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Evaluation of the Potential of Bacterial Cellulose in the Treatment of Oily Waters

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Cellulose is the most abundant biopolymer on the planet and has a wide range of applications in different industrial sectors. Environmental preservation policies, on the other hand, promote the development of research to replace vegetable cellulose (VC), due to the fact that its production causes several damages to the environment. In this way, bacterial cellulose (BC) appears as a promising alternative to VC, since it differs from its vegetal similar mainly because it presents fibers of a nanometric character against the micrometric of the vegetable, which gives it excellent mechanical properties like greater purity, higher index of crystallinity, higher water absorption power and higher tensile strength. Thus, the present study was carried out for the development of a filter base on the use of BC membrane for the treatment of oily waters. BC membranes were initially produced in an alternative medium based on corn steep liquor (industrial waste) due to the fact that the standard production medium (HS) represents a high cost for the production of BC on an industrial scale, since is formulated with synthetic compounds. Then, wet BC membrane samples were purified and tested as filters for the separation of oily waters with oil concentrations of 10 ppm, 150 ppm and 230 ppm. The experiments were performed in triplicate and showed 100% removal of the oil present in all samples. Variables such as flow rate, filter diameter (25 mm, 50 mm, 110 mm) and production time of the membranes (6 and 10 days) were evaluated in a filtration system constructed in PVC. By showing that the filtration rate increases proportionally to the filter diameter, it decreases from the 6-day membrane to the 10-day membrane. The mean water mass present in both 6 and 10-day BC membranes exceeded 98%. The wet BC membranes presented satisfactory results in the mechanical assays, however the 10-day membrane supported 100% more in strength (N) than the 6-day film. The results obtained in this study showed the potential of this new nontoxic and efficient biodegradable material in the separation of water/oil mixtures generated in industrial environments.
Keywords: Bacterial Cellulose, *Gluconacetobacter hansenii*; Industrial Waste; Filtrating Membranes; Filtration; Oily Water.

1. Introduction

To achieve the goals of sustainable development, it becomes essential to the rational utilization of natural resources management, which will require the use of new technologies. Among the technologies which have the potential to contribute to sustainable development, biotechnology has a lot to offer, especially in the fields of large-scale production of pharmaceuticals, food, energy and domestic waste water treatment plants. There is no doubt that biotechnology is the science that will revolutionize the production and supply of biomaterials (Rizwan et al., 2018). Among the possibility of delivering new biotechnological materials, is worth mentioning the cellulose production. Cellulose is a natural polymer produced by plants. It is widely used in biorefinery to produce second-generation biofuels (Giuliano et al., 2015). However, the increasing demand of VC, increased

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deforestation and global environmental issues. Although the plants are the largest sources of cellulose, several types of bacteria, that use carbon and nitrogen sources available in the culture medium, are capable of producing cellulose. Bacteria belonging to different genres, such as *Sarcina, Agrobacterium and Gluconacetobacter*, are capable of producing BC or biocellulose (Costa et al., 2018). It shows high purity, since it is not associated with other components such as hemicellulose and lignin as the VC, and due to your nanofibrillar 3D network, shows water absorption capacity (the initial membrane has approximately 99% water) and high tensile strength (Costa et al., 2017). These unique properties, in conjunction with other features, such as biocompatibility and biodegradability, resulting in a renewable character and in a wide range of applications in the biomedical area and several other technological areas (Vasconcellos et al, 2018). On the other hand, new perspectives of application appear due to nano-sized and hydrophilic properties of this biopolymer, as your possible use in separation of oily residues. Research to develop aerogels of BC obtained by addition of chemical additives for hydrophobization have been described in the literature for the separation of solvents and oils spilled on marine environment, although this technology still requires a high capital investment, which makes impracticable the process in industrial level (Carpenter et al., 2015). However, the use of the membrane as a filter of water and oil mixtures generated in industrial environments has not yet been described. In this sense, the objective of this work was to test the feasibility of application of BC films obtained in an alternative medium as an efficient filter for the separation of oily residues in water and oil mixtures generated in industrial environments.

2. Materials and Methods

2.1 Materials

Peptone, Glucose, citric acid monohydrate, yeast extract, sodium hydroxide and agar were purchased from Merck Ltd., United States. Corn steep liquor (CSL), was obtained from local companies in the state of Pernambuco, Brazil

2.2 Microorganism and Culture Conditions

A strain of *Gluconacetobacter hansenii* UCP1619, obtained from the culture collection of Nucleus of Resource in Environmental Sciences from the Catholic University of Pernambuco, Brazil, was used for the BC production, The strain was maintained in the HS medium described by Hestrin and Schramm (1954) and modified by Hungund and Gupta (2010). The liquid medium contained 2.0% glucose (w/v), 0.5% yeast extract (w/v), 0.5% peptone, 0.27% Na_2HPO_4 (w/v), and 0.15% citric acid (v/v). BC was produced in the modified HS medium, which has the following composition: 1.5% glucose, 2.5% CST, 0.27% Na_2HPO_4, 0.15% citric acid, pH 5 (Costa et al., 2017).

