I COBBIND
I CONGRESSO BRASILEIRO DE BIOTECNOLOGIA INDUSTRIAL
XXIV SINAFERM ·· XV SHEB ·· XV ENZITEC FLORIANÓPOLIS ·· 2024
Creating connections between biotechnology and industrial sustainability
August 25 to 28, 2024
Costão do Santinho Resort, Florianópolis, SC, Brazil
ENVIRONMENTAL BIOTECHNOLOGY

ASSESSMENT OF BOVINE MILK WHEY AS A SUBSTRATE FOR BACTERIAL CELLULOSE PRODUCTION

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ABSTRACT

This research explores the use of whey as a substrate for bacterial cellulose (BC) production with *Komagataeibacter hansenii* UCP1619, aiming to develop an efficient and economical alternative to traditional HS medium. The study found that whey media promotes the formation of BC films with less water retention, but with unique properties. The S2 medium, combining nutrients from HS and whey, achieved dry weight similar to HS, indicating its potential as an effective alternative. S1 and S3 demonstrated similar results, however it was observed that exclusion of peptone in S3 did not impact the final weight of CB, suggesting that whey together with yeast extract can replace it as a source of nitrogen. These findings highlight the importance of optimizing nitrogen and carbon sources in the culture medium, offering valuable information for formulating cost-effective media for BC production using whey.

Keywords: Bacterial cellulose. Milk whey. Agro-industrial waste. *Komagataeibacter hansenii*.

1 INTRODUCTION

The pursuit of transforming agro-industrial waste into value-added products has steadily grown over the years. Concepts such as the circular economy, environmental sustainability, and green marketing have become increasingly ingrained in the culture of large companies.[1] Dairy products, for instance, generate problematic waste if not disposed of properly. Whey, a by-product of cheese or curd production, contains numerous nutritionally rich components and has high chemical and biochemical oxygen demand. These properties make it an organic pollutant and a contributor to environmental impacts.[2]

Bacterial cellulose (BC) has emerged as a promising material, with significant potential for developing various food, pharmaceutical, and dermatological products, among others. This polymer can be produced by several species within the genera *Rhizobium*, *Agrobacterium, Komagataeibacter* (formerly known as *Gluconacetobacter*), and *Sarcina.*[3] Among these, the genus *Komagataeibacter* is particularly noted for its production efficiency.[4] However, yield and chemical structure depend on the medium in which the microorganism is cultivated and the cultivation conditions.[5] A notable limitation of bacterial cellulose is the high cost of the culture medium. Thus, current research focuses on finding cheaper alternatives.

The utilization of agro-industrial waste for cellulose development has increased due to its rich components and easy availability. Research involving dairy residue is still in its early stages, and optimizing conditions for bacterial polymer production is crucial. This research aims to use whey as a substrate for the production of bacterial cellulose with Komagataeibacter hansenii UCP1619, analyzing the composition of the culture medium to replace the HS components.

2 MATERIAL & METHODS

Microrganism, production medium and BC growth

The bacterium *Komagataeibacter hansenii* UCP1619, provided by the Catholic University of Pernambuco (UNICAP), was used to produce bacterial cellulose. Initially, the microorganisms were activated by transferring them to tubes containing sterilized HS [6] medium, which comprised 0.5% yeast extract, 2% D-glucose, 0.5% peptone, 0.27% disodium phosphate, 0.15% citric acid, and 100 mL of distilled water at pH 6. The cultures were then incubated at 30 ºC for 48 hours under static conditions.[7]

Fermentations for cellulose production were conducted in 250 mL Erlenmeyer flasks containing 100 mL of medium. The media were prepared according to the formulations presented in Table 1. After preparation, the media were autoclaved at 121°C for 20 minutes.

Table 1 Composition of culture media.

Component	HS	S1	S2	S3
D-glucose (2%)	X		X	
Yeast extract (0,5%)	X	X	X	X
Peptone (0,5%)	X	X	X	
Citric acid (0,15%)	X	X	X	X
Disodium phosphate (0,25%)	X	X	X	X
Water (100%)	X			
Milk whey (100%)		X	X	X

BC production followed the scheme shown in Figure 1. After activating the microorganism, the pre-inoculum was prepared by inoculating one section of the tube into 100 mL of HS medium and incubating it for 48 hours at 30°C. Then, 3 mL of the pre-inoculum was added to 100 mL of HS medium and incubated for 72 hours at 30°C to prepare the inoculum. From the inoculum, 3 mL was transferred to the alternative media (S1, S2, and S3) and the control (HS), and then placed in an oven at 30°C for 10 days.

