Nativa, Sinop, v. 12, n. 3, p. 567-573, 2024.
Pesquisas Agrárias e Ambientais
DOI: https://doi.org/10.31413/nativa.v12i3.17720
ISSN: 2318-7670

Production of bacterial cellulose from kombucha tea and coffee husk infusion

Augusta JIMÉNEZ-SÁNCHEZ [1], Linet HERNÁNDEZ-GIL [2], Yomara JIMÉNEZ-SÁNCHEZ [3], Mario A. GARCÍA PÉREZ [4*], Elianne RODRÍGUEZ-LARRABURU [5]

[1] Facultad de Ingeniería Química, Universidad de Guayaquil, Guayaquil, Ecuador.
[2] Universidad Carlos III de Madrid, Leganés, España.
[3] Escuela de Ingenierías, Pontificia Universidad Católica del Ecuador, Ambato, Ecuador.
[4] Universidad San Gregorio de Portoviejo, Manabí, Ecuador.
[5] Instituto Superior Universitario Bolivariano de Tecnología, Guayaquil, Ecuador.
*E-mail: marioifal@gmail.com

Submitted on: 05/23/2024; Accepted on: 09/08/2024; Published on: 09/27/2024.

ABSTRACT: This paper determined the best formulation of culture medium based on Kombucha tea and infusion of coffee husk for producing bacterial cellulose (BC) from *Acetobacter xylinum*. The highest BC production corresponded to the medium containing tea, coffee husk infusion, sugar and 0.005% methionine. This greater production occurred when the inoculum was kept in the dark, which allowed high multiplication of the microorganisms and, therefore, greater polymer production. Introducing a new, feasible, malleable material from an environmentally friendly process will allow the replacement of materials with a greater impact on pollution and cost.
Keywords: *Acetobacter xylinum*; biomaterials; bacterial cellulose; coffee husk; Kombucha.

Produção de celulose bacteriana a partir de infusão de chá de kombuchá e casca de café

RESUMO: Neste artigo foi determinada a melhor formulação de meio de cultura à base de chá de Kombuchá e infusão de casca de café para produção de celulose bacteriana (BC) de *Acetobacter xylinum*. A maior produção de CB correspondeu ao meio contendo chá, infusão de casca de café, açúcar e 0,005% de metionina. Essa maior produção ocorreu quando o inóculo foi mantido no escuro, o que permitiu alta multiplicação dos microrganismos e, portanto, maior produção do polímero. A introdução de um novo material viável, maleável e proveniente de um processo amigo do ambiente permitirá a substituição de materiais com maior impacto na poluição e no custo.
Palavras-chave: *Acetobacter xylinum*; biomateriais; celulose bacteriana; casca de café; Kombuchá.

1. INTRODUCTION

Population growth and current lifestyle contribute to the generation of plastic waste (AWOYERA; ADESINA, 2020); the global figure of 300 million metric tons (MT) of plastic waste generated during 2015 (SINGH; SHARMA, 2016), forces us to look for alternatives for the production of materials with adequate physical, mechanical and functional characteristics, that are biodegradable and, in addition, in their synthesis, renewable materials are used, they are accessible and do not generate toxic waste. For this reason, environmentally friendly and compatible alternatives are sought. In this context, we find bacterial cellulose (BC), an extracellular polysaccharide produced by bacteria of the genera *Achromobacter*, *Agrobacterium*, *Rhizobium*, *Sarcina*, *Zoogloea* (JARAMILLO et al., 2012) and *Gluconacetobacter* (ROSS et al., 1991), among which *Gluconacetobacter xylinum* (KUO et al., 2016) stands out. This natural polymer is similar to plant cellulose, although it has certain differences (SANTOS et al., 2015). BC does not contain lignin, pectin, hemicellulose (AVANTHI et al., 2017) or phytates, so it is purer and quicker to obtain.

The characteristics of BC membranes, such as high water retention, compressibility, elongation, high crystallinity (VANDAMME et al., 1998; AVANTHI et al., 2017), mechanical strength, large surface areas, high porosity, biocompatibility (CARREÑO et al., 2012; HUSSAIN et al., 2019), allow them to be used in different products (ABDELRAOF et al., 2019), for example, in the food, pharmaceutical, environmental, acoustics, paper sectors, among others (CARREÑO et al., 2012). However, the high costs of culture media and low productivity on an industrial scale are the obstacles BC production faces (HUSSAIN et al., 2019). This is why we are working on optimizing processes to reduce the use of expensive and low-performance commercial media (JANG et al., 2017), diversifying the use of raw materials such as agricultural waste, and improving fermentation, separation and purification processes (LEE; KIM, 2015).

Obtaining BC depends on several factors, such as carbon and nitrogen sources, pH, oxygen, sowing method and sowing surface area (JONAS; FARAH, 1998). Products such as brewer's yeast, fruit juices (VIANA et al., 2018), sugar,

glucose, fruit residues (KUROSUMI et al., 2009), as well as chemicals such as sodium dihydrogen phosphate (DE OLYVEIRA et al., 2017), citric acid, molasses, have been used, which have been studied with excellent results (BAE; SHODA, 2005; KSIĄŻEK, 2024), and others such as methionine and lactic acid (JONAS; FARAH, 1998). Methodologies for obtaining BC include static, rotational culture, bioreactors, rotating bioreactors, rotating disk bioreactors, and modified bioreactors (WANG et al., 2018). The production of BC in static and agitated form would seem similar (NGUYEN et al., 2008), however, there is evidence that the highest production of BC is in static culture in a minimum of 7 to 8 days and with 90 g/L of sucrose (KRYSTYNOWICZ et al., 2002). Therefore, alternative raw materials such as agroindustrial waste available in the environment are sought to obtain better yields at a lower cost.