2.3 Synthesis of Bacterial Cellulose

For the synthesis of BC was divided into four steps: activation, pre-inoculum, inoculum and cultivation in the modified medium. First, for the activation phase the strain was inoculated into HS-agar medium and incubated at 30°C for 48 h until growth. Then, the grown cells were transferred from the activation to the pre-inoculum in static conditions for at 30°C for 48 h in liquid HS medium, then, 3% of the pre-inoculum was transferred to the inoculum in the modified HS medium, under the same conditions, and further experiments were done after 6 and 10 days.

2.4 Percentage of Water Retention (PWR)

Three 6-day wet membranes, which was observed to be the minimum amount of days to obtain a uniform and filter-resistant membrane, and three 10-day wet membranes, which is the amount of days suggested by Hestrin and Schramm (1954), 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):

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

2.5. Construction of Filtration Systems and Preparation of Oil and Water Emulsions

In the experiments, filters were made of PVC with internal diameters of 25 mm, 50 mm and 100 mm. The filters were constructed with a steel screen as support for the membranes, avoiding the deformation and tearing of the biomaterial. The BC membranes previously prepared with the same filter diameter were coupled to the filtration system, which was filled with each type of oily solution to be treated. During the filtration procedure, the system pressure was set at 1 atm (gauge pressure). Since 20 ppm is the maximum limit allowed for oil value in industrial effluents, according to the Brazilian legislation (Brazil, 2011), emulsions with

10 ppm, 150 ppm and 230 ppm of oil were used in the experiments to test the membrane. These emulsions were evaluated in the forms stabilized with non-ionic non-polar surfactant.

2.6. Analytical Determinations

For the study of oil concentrations before and after filtration by the BC membrane, a curve of turbidity (NTU) vs. oil concentration (ppm) was plotted with the aid of the ALFAKIT Turbidimeter and Excel 2016. With the curve and the equation, it was possible to obtain the oil contraction before and after the filtration from the turbidity reading.

2.7. Scanning Electron Microscopy (SEM)

For SEM, pure BC samples of 6 and 10 days previously dried the dried was mounted on a copper stub using double adhesive carbon conductive tape and coated with gold for 30 s (SANYU ELECTRON). The SEM photographs were obtained using a scanning electron microscope (JEOL, JSM-5600 operating at 27 KV.)

2.8. Tensile Strength Testing of BC

To characterize the mechanical properties of BC (6 and 10 days), tensile strength (N), tensile strength at peak load (MPa) and elongation at break (%) were determined based on Rethwisch and William (2016). 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 using a universal testing machine (EMIC DL-500MF, Brazil) following ASTM Method D882.

2.9. Oil permeability and Membrane Flexibility

3 g of soybean oil were placed in a glass tube (internal diameter: 25 mm and outside diameter: 27 mm), and then a piece of BC (10 and 6 days) of 50x50 mm size was pressed firmly into the open end to seal the tube. The tube was inverted by placing a filter paper on the glass slide, and then observed whether the soybean oil permeated the membrane over time. Observations were made on at least five samples after 3 days of storage (Hu et al., 2009; Chen et al., 2013). For the membrane flexibility, the BC film was folded 100 times in the same stroke to test its flexibility. The film is evaluated as good if there is no visually observed slit. Folding times were recorded until ruptures appeared in the film. The flexibility of the film was correlated as poor (times <20), mean (20 ≤ times <50), good (50 ≤ times <100) and excellent (times ≥ 100) (Chen et al., 2013).

2.10. X-ray Diffraction (XRD) Analyses

X-ray diffraction patterns of the BC were measured with a Phillips X'pert MPD diffractometer using Cu Kα radiation. The crystallinity index (CI) was measured as x (%) = (Imax - Imin) / Imax x 100%, where Imax is the height of the peak at 2θ = 22.5 and Imin the minimum between the peak at θ = 22.5 and or peak at 2θ = 16.3 (Gomes et al., 2013).

3. Results and discussion

3.1. Production of BC Membranes

The average yield of BC hydrated membranes produced during 10 and 6 days was 475 and 194 g/L of medium, respectively, whereas in terms of dry membranes, yields of 6.82 and 2.69 g/L were obtained after 10 and 6 days, respectively. The NaOH purification step favored color uniformity, removal of the metabolites and possible residues from the culture medium that were adhered to the surface of the membranes.