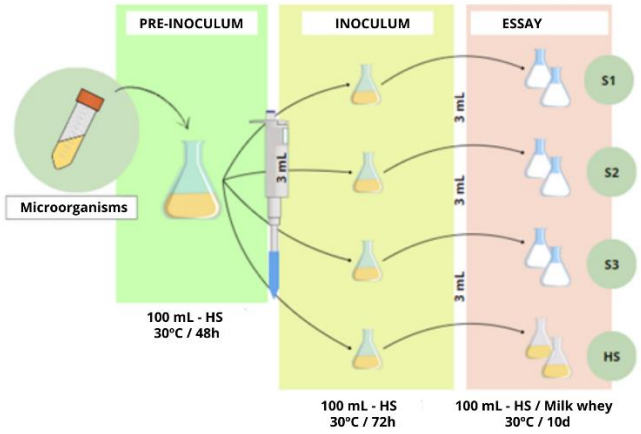

Figure 1 Schematic of *Komagataeibacter hansenii* UCP1619 activation and pre-inoculum preparation, preparation of the inoculum and addition of microorganisms to alternative media for producing CB film.

CB film washing, purification and weighing

After determining the cultivation and production conditions, the CB films were washed under running water. They were then immersed in a 4% NaOH solution at 80°C for 30 minutes and subsequently rinsed with distilled water until reaching a pH of 7. Once neutral pH was achieved, the membranes were weighed while wet, then dried in an oven at 40°C for 18 hours, and weighed again at the end of the process.

Calculation of water holding capacity (WHC)

After weighing the membranes, it was possible to calculate the water retention capacity (WHC) using equation 1. [7] Where M_a is the mass of water removed during drying (g) and MCB_s is the dry weight of the film (g).

$$WHC = \frac{M_a}{MCB_s} \quad \text{(Eq.1)}$$

3 RESULTS & DISCUSSION

The bacterial cellulose membranes obtained in each test varied in dry weight, water retention capacity, and appearance. Table 2 shows that HS and S2 have similar dry weights, as do S1 and S3. The similar production levels of HS and S2 are attributed to S2 contains all the nutrients of the HS medium, resulting in better performance of S2. In contrast, S1 and S3, which lack glucose, showed low production since glucose is theIt is the best source of energy for the microorganism. [8]

Table 2 Water holding capacity (WHC) of sample tests.

Sample	Dry weight	WHC
HS	0,16 (±0,05)	42,53 (±9,40)
S1	0,12 (±0,03)	7,04 (±4,33)
S2	0,18 (±0,01)	11,34 (±0,66)
S3	0,12 (±0,01)	5,74 (±2,47)

The absence of peptone in the S3 medium did not affect the final weight of CB, suggesting that peptone can be omitted from the whey formulation. This is consistent with the role of nitrogen sources, such as yeast extract, which do not directly contribute to cellulose synthesis but are crucial for bacterial growth and can substitute for peptone. [9,10] Additionally, it has been observed that the optimal nitrogen source depends on the specific carbon source used, a key consideration in optimizing conditions using dairy composition. [11]

Observing the wet weights, it is evident that the HS medium favors the formation of bacterial cellulose (BC) films with higher water retention compared to the whey-containing media, as shown in Table 2. The HS medium provides essential nutrients for the bacteria, resulting in BC with a high-water retention capacity. The presence of glucose as the primary carbon and energy source, along with yeast extract rich in B vitamins, amino acids, and trace elements, stimulates bacterial growth and metabolic activity, leading to BC with higher water content. This material is ideal for applications in food additives, cosmetics, and the biomedical industry due to its high-water retention capacity. [12] In contrast, BC produced in whey-containing media has a lower water retention capacity, which can be advantageous for certain applications. Lower water retention can result in a more stable structure, reduced water loss rate, and better anti-extrusion properties, making it useful in various industrial contexts where these characteristics are desirable. [12]

4 CONCLUSION

The results indicated that the HS medium promotes the formation of CB films with greater water retention, while the whey medium also produced viable CB with unique properties, however, with lower water retention. S2 medium, containing nutrients from HS and whey, reached dry weight similar to HS, suggesting its effectiveness as an alternative medium. The lower production in S1 and S3 was due to the absence of glucose, essential as an energy source. Deletion of peptone in S3 did not affect the final weight of CB, indicating that it can be omitted if yeast extract is used as a nitrogen source. These findings highlight the importance of optimizing nitrogen sources and considering the carbon source in the culture medium, providing valuable information for formulating efficient and economical media for CB production, especially using whey as substrate.

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ACKNOWLEDGEMENTS

We thank FINEP and IATI for their support.