A raw material could be coffee husk. Around 10 million MT of coffee were produced globally in 2018, and periodically, 85% is produced in Latin America, 10% in Asia and 5% in Africa; 85% corresponds to *Arabica coffee* and the rest to *Robusta coffee* (SKORUPA et al., 2023). The processing of coffee beans generates waste that usually becomes environmental contaminants (ULLOA et al., 2003; WANG et al., 2019) and a burden on the industrial process. The husk of *Arabica coffee* (*Coffea arabica* L.) contains a large amount of polyphenols (RANI; APPAIAH et al., 2013) and tannins that cause enormous contamination problems and prevent its use (LEIFA et al., 2000). However, it contains 26 to 27% sugars (HU et al., 2020) with 10 to 15% fructose (ANTIER et al., 1993) proteins and minerals; and could be a carbon source (RANI; APPAIAH et al., 2013) for BC production.

Considering the above, this paper determined the best formulation of culture medium based on Kombucha tea and coffee husk infusion to produce BC from *Acetobacter xylinum*. This improves yields and reduces elaboration costs, enhancing its applications.

2. MATERIAL E METHODS

2.1. Culture media

Culture media were prepared based on black tea (Hornimas, Cetca, Ecuador), sugar (Valdez, Compañía Azucarera Valdez S.A., Ecuador), methionine (Evonik Nutrition, Belgium), sugar cane molasses (Energías del Agro San Juan S.A., Ecuador) and coffee husks (*Coffea arabica* L.) var. Caturra (Loja, Ecuador).

2.2. Obtaining BC seed

A sample of Kombucha (Qinca Kombucha-Empresa de Foods y Bebidas, Guayaquil) was plated on GYC agar for 3 days. One colony was transferred to 100 mL of liquid medium (pH = 5) containing 10% sugar and 0.8% black tea and incubated for 72 h at 28 to 30 °C. Subsequently, 10% (v/v) of the infected liquid medium was seeded in 200 mL of sterile liquid medium. After 4 days of fermentation, they were transferred to 1.5 L capacity containers with 700 mL of sterile medium and incubated for 10 days between 28 and 30 °C until the BC seed was obtained.

2.3. BC membrane production

The production process of BC membranes consisted of inoculation, maintenance, harvest and purification. To do this, it began with the sowing of 20 g of moist BC seed membrane in 200 mL of the culture medium corresponding to the content in sterile flasks with a capacity of 300 mL. These were kept in the dark for 4 days between 28 and 30 °C (Procedure 1) and under normal daylight (Procedure 2). Then, the contents of the flasks from procedures 1 and 2 were transferred to 1.5 L bioreactors along with 700 mL of the corresponding freshly prepared medium. The bioreactors were kept covered with canvas to prevent the entry of dust, insects or contamination, ensuring that adequate hygienic-sanitary conditions existed during static fermentation and were kept under the normal effect of natural light for 10 days between 28 and 30 °C (Figure 1).

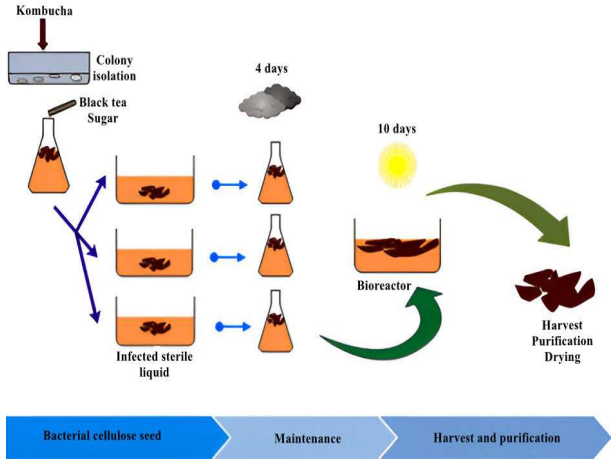

Figure 1. Laboratory-scale bacterial cellulose production under initial dark conditions.

Figura 1. Produção de celulose bacteriana em escala de laboratório sob condições iniciais de escuridão.

After 14 days from the beginning, the membrane was harvested and transferred to a container with hot water at 80 °C that was heated to boiling to eliminate microorganisms and safely handle the material. Afterward, it was washed with drinking water and boiled for 15 min with 1% (m/v) calcium carbonate to eliminate any remaining microbial cells. It was washed again with drinking water and immersed for 24 h with water exchange until the coloration disappeared. It was then immersed in 5% (v/v) acetic acid for 30 min, and its excess was removed with distilled water. The clean membrane was manually pressed to facilitate the removal of water and dried in a forced convection oven at 35 °C until constant mass, thus determining the performance of the process.

2.4. BC membrane production treatments

The best formulation of the culture medium (including coffee husks and additives) and the most favorable maintenance condition for obtaining BC by A. xylinum were determined. For the first, laboratory-scale productions were tested with different concentrations of black tea and coffee husks var. Caturra (Table 1) in infusion. In this trial, sugar and methionine were kept constant.

Next, the best culture maintenance condition was achieved by evaluating the best culture medium formulation in darkness (Procedure 1) and natural light (Procedure 2). After determining the best condition, the influence of sugar, sugar cane molasses and methionine was verified (Table 2).

2.5. Characterization of the microorganism

The morphology of colonies developed on GYC agar after 48 h was examined with an optical microscope (Olympus, Japan). The microorganism was isolated and

identified using Gram stain and catalase and oxidase tests (BRENNER, 2005).

