. Negut, I., Grumezescu, V. and Grumezescu, A. M. Treatment strategies for infected wounds. *Molecules* vol. 23 at <https://doi.org/10.3390/molecules23092392> (2018).
- [27]. Żywicka, A. et al. Argon plasma–modified bacterial cellulose filters for protection against respiratory pathogens. *Carbohydr. Polym.* 302, (2023).
- [28]. Fu, L. et al. Skin tissue repair materials from bacterial cellulose by a multilayer fermentation method. *J. Mater. Chem.* 22, 12349–12357 (2012).
- [29]. Ul-Islam, M., Khan, T., Khattak, WA. and Park, JK. Bacterial cellulose–MMTs nanoreinforced composite films: novel wound dressing material with antibacterial properties. *Cellulose* 20, 589–596 (2013).
- [30]. Andrade, FK. et al. Studies on the biocompatibility of bacterial cellulose. *J. Bioact. Compat. Polym.* 28, 97–112 (2013).
- [31]. Lustri, WR. et al. Microbial Cellulose — Biosynthesis Mechanisms and Medical Applications. in *Cellulose – Fundamental Aspects and Current Trends* vol. I 133–157 (InTech, 2015).
- [32]. Aquaroni, NAS. et al. Antibacterial activities and antiproliferative assays over a tumor cells panel of a silver complex with 4–aminobenzoic acid: Studies in vitro of sustained release using bacterial cellulose membranes as support. *J. Inorg. Biochem.* 212, 111247 (2020).
- [33]. Zhou, C. et al. De novo strategy with engineering a multifunctional bacterial cellulose–based dressing for rapid healing of infected wounds. *Bioact. Mater.* 13, 212–222 (2022).
- [34]. Ciecholewska–Juško, D., Junka, A. and Fijałkowski, K. The cross–linked bacterial cellulose impregnated with octenidine dihydrochloride–based antiseptic as an antibacterial dressing material for highly–exuding, infected wounds. *Microbiol. Res.* 263, (2022).
- [35]. Pita–Vilar, M., Concheiro, A., Alvarez–Lorenzo, C. & Diaz–Gomez, L. Recent advances in 3D printed cellulose–based wound dressings: A review on in vitro and in vivo achievements. *Carbohydr. Polym.* 321, 121298 (2023).
- [36]. Teixeira, MA. et al. Thermo–mechanical performance of nanostructured electrospun composites produced from poly(vinyl alcohol) and cellulosic compounds for potential uses as wound dressings. *Polymer (Guildf).* 281, 126131 (2023).
- [37]. Machado, RTA. et al. *Komagataeibacter rhaeticus* as an alternative bacteria for cellulose production. *Carbohydr. Polym.* 152, 841–849 (2016).