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Microbial Cellulose Production with Tomato (*Solanum lycopersicum*) Residue for Industrial Applications

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Bacterial cellulose (BC) is a natural polymer synthesized in a pure manner by different kinds of microorganisms. It is a material with a three-dimensional (3D) nanofibrillary network that has interesting chemical and mechanical properties. Due to its high polymeric degree and its characteristics, it can be applied in several technological fields, such as filtration membranes, packages, cosmetics, textile, and other industries. The usage possibility of agro-industrial residues as alternative nutrients source is a great economic advantage to not only lower BC production costs but also to improve its yield and diminish the disposal of such residues.

This work used the bacteria *Komagataeibacter hansenii* UCP1619, which was cultivated on alternative cultivation media, which were modified based on the standard media Hestrim-Schramm (HS). The best media was selected according to its cost benefit and BC's growth yield. The pellicles were obtained after 14 days incubation time. Flexibility and water holding capacity were calculated. It can be concluded that the production media modification with tomato extract resulted in a cost reduction of 76% on the used liquid media, with a great biocellulose production. This modification is ideal for its use in applications that require a denser fiber distribution, such as in emulsified effluent filtrations.

1. Introduction

Cellulose is a polymer produced mainly by plants. However, due to the growth in the production of its derivatives, the use of wood as a raw material has increased, leading to environmental problems. Because of that, the search for new production practices have been required. Cellulose can also be produced by different microorganisms. Among them, stand out the bacteria of the genera Acetobacter, Achromobacter, Aerobacter, Agrobacterium, Alcaligenes, Azotobacter, Escherichia, Komagataeibacter, Pseudomonas, Rhizobium e Sarcina (Silva et al., 2021).

Bacterial cellulose (BC) is secreted extracellularly by microorganisms in a pure form, in contrast to vegetable cellulose (VC) that are naturally produced with other components such as lignin and hemicellulose. Its three-dimensional (3D) nanofibrils allow for it to have a high tensile strength and large surface area which results in excellent water retention capacity (WRC). In addition to these properties, it is also biodegradable and renewable, thus being a polymer with possible applications in various technological fields, such as packaging (Albuquerque et al., 2020), filtering membranes (Medeiros et al., 2022), textiles (Galdino et al., 2022) and medicine (Amorim et al., 2020). However, large-scale industrialization and commercialization is still a challenge, due to the delicate fermentation process and the high cost of its synthetic culture medium (Hussain et al., 2019).

Hestrin-Schramm (HS, 1954) culture medium is the established standard medium for bacterial cellulose production. However, it requires the supplementation of glucose, peptone, and yeast extract, which are reagents with a high market value.

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Currently, many research are focused on trying to produce BC using different microorganisms strains and alternative sources of carbon and nitrogen from agro-industrial residues. Thus, the cellulose produced with these modified media has a high added value as it can have a more accessible cost due to its ecofriendly production (Costa et al., 2019; UI-Islam, 2020).

The production of BC from agricultural and industrial by-products, including food (Nascimento et al., 2022), fruit (Amorim et al., 2019) and corn waste (Galdino et al., 2020), has been reported. Their use not only has the possibility of improving the cellulosic production yields, but also to reduce environmental pollution that would later be disposed (Medeiros et al., 2021). This possibility provides the expansion of BC's applications (Costa et al., 2017).

In this work, BC was produced in HS standard medium and in tomato juice-based media. After the production process, the yield, flexibility, and water retention capacity (WRC) of the produced pellicles were evaluated. The present paper intends to show the discovery of alternative forms of production of microbial cellulose with fruit waste, aiming at obtaining new biotechnological materials to be applied in different industrial segments.

2. Material and methods

2.1 Bacterial cellulose (BC) producing microorganism and maintenance media

The bacterium Komagataeibacter hansenii UCP1619 from the Bank of Cultures of the Center for Research in Environmental Sciences (NPCIAMB) of the Catholic University of Pernambuco, was used. *K. hansenii* was maintained in solid HS medium (20.00 g/L of agar, (20.00 g/L of glucose, 5.00 g/L of peptone, 5.00 g/L of yeast extract, 1, 15 g/L of citric acid and 2.70 g/L of disodium phosphate, pH 6) under refrigeration at 4ºC (Hestrin and Schramm, 1954).

2.2 BC production, cultivation, purification, and yield conditions

The pre-inoculum was prepared from the growth of the bacterium at 30 °C for 48 h, under static conditions, in 100 mL of HS liquid maintenance media.