Table 1. Variation of the components of the culture medium for the production of bacterial cellulose depending on the treatments
Tabela 1. Variação dos componentes do meio de cultura para produção de celulose bacteriana em função dos tratamentos

Product (%)	T1	T2	T3	T4	T5
Black tea	0	25	50	75	100
Coffee	100	75	50	25	0
Sugar	10	10	10	10	10
Methionine	0.005	0.005	0.005	0.005	0.005

Table 2. Additives in the culture medium for the production of bacterial cellulose.
Tabela 2. Aditivos no meio de cultura para produção de celulose bacteriana.

Additive (%)	Culture medium formulation				
	A	B	C	D	E
Methionine	0	0	0	0.5	0.5
Sugar	5	2	10	5	2
Sugar cane molasses	5	8	0	5	8

2.6. Characterization of cellulose membranes

The pH (DU et al., 2018) was evaluated in the fermentation medium at the beginning and end of fermentation. The dimensions of the membranes were determined and the performance was calculated through an equation that relates the mass of cellulose produced per mass of sugar in a volume of 1 L (GOH et al., 2012):

$$\text{Performance (\%)} = \frac{\text{Cellulose } (\frac{g}{L})}{\text{Sugar } (\frac{g}{L})} \cdot 100 \qquad (01)$$

The cellulose fibers that comprise the bacterial membrane were observed through an Inspect S50 scanning microscope (FEI, USA). The sample was coated with gold-palladium to improve its conductivity.

Furthermore, the presence of cellulose in the membranes was verified using a Nicolet 6700 FTIR spectrophotometer (Thermo, USA) in the range of 4000 to 400 cm^{-1} in ATR mode. Spectra were obtained with a resolution of 4 cm^{-1} and an accumulation of 64 measurements. Before measurement, the samples were dried at 40 °C for 2 h to remove moisture.

2.6. Statistical analysis

Analysis of variance was carried out to determine whether there were significant differences between cellulose yields for each formulation. The Tukey test was used to compare means, while the Student's t-test was used to verify the influence of light. In all cases, the SPSS program (v. 22, 2013, IMB Corporation) was used with a confidence level of 95%.

3. RESULTS

The isolated colonies were off-white and creamy, between 1 and 5 µm in size, and the shape of a round bacillus or rod, similar to those found by DU et al. (2018). They were Gram-negative bacilli with positive catalase activity and negative oxidase activity. They grew in a medium with acetic acid and alcohol and were cellulose producers; the isolates were identified as *Gluconacetobacter* (DU et al., 2018).

After 14 days of production, it was harvested, and it was found that the formulation with 25% tea and 75% coffee husk, corresponding to 8 g of substrate/L of infusion, presented the highest cellulose production (Figure 2). The highest yield was 18.9 g/L/d of cellulose. There was a direct relationship between the presence of coffee husk and cellulose production, although this decreased when the medium consisted only of black tea.

Figure 3 shows the results of BC synthesis considering the inclusion of cane molasses, methionine and the two sowing processes: in darkness and natural light. It is observed that the highest value ($p \leq 0.05$) corresponded to formulation C of the first process, that is, to the infusion of sugar, tea and coffee husks planted in darkness and subsequently exposed to light. The masses of the membranes obtained under lighting conditions showed significant differences; the highest production value was for the dark growth process.

Formulations with methionine exhibited the lowest BC production values. The formulations' analysis of variance showed significant differences between the treatments (A, B, C, D, E, F). The 95% Tukey test showed that concerning the wet mass or yield variable, treatments F and E, B and D, and the previous ones had similarities to treatments A and C.

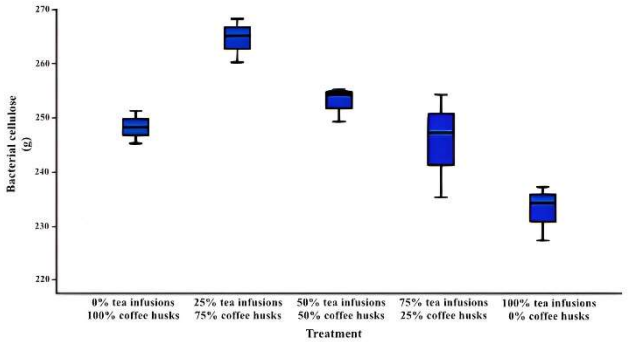

Figure 2. Bacterial cellulose production in the medium of tea infusions and coffee husks.
Figura 2. Produção de celulose bacteriana em meio de infusões de chá e casca de café.

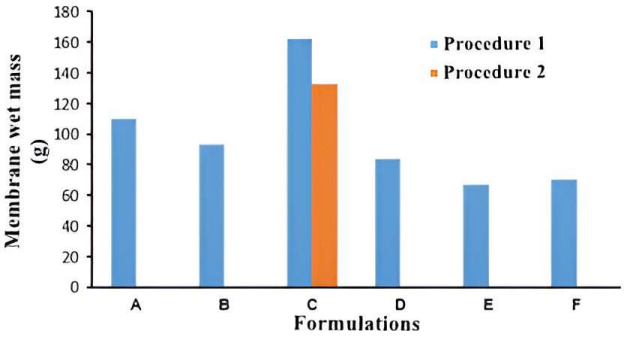

Figure 3. Mass of bacterial cellulose synthesized under dark and light conditions. Procedure 1: dark for 4 days between 28 and 30 ºC; and Procedure 2: under normal daylight.
Figura 3. Massa de celulose bacteriana sintetizada em condições de claro e escuro. Procedimento 1: escuro por 4 dias entre 28 e 30 ºC; e Procedimento 2: sob luz natural.