3.2. Percentage of Water Retention (PWR)

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 calculated as described in Table 1.

Table 1 – Wet, dry weights and water retention percentage of BC membranes obtained after 6 and 10 days

	BC obtained after 6 days		**BC obtained after 10 days**	
	Wet weight (g)	Dry weight (g)	Wet weight (g)	Dry weight (g)
Mean	19.406	0.269	47.509	0.682
PWR (%)	**98.61 (%)**		**98.56 (%)**	

The results confirm that the BC membranes present a high-water activity, as described by Costa et al. (2017), reaching over 98%, being this property one of the fundamental characteristics for its efficiency in the separation of oily emulsions, giving it a hydrophilic and oleophobic characteristic.

3.3. Oil Permeability and Flexibility

BC membranes have been tested for oil permeability, otherwise the oil will exude through the membranes over time when they are used for filtration of oily mixtures, thus affecting filtration efficiency. The tubes containing soybean oil were covered with the films and placed inversely on the filter paper at room temperature and humidity. The results showed that the soybean oil showed no trace of permeation through the films. In fact, BC membranes have many hydrophilic hydroxyl groups and carboxyl groups, which prevent the adsorption of oil molecules on the surface of the membrane. Similar results were observed by Hu et al. (2009) and by Chen et al., (2013) for glycerol-oxidized potato starch-based films as plasticizer and cellulose sulphate (NaCS) plasticized with glycerol incorporated with starch, respectively. BC membranes also exhibited excellent flexibility as they remained intact for folding for much more than 100 times.

3.4. Characterization of BC Membranes by SEM

Figure 1 shows images of the pure dry BC membranes. Figure 1a shows a BC membrane screen of 6 days, in which a network of nanofibres of cellulose arranged in a random way is observed. The visualization of the fiber network increased 14,000 and 17,000 times and exhibited the images of the surface of BC of 6 days (Figure 1a) and 10 days (Figure 1b), showing fiber dispersion and interfacial adhesion. Both films exhibited a cross-linked structure consisting of ultrafine nanofibrils and a difference in structure is observed, the 10-day membrane (Figure 1B) having a more linked and closed structure as shown in SEM images below.
It was found that the width of the BC nanofibers changed as the harvest time increased, and the film thickness obtained also increased due to the larger mass of BC being formed. According to Hassan et al. (2017), the fabric structure of BC is unique in terms of porosity, compaction, watertightness and wet strength. It is important to note that due to these characteristics, the filtration of the oily solutions was possible, and it is also possible to understand the difference of the flow rates obtained between the membranes obtained after 6 days (higher flow) from the one obtained after 10 days.

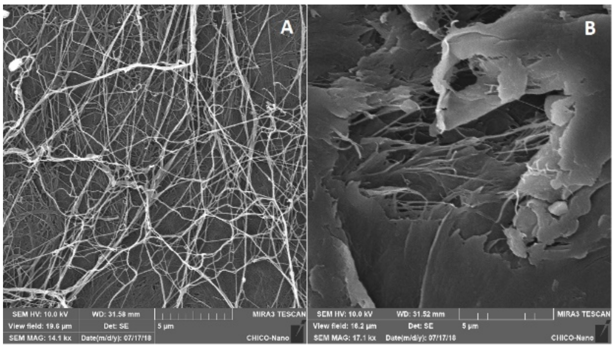

Figure 1. Dry film scanning electron micrograph of BC obtained after (A) 6 days and (B)10 days.

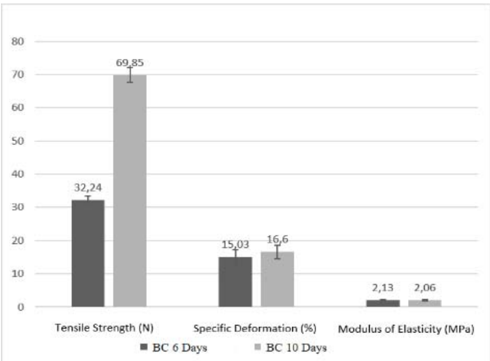

Figure 2. Tensile Strength (N), Specific Deformation (%) and Modulus of Elasticity (MPa) of samples of BC obtained after 6 and 10 days.

3.5. Tensile Strength Testing of BC

The results of the maximum tensile strength, specific deformation and modulus of elasticity of BC membranes are shown in Figure 2. The maximum tensile strength (N) of the 10-day BC membrane was increased by

100% in relation to the 6-days membrane. However, the values of modulus of elasticity (MPa) and specific deformation (%) did not differ much, as can be seen in Figure 2. As can be evidenced by SEM images, shown earlier (Figure 1), the 10-day BC membrane has a denser fiber structure, resulting in improved mechanical strength due to good interfacial adhesion and the formation of strong interactions between its fibers, which justifies the tensile strength result observed in the graph to be twice the value presented for the 6-days membrane.

