



Bacterial Cellulose Production Using Fruit Residues as Substrat to Industrial Application

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The term cosmeceutical comes from the combination of cosmetics and pharmaceuticals, which has been emerged due to the CPC's market's (Cosmetics and Personal Care) necessity of combining body and skin care with products that has the usage of bioactive ingredients, ensuring a more sustainable development with a new kind of biotechnology. Regarding this idea, this project consisted on studying the usage of a bacterial cellulose (BC) producing bacteria, *Glucanacetobacter hansenii*, which has been cultivated in a media made out of juice from tropical fruit residue, used as a plant alternative for the production of a BC with beneficial characteristics for the skincare. The tropical fruit, with its enzymatic and non-enzymatic sites and its high nutrient and minerals content, contributes for the prevention of free radicals of the skin. Besides from serving as a support for incorporation of the active principles of tropical fruit, BC has high water activity when applied to the skin, helping to retain its natural moisture. For the experiments, the microorganism was cultivated on eleven different cultivation media, which were modified from the standard media HS (Hestrim-Schramm). The working media was selected according to its cost benefit and BC's growth yield. The experiments were performed after 10 days of the bacteria's cultivation on the media cited previously. With the obtained BC pellicle, both its water activity and ascorbic acid were quantified, and its mechanical properties were determined. A BC face mask was also constructed. It can be concluded that the addition of tropical fruit residue in the bacteria's cultivation media, grants ideal skin hydration characteristics to the BC pellicle, enabling the development of a new biotechnological product for the pharmaceutical and cosmetics industries.

1. Introduction

Cellulose is the most abundant biopolymer on the surface of earth with 1.5×10^{12} tons annual world production (Klemm *et al.*, 2005; Czaja *et al.*, 2006), and it is most commonly obtained from plants. In addition to plant cellulose (PC), cellulose can also be obtained through various aerobic non-pathogenic bacteria such as *Agrobacterium*, *Sarcina*, *Glucanacetobacter* and others.

Bacterial cellulose (BC) is a biopolymeric material, made out of renewable natural sources, with unique properties, such as biocompatibility, biodegradability, high crystalline degree and porosity, great water retention and non-toxicity. BC has a high degree of purity, since it is not tied to other components such as lignin and hemicellulose, like vegetal cellulose (Gomes *et al.*, 2013). Regarding these excellent properties, the BC becomes attractive for various biotechnological applications, including biomedical, cosmetics, food, and even electronic devices (Iguchi *et al.*, 2010). Applications in cosmetics include facial mask, facial scrub, personal cleansing formulations and contact lenses. BC can even also be used for controlled drug delivery (Weyell *et al.*, 2019).

2.4 Percentage of Water Retention (PWR)

All eleven wet membranes of BC were weighed and dried in the oven in order to completely remove the water to constant weight. Then the PWR was obtained using Eq. (1):

$$\text{PWR (\%)} = \left(\frac{\text{Mean of the wet weights} - \text{Mean of the dry weights}}{\text{Mean of the wet weights}} \right) \times 100\% \quad (1)$$

2.5 Characterization Analyses

The characterization was done with both standard HS media pellicle and modified HS media pellicle. The determination of water activity was done by using an Aqualab 4TE equipment, being measured at 24,9°C. The soluble solids quantity was found with the help of a bench refractometer, Reichert, model r²i300. The determination of total acidity was done by titratable acidity method. The Tillmans method was used to quantify the concentration of ascorbic acid.

2.6 Mechanical Properties Testing

The mechanical properties of the pellicles were characterized by its tensile strength (N/m²), tensile strength at peak load (MPa) and elongation at break (%) by a method described by Rethwisch and William (2016) with the usage of a universal testing machine (EMIC DL-500MF, Brazil). Samples were cut into rectangular strips (2 x 7 cm). The tensile test was performed at room temperature at a speed of 5 m/min and a static load of 0.5 N following ASTM Method D882. The Bluehill LiteTM software program was used to calculate the stress-strain ratio and modulus of elasticity.

2.7 Prototype BC Face Mask Confection

A face mask was done so that it could work by skin occlusion effect. After being obtained on the modified media, the pellicle was washed on tap water and were purified in a 0,1 M solution of NaOH, at 90°C for 20 minutes, in order to eliminate all retained bacterial cells, as shown on Figure 1.

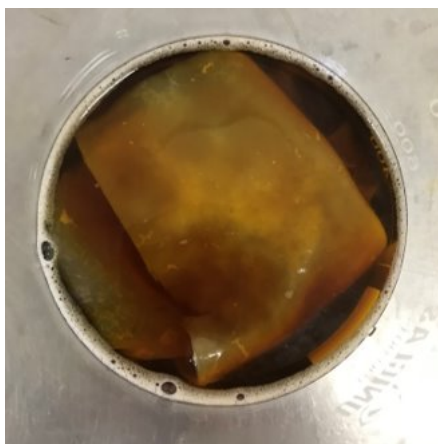


Figure 1: BC membrane being purified on NaOH.



Figure 2: Prototype of the face mask made out of BC

The pellicle was washed in deionized water until neutral pH was achieved and were sterilized in an autoclave at 121°C for 15 minutes. Afterwards, the pellicles were dried out at room temperature for 48h while slightly covered on coconut oil. The mask confection consisted on cutting the pellicle as the shape of other face masks on the market, as it can be seen on Figure 2.