The final pH of all treatments was between 3.3 and 4.1; there were no significant differences between treatments A, B, C and A, C, D, E, and F (Table 3). The dry mass variable shows that treatments A and B; B, D and F; D, E and F did not present significant differences. Treatment C presented significant differences compared to the other treatments and greater mass ($p \leq 0.05$) (Table 3). The production yield

showed similarities between treatments D, F, E, and F and differences between the others. Table 4 brings together the physical and mechanical characteristics of the BC of each formulation. The thickness of the membranes measured at 10 different points ranged between 0.092 and 0.512 mm. The physical characteristics of the membranes varied. The membranes with coffee husk had a whiter color than the rest. Membrane C presented the highest ($p \leq 0.05$) tensile strength, and sample D presented the highest ($p \leq 0.05$) elongation at break. The membranes with methionine were thinner, more fragile and more elastic ($p \leq 0.05$).

Electron microscopy (Figure 4) allowed us to observe the formed and interwoven fibers that form the membranes. The microorganisms producing the nanofibers are observed (Figure 4a). You can see the pores formed by the cellulose-producing microorganisms housed in the membrane. The surface is not completely smooth or flat (Figure 4b). The grouping of the fibers forms nanometric fabrics to form membranes several mm thick.

The FTIR spectrum of BC produced with the infusion of coffee husks and sugar is observed in Fig. 5. The bands at specific wavelengths show similarity to plant cellulose. The band between 3482 and 3200 cm^{-1} could indicate inter- and intramolecular bonds;[31] between 3450 and 3420 cm^{-1} peaks corresponding to the O-H vibration are observed (DAYAL et al., 2013); the band bendings from 2912 to 2960 cm^{-1} could be due to the presence of the C-H and CH_2 groups (DAYAL et al., 2013). The peak at 2960 cm^{-1} represents the amorphous nature of BC (BAGEWADI et al., 2020). The band at 1647 cm^{-1} is attributed to a hydroxyl (AN et al., 2017; BULDUM et al., 2018), and at 1333 cm^{-1} indicates OH bending and the crystallinity of BC. At 1067 cm^{-1}, the characteristic peak of the C-O-C bond of BC is observed (JOSEPH et al., 2003; SHAO et al., 2016).

Table 3. Mass and production yield of bacterial cellulose.
Tabela 3. Massa e rendimento de produção de celulose bacteriana.

Treatment	pH	Wet mass (g)	Dry mass (g)	Performance ($g\ L^{-1}\ d^{-1}$)*
A	3.4 ± 0.5 ab	110.0 ± 3.6 c	2.8233 ± 0.1 c	7.87 ± 0.2 e
B	3.3 ± 0.5 a	87.7 ± 9.0 b	2.0911 ± 0.5 bc	6.63 ± 1.3 d
C	3.7 ± 0.6 ab	162.0 ± 2.0 d	5.4875 ± 0.8 d	11.57 ± 0.1 f
D	4.1 ± 0.1 b	85.3 ± 4.5 b	1.6734 ± 0.2 ab	5.97 ± 0.7 bc
E	4.1 ± 0.1 b	67.0 ± 3.0 a	1.2640 ± 0.09 a	4.8 ± 0.2 a
F	4.1 ± 0.1 b	70.0 ± 1.0 a	1.3471 ± 0.1 ab	5.0 ± 0.1 ab

Mean ± standard deviation; n= 3.
Different letters indicate significant differences ($p \leq 0.05$).
*Mass of bacterial cellulose per volume of medium and fermentation time.

Table 4. Mechanical properties and cost of BC membranes according to the formulation.
Tabela 4. Propriedades mecânicas e custo das membranas BC de acordo com a formulação.

Treatment	Thickness (mm)	Tensile strength (MPa)	Elongation at break (mm)	Cost ($ USD)
A	0.52 ± 0.1 a	55.0 ± 3.0 d	0.014 ± 0.001 a	0.116
B	0.19 ± 0.1 b	48.0 ± 2.0 c	0.018 ± 0.001 b	0.093
C	0.23 ± 0.02 b	64.0 ± 2.0 e	0.021 ± 0.001 c	0.153
D	0.15 ± 0.01 b	18.0 ± 1.0 b	0.068 ± 0.002 d	0.266
E	0.12 ± 0.01 b	15.0 ± 4.4 ab	0.056 ± 0.002 e	0.243
F	0.097 ± 0.008 b	13.0 ± 2.0 a	0.047 ± 0.001 f	0.303

Mean ± standard deviation; n= 3.
Different letters indicate significant differences ($p \leq 0.05$).

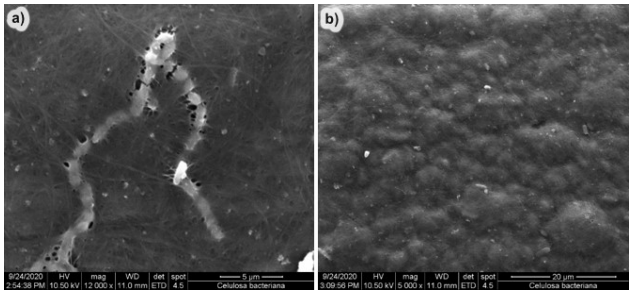

Figure 4. Scanning electron microscopy images. a) Microorganism with nanofiber production; b) Surface of cellulose membranes.
Figura 4. Imagens de microscopia eletrônica de varredura. a) Microrganismo com produção de nanofibras; b) Superfície das membranas de celulose.

The calcium carbonate used to wash the membranes cleaned away cellular debris and other substances, allowing for safer handling of the membranes. Once dry, this material was soft to the touch and flexible, in addition to being light and difficult to break by hand, dyeable and easy to manipulate, which led to the creation of objects.