3.6. X-ray Diffraction (XRD) Analyses

The crystallinity index (CI) was calculated based on the peak intensity, as shown in Figure 3. The CI obtained for the 6-days BC was 71% and while that of 10-days BC was 84.4%. It shows that the increase in the days of culture increased the CI of the BC membrane. CI has a ratio inversely proportional to the porosity of the cellulose surface. The presence of larger pores allows a greater penetration of the water molecules, which are absorbed by the membrane, thus increasing the degree of hydrophilicity (citing cellulose work as a filter). In the case of BC membranes, the increase in CI indicates the reduction of pores, and consequently a lower water flow, thus favoring the use of the 6-days BC as a filter membrane, because greater porosity increases the permeability of the material, that is, we have a membrane that presents greater number of pores in relation to the 10-days BC.

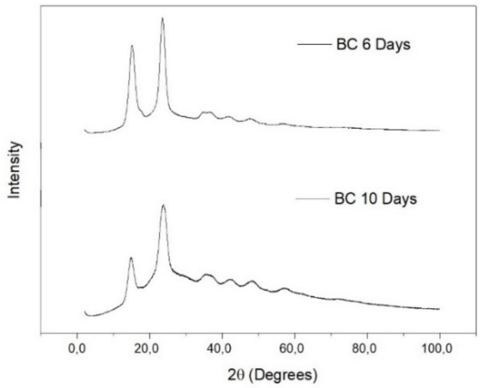

Figure 3. XRD of samples of BC obtained after 6 and 10 days.

4. Filtration System

Table 2 shows the mean volumes filtered in milliliters (mL) and the amount of oil present in the filtrate (ppm). For each filtration the time of 4 minutes was set and the system pressure was 1 atm.

Table 2 – Mean of the filtered volume (mL), mean of the amount of oil present filtered (ppm) and standard deviation of the mean filtered volume of the 6-day and 10-day BC filtrations

		Mean (mL)/ Mean (ppm)	**Filtered Std. Deviation**	**Mean (mL)/ Mean (ppm)**	**Filtered Std. Deviation**	**Mean (mL) / Mean (ppm)**	**Filtered Std. Deviation**
		25 mm		50 mm		100 mm	
6-day BC	10 ppm	14.58 / 0.0	0.52	46.68 / 0.0	2.29	149.79 / 0.0	2.50
	150 ppm	13.49/ 0.0	0.88	37.38 / 0.0	0.72	145.00/ 0.0	1.47
	230 ppm	13.36 / 0.0	1.16	36.60 / 0.0	1.20	147.147 / 0.0	1.62
10-days BC	10 ppm	9,28 / 0,0	0,33	29,13 / 0,0	2,04	88,45 / 0,0	2,03
	150 ppm	9,12 / 0,0	0,14	33,46 / 0,0	2,09	89,58 / 0,0	1,31
	230 ppm	9,00/ 0,0	0,10	31,92 / 0,0	1,53	90,50 / 0,0	0,46

With the results of the filtration it is observed that the 6-days BC membrane of present a greater filtration flow, being 1.7 times higher, with the same efficiency of the 10-days BC membrane, allowing a total removal of the oil present and proving to be efficient in oil removal.

5. Conclusions

The experiments performed showed that the removal of the oil present in all samples was of 100%. It is also possible to note that the filtration rate increases proportionally to the filter diameter and it decreases from the 6-day membrane to the 10-day membrane, because the CI obtained for the 6-days BC was 71% and while

that of 10-days BC was 84.4%, these results show that CI has a ratio inversely proportional to the porosity of the cellulose surface, and the presence of larger pores allows a greater penetration of the water molecules, favoring the use of the 6-days BC as a filter membrane. The percentage of water retention present in both BC membranes exceeded 98%, showing the enormous presence of water in their compositions, making it difficult to pass the oil and facilitating its use as a filter. Thus, the BC membrane is an efficient hydrophilic barrier to prevent the passage of nonpolar components like oils and fats.
The studies on the application and efficiency of the BC membrane as a filter for oily waters will continue to identify physical chemical parameters as reference for this type of effluent. For a future research the study will focus on the difference between a filtration of a stabilized emulsified oily solution against a non-emulsified. Further characterization of the BC membranes will support the use of the biomaterial as a biotechnological filter. However, with the execution of these experiments the potential in the application of BC membrane obtained in alternative medium as an efficient filter for separation of water and oil mixtures was demonstrated.

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