

VOL. 74, 2019



Guest Editors: Sauro Pierucci, Jiří Jaromír Klemeš, Laura Piazza Copyright © 2019, AIDIC Servizi S.r.l. ISBN 978-88-95608-71-6; ISSN 2283-9216

# Bacterial Cellulose Production Using Fruit Residues as Substract 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  $1.5 \times 10^{12}$  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*, *Gluconacetobacter* 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).

Paper Received: 2 May 2018; Revised: 25 August 2018; Accepted: 25 December 2018

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The current challenges limiting the application of BC in a broader and larger scale is related to bioprocess developments to increase its production and also its high cost. The process used to produce BC affects directly its properties and consequently its application (Vasconcellos *et al.*, 2018).

Finding a new cost-effective culture media to achieve the highest yield of BC in large-scale is paramount and requires new sources of carbon and nitrogen. The Hestrin–Schramm (HS) medium, described on 1954, is commonly used in the cultivation of BC, however, it is expensive. In recent years, studies have focused on a variety of cellulose-producing bacterial strains, inexpensive nutrient sources, and supplementary materials for the production of inexpensive BC such as residues from sugar cane (Costa *et al.*, 2018) and others. The production of BC from industrial and agricultural residues, not only makes the process less expensive, but also makes it sustainable.

Tropical fruits have high quantities of nutrients, such as vitamins and minerals. Those properties can be ideal on the fermentation of the bacteria, also, it can grant beneficial characteristics to its final use as a cosmetic product. Regarding this idea, this work consists on the evaluation of the usage of tropical fruit residue as an alternative nutrient source for the BC pellicles production for future application on the cosmeceutical industry.

#### 2. Materials and Methods

#### 2.1 Microorganism and Cultivation Condition

For BC production, a strain of *Gluconacetobacter hansenii*, (ATCC 53582) obtained from the culture collection of Nucleus of Resource in Environmental Sciences, from the Catholic University of Pernambuco, Brazil, was used. The methodology for the synthesis of BC was divided into four steps: activation, pre-inoculum, inoculum and cultivation in the modified medium. The strain was maintained in the HS liquid medium described by Hestrin and Schramm (1954) which contained 2.0% glucose (w/v), 0.5% yeast extract (w/v), 0.5% peptone, 0.27% Na<sub>2</sub>HPO<sub>4</sub> (w/v), and 0.15% citric acid (v/v). BC was then produced in the modified HS medium, which has in its composition juice from the tropical fruit residue, Na  $_2$ HPO<sub>4</sub>, and citric acid, which was adjusted to pH 6.

#### 2.2 Activation, Pre-inoculum and Inoculum

In the activation phase the bacteria was inoculated into HS-agar medium and incubated at 30°C until growth, for 48 h. Afterwards, the grown cells were transferred from the activation to the pre-inoculum, which was prepared in liquid HS medium, in static conditions for 48 h, at 30°C, then, 3% of the pre-inoculum was transferred to the inoculum in the modified HS media, under the same conditions, and further experiments were done after 10 days.

#### 2.3 Selection of the modified media

To determine the best BC producing media, eleven different fermentations were conducted. All went under the same conditions (30°C, 10 days, static condition), only the sources of carbon and nitrogen were modified, according to the table 1. The best producing media was the one that had the highest production yield of BC, taking into consideration its cost. The media entitled as number 1 is the standard HS media.

Media	1	2	3	4	5	6	7	8	9	10	11
Compounds											
Glucose (20g/L)	Х	Х							Х		
Peptone (5g/L)	Х	Х	Х			Х					
Yeast Extract (5g/L)	Х	Х	Х			Х					
Dissodium Phosphate (2,7g/L)	Х	Х	Х	Х	X	X	Х				
Citric Acid (1,5g)	Х	Х	Х	Х	Х	Х	Х				
Sugar (20g)			Х		Х					Х	
Corn Steep Liquor (20g)						Х	Х				Х
Tropical Fruit Residue (1L)		X	X	X	X	X	X	X	X	X	X
Water (1L)	X										

Table 1 – A described comparison of the modified media, according to its supplementation

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#### 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):

DWD(06) -	Mean of the wet weights – Mean of the dry weights	$-) \times 100\%$	(1)
FWR(90) =	Mean of the wet weights	) × 100%	(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  $24,9^{\circ}$ C. The soluble solids quantity was found with the help of a bench refractometer, Reichert, model  $r^{2}i300$ . 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/m<sup>2</sup>), 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 Lite<sup>TM</sup> 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.

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

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

## 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.

	PWR (%)
Experiments	
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

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

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

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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% <sup>o</sup>Bx of sugar content (whereas the pellicle obtained from the standard HS media was found of 4 $\pm$ 0,3°Bx), 13 $\pm$ 1g of citric acid/100g cellulose and 24,4 $\pm$ 1,10mg 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.

#### Acknowledgments

This study was funded by the National Council for Scientific and Technological Development (CNPq), the Coordination for the Improvement of Higher-Level Education Personnel (CAPES) and the Advanced Institute of Technology and Innovation (IATI).

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