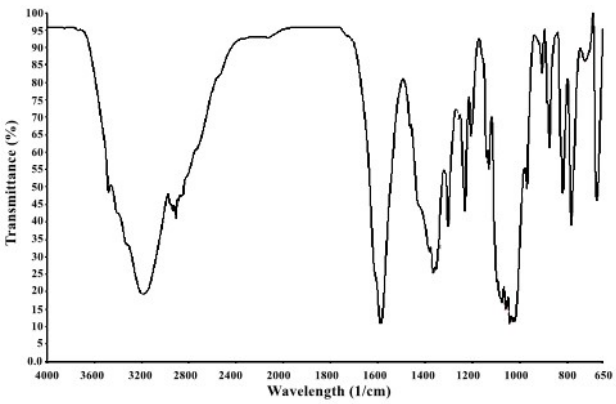

Figure 5. FTIR spectrum of the bacterial cellulose from Kombucha tea and coffee husk infusion.
Figura 5. Espectro FTIR da celulose bacteriana do chá de Kombuchá e infusão de casca de café.

4. DISCUSSION

The production of BC with coffee husks in this work gave a production of 18.8 and 11.7 g/L, while for RANI; APPAIAH (2013) with coffee husks, informed values of 5.6 g/L; additional with urea and fermented corn liquor gave values of 8.2 g/L, both lower values, which could be due to the sugar in the pulp adhered to the coffee husks. The pulp has about 27% sugar (HU et al., 2020) and 10% fructose (ANTIER et al., 1993), so the husks could be an attractive raw material for BC production.

The transformation of glucose into gluconic acid due to the presence of glucose dehydrogenase causes a decrease in pH to approximately 3 which could inhibit the production of BC (KRYSTYNOWICZ et al., 2002), which is why pH control is critical in the production of BC with *A. xylinum*, (JOSEPH et al., 2003) which suggests the use of buffered media (NORO et al., 2004), and it has been observed that with the use of sucrose the pH drops only to 4.1 although the optimal pH of *A. xylinum* is between 4.5 to 5.5 (NORO et al., 2004); however in this paper, it is observed that the pH drops to 3.3.

Molasses, a byproduct of the final state of crystallization in sugar production, constitute one of the most economical carbon sources in the microbiological industry (BAE; SHODA, 2004). The sugar composition does not affect BC production as long as fructose is in the medium (BAE; SHODA, 2005), most likely because fructose activates a phosphoenolpyruvate-dependent phosphotransferase present in *A. xylinum* (TONOUCHI et al., 1996). However, these tests showed that the formulations with more molasses presented low BC production.

The results show the significant influence of pH on bacterial cellulose production (ZAHAN et al., 2015). Treatments with a more acidic pH, such as those observed in treatments A and B (around 3.4 and 3.3, respectively), decreased mass production of wet and dry BC compared to treatment C, which had a higher pH of around 3.7. This is because acidic conditions can negatively affect the metabolic activity of cellulose-producing bacteria, such as *Gluconacetobacter xylinus*, which is sensitive to the pH of the medium. Lower pH may inhibit cellulose synthesis or affect cell viability, reducing BC production. In contrast, as in treatment C, a slightly higher pH may be more optimal for bacterial cellulose growth and production, resulting in greater BC wet and dry mass (ZAHAN et al., 2015; HASANIN et al., 2023).

Treatment C, with a pH of around 3.7, showed the best results regarding wet (162.0 g) and dry (5.4875 g) mass of BC. These observations suggest that slightly alkaline conditions favor the metabolic activity of cellulose-producing bacteria, probably *Gluconacetobacter xylinus*, resulting in increased BC production. An optimal pH can facilitate the synthesis and accumulation of extracellular cellulose, thus contributing to a higher wet and dry mass of BC than in acidic conditions.

The differences in the thickness of the membranes produced under different treatments could be because the treatments used specific culture conditions that affect the production and quality of bacterial cellulose, which can influence the structure and properties of the cellulose (KACZMAREK et al., 2022). The composition of the culture medium, including nutrients and carbon sources, can vary between treatments and affect the production and characteristics of bacterial cellulose (FATIMA et al., 2023). Fermentation conditions such as temperature, pH and fermentation time between treatments directly impact the formation of bacterial cellulose, including its structure and physical properties (GORGIEVA et al., 2023).

The increase in 1,4 covalent bonds (KESHK, 2006) from the fibers' crossing produces the membranes' mechanical characteristics (RANI; APPAIAH, 2013). In this work, the mechanical properties increased with the medium that used only sugar and coffee.

The suitable nitrogen source for BC production depends on the carbon source, preferably casein and peptone (RAMANA et al., 2000). Casein is presented as indispensable at the beginning of microbial growth (MATSUOKA et al., 1996) with increased cellulose production. However, with methionine, low BC production and disordered cellulose growth with an unpleasant appearance were observed in this paper, possibly due to the excess added amino acid.

An important effect of a culture medium parameter on BC production was dark conditions during sowing. In the darkness, microorganisms grow, and cellulose production increases since it is generated to protect from UV rays (SCOTT; CANNON, 1989).

5. CONCLUSIONS

The highest BC production corresponded to the medium containing tea, coffee husk infusion, sugar and 0.005% methionine. This greater production occurred when the inoculum was kept in the dark, which allowed the microorganisms to multiply highly and, therefore, greater polymer production. Introducing a new, feasible, malleable material from an environmentally friendly process will allow materials to be replaced with a greater impact on pollution and cost.