The *K. hansenii* inoculum was prepared by transferring 3 % of the pre-inoculum in HS medium in 250mL Erlenmeyer flasks with 100mL of liquid production media, for 14 days. The membranes were washed in running water and purified by immersion in a 0.1 M NaOH solution for 1 h at 100 ºC to eliminate retained cells and impurities during the fermentation. Subsequently, the membranes were weighed, and the production yields per volume of culture medium (g/L) in dry and wet weight were determined.

2.3 BC production with the alternative culture medium and yield

To prepare the alternative medium, tomato fruit (*Solanum lycopersicum*) was used. It is a food easily found in the region of the city of Recife, Pernambuco, Brazil. This fruit has a high discard rate after ripening, even though it is still rich in nutrients, including about 46 % carbohydrates, 9 % citric acid, among others, which are ideal for BC's fermentation media (Diane, 2012).Different concentrations of the tomato extract used in the alternative media were made. T1 and T2 were done with the concentration of four ripe tomatoes per 100mL of distilled water (4/100). T3 and T4 media were used with the concentration of 2 ripe tomatoes per 100mL of distilled water (2/100). Alternative media T5 and T6 were prepared at the concentration of 1 ripe tomato per 100mL of distilled water (1/100). With the help of an industrial blender, the tomatoes were blended at 18,000 rpm for 2 minutes to form a homogeneous mass. After homogenization, it was necessary to strain to remove excess bagasse and the juice was reserved for production of culture media, according to Table 1. After sterilization in an autoclave (120ºC for 20 minutes), the bacteria were inoculated and incubated statically at 30°C for 14 days for BC production. All experiments were done in triplicate.

Table 1: Alternative growing media made with tomato pulp

Growing media	Content							
	Tomato (unit)	Water (mL)	Sacarose (g)	Glucose (g)	Peptone (g)	Yeast extract (g)	Disodium phosphate (g)	Citric acid (g)
T1	4	100	-	-	-	-	-	-
T2	4	100	6.00	-	-	-	-	-
T3	2	100	-	-	-	-	-	-
T4	2	100	6.00	-	-	-	-	-
T5	1	100	-	-	-	-	-	-
T6	1	100	6.00	-	-	-	-	-
HS	-	100	-	6.00	1.50	1.50	0.81	0.34

2.4 Flexibility test

The dry cellulose membranes were hand-folded 100 times along the same point. Flexibility classification was based on the number of folds until reaching 100 folds or exceeding them, with a classification of: poor (<20), fair (20-49), good (50-99) and excellent (Chen et al. 2013).

2.5 Water holding capacity (WHC)

The wet BC membranes were weighed and dried in an airflow oven at 40°C until constant weight was reached (evaporation of water). The WHC (%) was then determined using the equation:

$$WHC\% = \left(\frac{BC\ wet\ mass - \ BC\ dry\ mass}{BC\ wet\ massa} \right).100\% \qquad (1)$$

3. Results and discussion

Table 2 shows that all alternative media produced cellulose. It was observed that tomatoes have 46% of sugars in their mass composition, being a great alternative source of carbon, as glucose is the structural carbohydrate of BC (Diane, 2012).

Table 2: Water holding capacity (WHC), wet cellulose yield (Wy), dry cellulose yield (Dy) and production cost

Growing media	WHC (%)	Wy (g/L)	Dy (g/L)	Production cost (R$/L)
T1	96.59	242.76	8.33	3.21
T2	97.37	389.03	10.70	3.26
T3	98.13	171.93	2.73	1.92
T4	98.31	201.03	3.90	1.97
T5	97.52	392.86	9.73	1.28
T6	97.39	409.96	10.73	1.33
HS	98.68	265.90	8.70	9.50

Media T1 (not supplemented with sucrose) and T2 (supplemented with sucrose) are shown in Figure 1. The membranes produced showed excellent flexibility. The T1 medium presented a Wy of 242.76 g/L, Dy of 8.33 g/L and a WHC of 96.59 %. It was noted the production of a thicker pellicle for the T2 medium in contrast to T1. T2 presented a Wy of 389.03 g/L, Dy of 10.70 g/L and a WHC of 97.37 %, resulting in a pellicle with 46.30 % greater in terms of wet mass and 22.98 % in dry mass when compared to the HS medium. Taking this comparison into account, when analyzing the production cost, the T2 medium had a standard cost reduction of R$ 6.24 (65.68 %), according to an average price market at January 2023 at Recife (Brazil).