3. Results and discussion

3.1 Selection of the modified media

According to the experiments results, as shown on table 2, the best BC producing media was the number four. Its BC production yield was of 165,24 g of cellulose (wet weight) /L of medium and 6,98 g (dry weight) of cellulose/L of medium. It was observed that the carbon and nitrogen sources from the standard HS media could be eliminated, resulting in a major impact on the cost reduction. The media number 4 had a production yield 31,4% lower of wet BC mass and 27,8% lower of dry mass when compared with the standard HS media (number 1). Although, still its cost was considerably lower, resulting in a reduction of 87,89%.

With that being said, the selected media takes a step further to a large-scale production and cost reduction of the biopolymer.

Table 2 - Yields expressed both in wet and dry weight of the modified media

Media	Yield (g wet BC mass /L medium)	Yield (g dry BC mass /L medium)
1	240,83	9,67
2	276,43	13,2
3	11,31	0,62
4	165,24	6,98
5	125,32	4,67
6	73,88	3,56
7	93,31	3,36
8	43,35	2,79
9	122,99	5,42
10	49,47	3,11
11	34,22	1,52

3.2 Percentage of Water Retention (PWR)

As mentioned previously, the wet BC membranes were weighed and dried in the oven in order to completely remove the water to constant weight, the mass values were obtained and then the PWR was obtained as described in Table 3.

Table 3 – Water retention percentage of BC membranes obtained with the modified media

Experiments	PWR (%)
1	96,01
2	95,22
3	94,52
4	95,77
5	96,27
6	95,18
7	96,39
8	93,56
9	95,60
10	93,71
11	95,55

The results confirm that the BC membranes present a high-water activity, as described by Costa et al. (2017), being this property of extreme important characteristic for its usage as a material for a hydration face mask.

3.3 Production of the BC

The antioxidizing capacity of the vegetable tissue can be associated to its high quantity of non-enzymatic antioxidants, such as ascorbic acid. The tropical fruit residue possesses a considerable amount of vitamins

and minerals, being considered as an attractive substrate not only for the BC production, but also as a raw material of added value to the cosmetics industry. The selected working media content is made out of 86,58% of water, 13% of tropical fruit residue, 0,27% of disodium phosphate and 0,15% of citric acid. The obtained biofilm is shown on Figure 3.

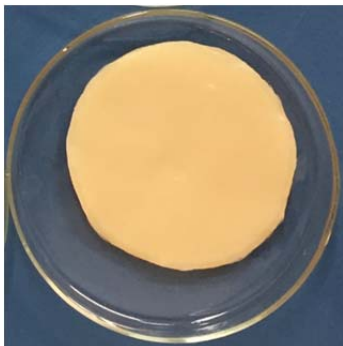


Figure 3 – BC membrane obtained from the selected modified media

The productive capacity of BC is closely linked to the parameters of the bioprocess, so that different variables must be attempted to be explored. It was noted that due its high ascorbic acid content, the acid enabled a higher production of the BC membrane. This can be said because vitamin C lowers the production of gluconic acid, which is produced by the bacteria during the formation of the pellicle (Keshk, 2014). According to the study made by Lin et al. (2014), gluconic acid can serve as an inhibitor of the cellulose formation. Taking this into consideration, the ascorbic acid retained in the tropical fruit residue, when added in an indirect way, it served as a potentiator for BC production.

3.4 Characterization of the BC

The experiments were performed after 10 days of the bacteria's cultivation on the medium cited previously. The results showed a BC pellicle containing $99,13 \pm 0,09\%$ of water activity, $2,0 \pm 0,1\%$ °Bx of sugar content (whereas the pellicle obtained from the standard HS media was found of $4 \pm 0,3\%$ °Bx), $13 \pm 1\text{g}$ of citric acid/100g cellulose and $24,4 \pm 1,10\text{mg}$ of ascorbic acid/100g cellulose. The tensile strength of the pellicle was found to be of 247 MPa, with its elastic modulus of 1940 MPa and its specific deformation of 20%.

4. Conclusions

Plant and microbial raw materials have been used as an effective alternative in the cosmetic industry, because they present active natural compounds. This sector has explored nature in search of new raw materials of natural and organic origin in order to guarantee satisfactory results to the consumer, contributing to the development of sustainable technology in the cosmetic sector.

Due to its high-water content and activity, the biofilm can be considered a great material for the construction of a hydrating face mask. The tropical fruit, with its enzymatic and non-enzymatic sites and its high nutrient and minerals content, contributes for the prevention of free radicals of the skin. Besides from serving as a support for incorporation of the active principles of tropical fruit, BC has high water activity when applied to the skin, helping to retain its natural moisture. Cellulose masks made out of natural sources, such as bacteria, are of high importance on the cosmetics industry, because of its low toxicity and high biodegradability.

In this work it was shown the capacity of the bacteria *Gluconacetobacter hansenii* to grow on alternative media, as a sustainable design, making the production process cheaper. The production of the biodegradable biomaterial is connected with the reduction of environmental impacts. Also, it can be concluded that the components on the tropical fruit residue were still found on the final BC pellicle, granting an excellent characteristic to posterior use as the proposed purpose of this work, a biotechnological cosmeceutical product.

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