6. REFERENCES

ABDELRAOF, M.; HASANIN, M. S.; EL-SAIED, H. Ecofriendly green conversion of potato peel wastes to high productivity bacterial cellulose. **Carbohydrate Polymers**, v. 211, p. 75-83, 2019. https://doi.org/10.1016/j.carbpol.2019.01.095

AN, S. J.; LEE, S. H.; HUH, J. B.; JEONG, S. I.; PARK, J. S.; GWON, H. J.; KANG, E. S.; JEONG, C. M.; LIM, Y. M. Preparation and characterization of resorbable bacterial cellulose membranes treated by electron beam irradiation for guided bone regeneration. **International Journal of Molecular Sciences**, v. 18, n. 11, e2236, 2017. https://doi.org/10.3390/ijms18112236

ANTIER, P.; MINJARES, A.; ROUSSOS, S.; RAIMBAULT, M.; VINIEGRA-GONZALEZ, G. Pectinase-hyperproducing mutants of *Aspergillus niger* C28B25 for solid-state fermentation of coffee pulp. **Enzyme and Microbial Technology**, v. 15, n. 3, p. 254-260, 1993. https://doi.org/10.1016/0141-0229(93)90146-s

AVANTHI, A.; KUMAR, G. L.; BANERJEE, R. Partially consolidated bioprocessing of mixed lignocellulosic feedstocks for ethanol production. **Bioresource Technology**, v. 245, p. 530-539, 2017. https://doi.org/10.1016/j.biortech.2017.08.140

AWOYERA, P. O.; ADESINA, A. Plastic wastes to construction products: Status, limitations and future perspective. **Case Studies in Construction Materials**, v. 12, e00330, 2020. https://doi.org/10.1016/j.cscm.2020.e00330

BAE, S. O.; SHODA, M. Production of bacterial cellulose by *Acetobacter xylinum* BPR2001 using molasses medium in a jar fermentor. **Applied Microbiology and Biotechnology**, v. 67, n. 1, p. 45-51, 2005. https://doi.org/10.1007/s00253-004-1723-2

BAE, S.; SHODA, M. Bacterial cellulose production by fed-batch fermentation in molasses medium. **Biotechnology Progress**, v. 20, n. 5, p. 1366-1371, 2004. https://doi.org/10.1021/bp0498490

BAE, S.; SHODA, M. Statistical optimization of culture conditions for bacterial cellulose production using Box-Behnken design. **Biotechnology and Bioengineering**, v. 90, n. 1, p. 20-28, 2005. https://doi.org/10.1002/bit.20325

BAGEWADI, Z. K.; BHAVIKATTI, J. S.; MUDDAPUR, U. M.; YARAGUPPI, D. A.; MULLA, S. I. Statistical optimization and characterization of bacterial cellulose produced by isolated thermophilic *Bacillus licheniformis* strain ZBT2. **Carbohydrate Research**, v. 491, e107979, 2020. https://doi.org/10.1016/j.carres.2020.107979

BRENNER, D. J.; KRIEG, N. R.; STALEY, J. T.; GARRITY, G. M. (Eds.). **Bergey's Manual of Systematic Bacteriology**. 2nd Edition, v. 2 (The Proteobacteria), part C (The Alpha-, Beta-, Delta-, and Epsilonproteobacteria), Springer, New York, 2005.

BULDUM, G.; BISMARCK, A.; MANTALARIS, A. Recombinant biosynthesis of bacterial cellulose in genetically modified *Escherichia coli*. **Bioprocess and Biosystems Engineering**, v. 41, n. 2, p. 265-279, 2018. https://doi.org/10.1007/s00449-017-1864-1

CARREÑO, L.; CAICEDO, L.; ALFONSO, L.; MARTÍNEZ, C. Técnicas de fermentación y aplicaciones de la celulosa bacteriana: una revisión. **Ingeniería y Ciencia**, v. 8, n. 16, p. 307-335, 2012. http://www.redalyc.org/articulo.oa?id=83524625012

DAYAL, M. S.; GOSWAMI, N.; SAHAI, A.; JAIN, V.; MATHUR, G.; MATHUR, A. Effect of media components on cell growth and bacterial cellulose production from *Acetobacter aceti* MTCC 2623. **Carbohydrate Polymers**, v. 94, n. 1, p. 12-6, 2013. https://doi.org/10.1016/j.carbpol.2013.01.018

DE OLYVEIRA, G. M.; BASMAJI, P.; COSTA, L. M. M.; DOS SANTOS, M. L.; DOS SANTOS, C.; GUASTALDI, F. P. S.; SCAREL-CAMINAGA, R. M.; DE OLIVEIRA, T. S.; PIZONI, E.; GUASTALDI, A. C. Surface physical chemistry properties in coated bacterial cellulose membranes with calcium phosphate. **Materials Science and Engineering: C**, v. 75, p. 1359-1365, 2017. https://doi.org/10.1016/j.msec.2017.03.025

DU, R.; ZHAO, F.; PENG, Q.; ZHOU, Z.; HAN, Y. Production and characterization of bacterial cellulose produced by *Gluconacetobacter xylinus* isolated from Chinese persimmon vinegar. **Carbohydrate Polymers**, v. 194, p. 200-207, 2018. https://doi.org/10.1016/j.carbpol.2018.04.041

FATIMA, A.; ORTIZ-ALBO, P.; NEVES, L. A.; NASCIMENTO, F. X.; CRESPO, J. G. Biosynthesis and characterization of bacterial cellulose membranes presenting relevant characteristics for air/gas filtration. **Journal of Membrane Science**, v. 674, e121509, 2023. https://doi.org/10.1016/j.memsci.2023.121509

GOH, W. N.; ROSMA, A.; KAUR, B.; FAZILAH, A.; KARIM, A. A.; BHAT, R. Fermentation of black tea broth (Kombucha): I. effects of sucrose concentration and fermentation time on the yield of microbial cellulose. **International Food Research Journal**, v. 19, n. 1, p. 109-117, 2012. http://www.ifrj.upm.edu.my/19%20(01)%202011/(15)IFRJ-2011-105%20Rajeev.pdf

GORGIEVA, S.; JANČIČ, U.; CEPEC, E.; TRČEK, J. Production efficiency and properties of bacterial cellulose membranes in a novel grape pomace hydrolysate by *Komagataeibacter melomenusus* AV436T and *Komagataeibacter xylinus* LMG 1518. **International Journal of Biological Macromolecules**, v. 244, e125368, 2023. https://doi.org/10.1016/j.ijbiomac.2023.125368