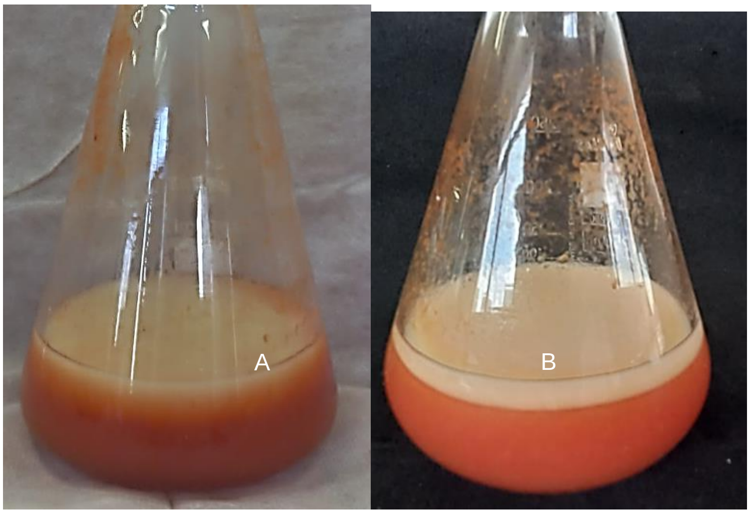

Figure 1: A shows T1 medium and B T2 medium after cellulosic growth of 14 days

Media T3 (not supplemented with sucrose) and T4 (supplemented with sucrose) are shown in Figure 2. Both showed excellent results on the flexibility test. The T3 medium presented a Wy of 171.93 g/L, Dy 2.73 g/L and a WHC of 98.13 %. The sucrose supplementation in the T4 medium was responsible of the increase in the cellulosic Wy of 201.03g/L, Dy of 3.90g/L and a WHC of 98.31%.

Comparing with the HS medium, the T4 medium had a reduction in the cellulose yield of 24.39 % of the wet mass and 55.17 % of the dry mass. There was a reduction of R$ 7.53 (79.26%) of the standard medium production cost.

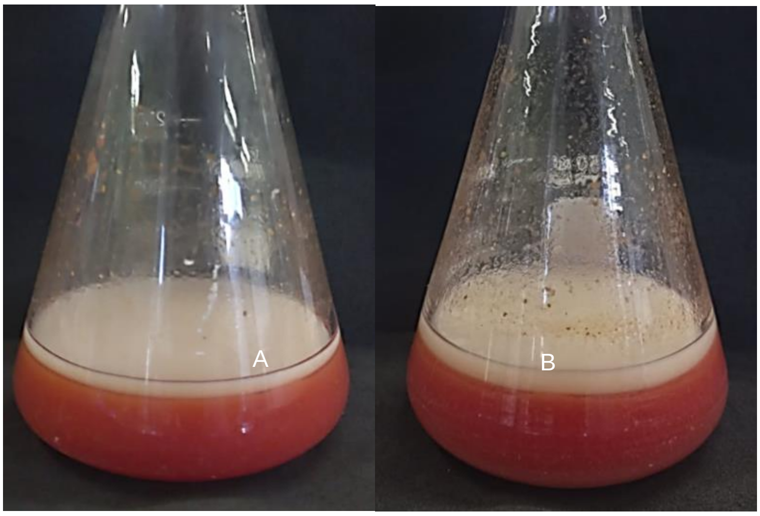

Figure 2: A shows T3 medium and B T4 medium after cellulosic growth of 14 days

Media T5 (not supplemented with sucrose) and T6 (supplemented with sucrose) are shown in Figure 3. The pellicle produced with the T5 medium presented a Wy of 392.86 g/L, Dy 9.73 g/L and a WHC of 97.52 %. T6 had a better production yield than T5, with a Wy of 409.96 g/L, Dy 10.76 g/L and a WHC of 97.39 %. Both pellicles showed excellent results on the flexibility test are shown in Figure 4. When compared to the HS pellicle, T6 showed a yield 54.17 % higher of wet weight and 23.33 % of dry weight. As for the production cost, T6 showed to be 86 % cheaper than HS.