HASANIN, M. S.; ABDELRAOF, M.; HASHEM, A. H.; SAIED, H. E. Sustainable bacterial cellulose production by *Achromobacter* using mango peel waste. **Microbial Cell Factories**, v. 22, e24, 2023. https://doi.org/10.1186/s12934-023-02031-3

HU, G.; PENG, X.; WANG, X.; LI, X.; LI, X.; QIU, M. Excavation of coffee maturity markers and further research on their changes in coffee cherries of different maturity. **Food Research International**, v. 132, 109121, 2020. https://doi.org/10.1016/j.foodres.2020.109121

HUSSAIN, Z.; SAJJAD, W.; KHAN, T.; WAHID, F. Production of bacterial cellulose from industrial wastes: a review. **Cellulose**, v. 26, p. 2895-2911, 2019. https://doi.org/10.1007/s10570-019-02307-1

JANG, W. D.; HWANG, J. H.; KIM, H. U.; RYU, J. Y.; LEE, S. Y. Bacterial cellulose as an example product for sustainable production and consumption. **Microbial Biotechnology**, v. 10, n. 5, p. 1181-1185, 2017. https://doi.org/10.1111/1751-7915.12744

JARAMILLO, R.; TOBIO, W.; ESCAMILLA, J. Efecto de la sacarosa en la producción de celulosa por *Gluconacetobacter xylinus* en cultivo estático. **Revista MVZ Córdoba**, v. 17, n. 2, p. 3004-3013, 2012. http://www.redalyc.org/articulo.oa?id=69323751008

JONAS, R.; FARAH, L. F. Production and application of microbial cellulose. **Polymer Degradation and Stability**, v. 59, n. 1-3, p. 101-106, 1998. https://doi.org/10.1016/S0141-3910(97)00197-3

JOSEPH, G.; ROWE, G. E.; MARGARITIS, A.; WAN, W. Effects of polyacrylamide-co-acrylic acid on cellulose production by *Acetobacter xylinum*. **Journal of Chemical Technology and Biotechnology**, v. 78, n. 9, p. 964-970, 2003. https://doi.org/10.1002/jctb.869

KACZMAREK, M.; JĘDRZEJCZAK-KRZEPKOWSKA, M.; LUDWICKA, K. Comparative Analysis of Bacterial Cellulose Membranes Synthesized by Chosen *Komagataeibacter* Strains and Their Application Potential. **International Journal of Molecular Sciences**, v. 23, n. 6, e3391, 2022. https://doi.org/10.3390/ijms23063391

KESHK, S. Physical properties of bacterial cellulose sheets produced in presence of lignosulfonate. **Enzyme and Microbial Technology**, v. 40, p. 9-12, 2006. https://doi.org/10.1016/j.enzmictec.2006.07.038

KRYSTYNOWICZ, A.; CZAJA, W.; WIKTOROWSKA-JEZIERSKA, A.; GONÇALVES-MIŚKIEWICZ, M.; TURKIEWICZ, M.; BIELECKI, S. Factors affecting the yield and properties of bacterial cellulose. **Journal of Industrial Microbiology and Biotechnology**, v. 29, n. 4, p. 189-195, 2002. https://doi.org/10.1038/sj.jim.7000303

KSIĄŻEK, E. Citric Acid: Properties, Microbial Production, and Applications in Industries. **Molecules**, v. 29, e22, 2024. https://doi.org/10.3390/molecules29010022

KUO, C.H.; CHEN, J. H.; LIOU, B. K.; LEE, C. K. Utilization of acetate buffer to improve bacterial cellulose production by *Gluconacetobacter xylinus*. **Food Hydrocolloids**, v. 53, p. 98-103, 2016. https://doi.org/10.1016/j.foodhyd.2014.12.034

KUROSUMI, A.; SASAKI, C.; YAMASHITA, Y.; NAKAMURA, Y. Utilization of various fruit juices as carbon source for production of bacterial cellulose by *Acetobacter xylinum* NBRC 13693. **Carbohydrate Polymers**, v. 76, n. 2, p. 333-335, 2009. https://doi.org/10.1016/j.carbpol.2008.11.009

LEE, S. Y.; KIM, H. U. Systems strategies for developing industrial microbial strains. **Nature Biotechnology**, v. 33, n. 10, p. 1061-1072, 2015. https://doi.org/10.1038/nbt.3365

LEIFA, F.; PANDEY, A.; SOCCOL, C. R. Solid state cultivation - An efficient method to use toxic agro-industrial residues. **Journal of Basic Microbiology**, v. 40, n. 3, p. 187-197, 2000. https://doi.org/10.1002/1521-4028(200007)40:3<187::AID-JOBM187>3.0.CO;2-Q

MATSUOKA, M.; TSUCHIDA, T.; MATSUSHITA, K.; ADACHI, O.; YOSHINAGA, F. A Synthetic Medium for Bacterial Cellulose Production by *Acetobacter xylinum* subsp. *sucrofermentans*. **Bioscience, Biotechnology and Biochemistry**, v. 60, n. 4, p. 575-579, 1996. https://doi.org/10.1271/bbb.60.575

NGUYEN, V. T.; FLANAGAN, B.; GIDLEY, M. J.; DYKES, G. A. Characterization of cellulose production by a *Gluconacetobacter xylinus* strain from Kombucha. **Current Microbiology**, v. 57, n. 5, p. 449-453, 2008. https://doi.org/10.1007/s00284-008-9228-3

NORO, N.; SUGANO, Y.; SHODA, M. Utilization of the buffering capacity of corn steep liquor in bacterial cellulose production by *Acetobacter xylinum*. **Applied Microbiology and Biotechnology**, v. 64, p. 199-205, 2004. https://doi.org/10.1007/s00253-003-1457-6