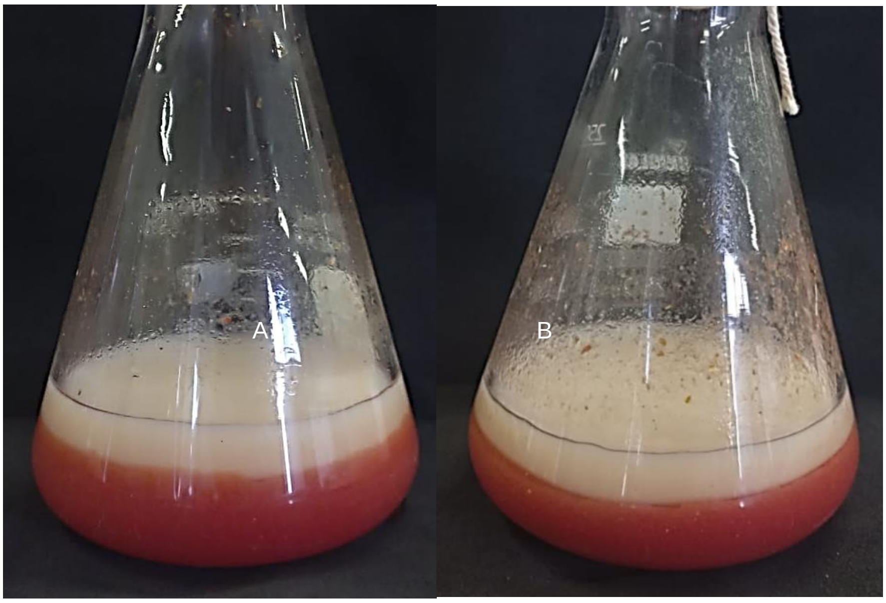

Figure 3: A shows T5 medium and B T6 medium after cellulosic growth of 14 days

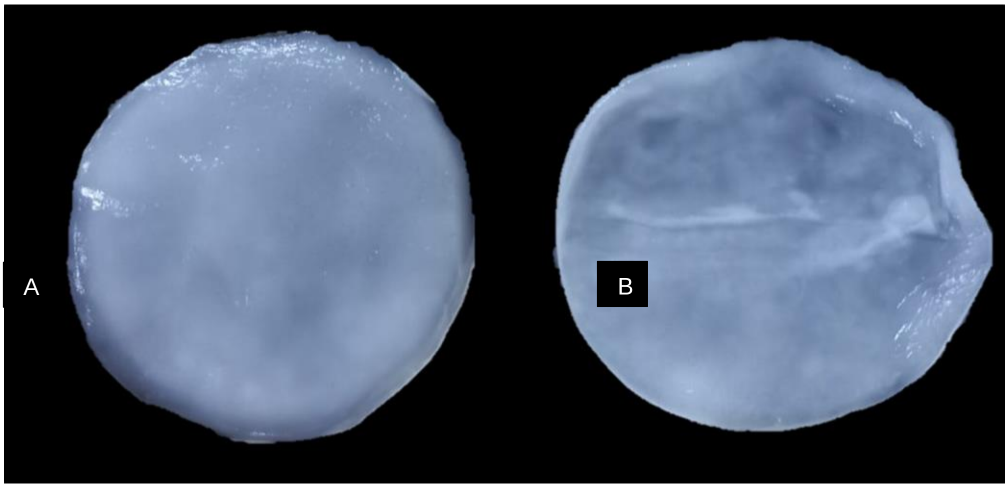

Figure 4: T6 medium flexibility test, A before the test and B after the test

The BC obtained from the standard medium was used for comparison in relation to the properties and costs of the alternative media. All tested alternative media showed cellulose growth due to the presence of nutrients present in the tomatoes. All pellicles showed excellent flexibility, as they remained intact even after being bent by hand more than 100 times in the same line. It is worth noting that the media that had the best yields were those supplemented with sucrose, which have an additional cost compared to non-supplemented media. The medium that showed the best cellulose production was T6 tomato juice supplemented with sucrose and made at a concentration of 1 tomato per 100mL of distilled water, with a higher reduction in production cost compared to standard HS.

4. Conclusion

This work analyzed the ability of the bacteria Komagataeibacter hansenii to grow in alternative culture media and produce cellulose, aiming at its application in industry. The experiments revealed that the production in tomato juice was positive. All media had a low production cost, being promising for large-scale production. The media that best developed were those supplemented with sucrose. It was observed that the tomato concentration in the medium is inversely proportional to its yield. The best medium among those tested was the T6 medium, having the highest overall yield and a low production cost. Further characterizations still need to be done; however, the current study of alternative culture media is very promising to applications within the microbial cellulose industry.

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