RAMANA, K. V.; TOMAR, A.; SINGH, L. Effect of various carbon and nitrogen sources on cellulose synthesis by *Acetobacter xylinum*. **World Journal of Microbiology and Biotechnology**, v. 16, n. 3, p. 245-248, 2000. https://doi.org/10.1023/A:1008958014270

RANI, M. U.; APPAIAH, K. A. A. Production of bacterial cellulose by *Gluconacetobacter hansenii* UAC09 using coffee cherry husk. **Journal of Food Science and Technology**, v. 50, n. 4, p. 755-762, 2013. https://doi.org/10.1007/s13197-011-0401-5

ROSS, P.; MAYER, R.; BENZIMAN, A. N. D. M. Cellulose Biosynthesis and Function in Bacteria. **Microbiological Reviews**, v. 55, n. 1, p. 35-58, 1991. https://doi.org/10.1128/mr.55.1.35-58.1991

SANTOS, S. M.; CARBAJO, J. M.; QUINTANA, E.; IBARRA, D.; GÓMEZ, N.; LADERO, M.; EUGENIO, M. E.; VILLAR, J. C. Characterization of purified bacterial cellulose focused on its use on paper restoration. **Carbohydrate Polymers**, v. 116, p. 173-81, 2015. https://doi.org/10.1016/j.carbpol.2014.03.064

SCOTT, W. S.; CANNON, R. E. Alternative environmental roles for cellulose produced by *Acetobacter xylinum*. **Applied and Environmental Microbiology**, v. 55, n. 10, p. 2448-2452, 1989. https://doi.org/10.1128%2Faem.55.10.2448-2452.1989

SHAO, W.; LIU, H.; WANG, S.; WU, J.; HUANG, M.; MIN, H.; LIU, X. Controlled release and antibacterial activity of tetracycline hydrochloride-loaded bacterial cellulose composite membranes. **Carbohydrate Polymers**, v. 145, p. 114-120, 2016. https://doi.org/10.1016/j.carbpol.2016.02.065

SINGH, P.; SHARMA, V. P. Integrated plastic waste management: environmental and improved health approaches. **Procedia Environmental Sciences**, v. 35, p. 692-700, 2016. https://doi.org/10.1016/j.proenv.2016.07.068

SKORUPA, A.; WORWĄG, M.; KOWALCZYK, M. Coffee Industry and Ways of Using By-Products as Bioadsorbents for Removal of Pollutants. **Water**, v. 15, e112, 2023. https://doi.org/10.3390/w15010112

TONOUCHI, N.; TSUCHIDA, T.; YOSHINAGA, F.; BEPPU, T.; HORINOUCHI, S. Characterization of the biosynthetic pathway of cellulose from glucose and fructose in *Acetobacter xylinum*. **Bioscience, Biotechnology and Biochemistry**, v. 60, n. 8, p. 1377-1379, 1996. https://doi.org/10.1271/bbb.60.1377

ULLOA, J. B.; VERRETH, J. A. J.; AMATO, S.; HUISMAN, E. A. Biological treatments affect the chemical composition of coffee pulp. **Bioresource Technology**, v. 89, n. 3, p. 267-274, 2003. https://doi.org/10.1016/s0960-8524(03)00070-1

VANDAMME, E. J.; DE BAETS, S.; VANBAELEN, A.; JORIS, K.; DE WULF, P. Improved production of bacterial cellulose and its application potential. **Polymer Degradation and Stability**, v. 59, n. 1-3, p. 93-99, 1998. https://doi.org/10.1016/S0141-3910(97)00185-7

VIANA, R. M.; SÁ, N. M. S. M.; BARROS, M. O.; BORGES, M. F.; AZEREDO, H. M. C. Nanofibrillated bacterial cellulose and pectin edible films added with fruit purees. **Carbohydrate Polymers**, v. 196, p. 27-32, 2018. https://doi.org/10.1016/j.carbpol.2018.05.017

WANG, Y.; JIA, J.; TIAN, Y.; SHU, X.; REN, X. -J.; GUAN, Y.; YAN, Z. -Y. Antifungal effects of clove oil microcapsule on meat products. **LWT - Food Science and Technology**, v. 89, p. 604-609, 2018. https://doi.org/10.1016/j.lwt.2017.11.042

WANG, Z.; DADI, L.; QIU, Y.; DAI, Y.; ZHU, S. SARSAIYA, S.; CHEN, J. Preparation and characterization of coffee hull fiber for reinforcing application in thermoplastic composites. **Bioengineered**, v. 10, n. 1, p. 397-408, 2019. https://doi.org/10.1080/21655979.2019.1661694

ZAHAN, K. A.; PA'E, N.; MUHAMAD, I. I. Monitoring the Effect of pH on Bacterial Cellulose Production and *Acetobacter xylinum* 0416 Growth in a Rotary Discs Reactor. **Arabian Journal for Science and Engineering**, v. 40, p. 1881-1885, 2015. https://doi.org/10.1007/s13369-015-1712-z

Authors contribution: Conceptualization, A.J. and L.H.; methodology, A.J.; investigation or data collection, A.J., L.H., Y.J. and E.R.; statistical analysis, A.J. and M.G.; administration or supervision: A.J.; validation, A.J. and M.G.; writing (original draft), A.J., L.H., Y.J., M.G. and E.R.; writing (review and editing), A.J. and M.G.; translation, M.G. All authors read and agreed to the published version of the manuscript.

Financing: This research received no external funding.

Data availability: The corresponding author can obtain study data by e-mail.

Conflicts of Interest: The authors declare no conflict of interest. Supporting entities had no role in the study's design; in the collection, analysis, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.