

# Effect Of Culture And Purification Conditions On Physicochemical And Transport Properties In Bacterial Cellulose Membranes

Luz Dary Carreño Pineda<sup>1</sup>, Luis Alfonso Caicedo Mesa<sup>1</sup>,  
Alberto Claudio Habert<sup>2</sup>

<sup>1</sup>Grupo de Procesos Químicos y Bioquímicos, Departamento de Ingeniería Química.  
Facultad de Ingeniería, Universidad Nacional de Colombia. Bogotá, Colombia.

<sup>2</sup>Laboratorio de Processos con Membranas. Universidade Federal do Rio de Janeiro, Brazil.  
ldcarrenop@unal.edu.co, lacaicedom@unal.edu.co, habert@peq.coppe.ufrj.br

Bacterial Cellulose (BC) is a polymer which has similar composition than plant cellulose, but its morphological structure and properties are different. In this work, were obtained several bacterial cellulose membranes from three carbon sources (sucrose, glucose and glycerol) using as culture temperatures 25°C and 30°C. The cellulose was purified varying the chemical agent, concentration, temperature and exposition time. SEM, DSC, Hydraulic permeability and solute rejection were performed and it was observed the purification conditions have influence on the structure and performance of the membranes, which is promissory in a further design of a membrane for a specific membrane separation process application.

## 1. Introduction

Bacterial cellulose (BC) is an extracellular polymer synthesized by bacteria belonging to *Sarcina Rhizobium*, *Agrobacterium* and specially the *Acetobacter* genera (CZAJA et al, 2006., HIRAI et al, 1987). This cellulose has the same chemical composition than plant cellulose; however, the structural conformation, purity, and size of microfibril are different. BC obtained from static culture forms a gel which once purified and dried is converted into a membrane capable to be used in separation processes like ultrafiltration, gas permeation and pervaporation (SHIBAZAKI, H, 1993., DUVEY et al, 2002., PANDEY et al, 2005., SOKOLNICKI et al, 2006). It was observed that culture conditions (microorganism, temperature, carbon source, pH) and gel purification (chemical agent, concentration, temperature and exposition time) can change the physical properties of the membranes (WANICHAPICHART et al, 2002., GEORGE et al, 2005). Literature shows potential applications of these membranes in separation processes. The aim of this work is to evaluate the effect of culture and purification conditions on the physicochemical and transport properties of the membranes. It is

expected this results allow designing a membrane for a specific membrane separation process.

## 2. Experimental

### 2.1 Fermentations

Microorganism used in this study was an *Acetobacter* strain isolated from a local tea fungus culture. The culture medium used for the inoculum was composed for sucrose (10g/l), yeast extract (2 g/l), and tea (4g/l). pH was adjusted 5,5 with HCl 1N.

For the membranes production, the culture media was composed for: carbon source (glucose, sucrose or glycerol) 30 g/l, yeast extract 10 g/l, pH 5,5. The media was autoclaved to 121°C for 15 minutes, inoculated with and incubated to 25 and 30 °C, taking samples each 3 days during 30 days in order to obtain the production of cellulose and membrane thickness curves. For the membranes used in characterization tests, the same culture proceedings was followed, adjusting the culture time until the membranes reach 50 µm of thickness.

### 2.2 Purification

Cellulose was removed of culture medium, washed with distilled water. After that, membranes were processed using conditions summarized in table 1.

Table 1. Purification Conditions

Chemical Agent	Concentration	Temperature	Exposition Time (min)
Distilled Water		90°C	10
NaOH	1N	60°C	10
	5N	90°C	30

When thermal treatment was finished, cellulose was washed several times until the NaOH was completely removed. After that, cellulose was dried at 30°C until constant weight.

### 2.3 Characterization

*SEM*: It was used a FEI- Quanta 2000 equipment to obtain the micrographs of the samples previously coated with gold.

*DSC*: DSC 2910 (TA Instruments) was used to determine the glass transition temperature ( $T_g$ ) and crystalline fusion temperature <sup>TM</sup>. The tests were carried out with a sealed empty pan as a reference and N2 as gas flushing from -50 a 270°C a 10°C/min.

*Hydraulic Permeability*: It was used a permeation equipment, with distilled water. The pressure interval was from 3 to 12 bar. Pressure was adjusted, and after 30 minutes as stabilization time it was started data collecting.

*Solute Rejection*: The test is similar to the hydraulic permeability test, but it was used a Dextran T500 1% (p/v) solution instead water. Permeate concentration was estimated by refraction index.

### 3. Results and Discussion

#### 3.1 Thickness

Figure 1 shows the results of membrane thickness with the culture time. It was found that the membranes with the lowest thickness are from glicerol while the higher was obtain using sucrose as carbon source. After 30 days of cultivation, no significant increase of thickness is presented for all carbon sources. Thus, for obtaining membranes with 50  $\mu\text{m}$ , the necessary time was 20, 13 and 24 days using glucosa, sucrose and glicerol as carbon sources respectively.

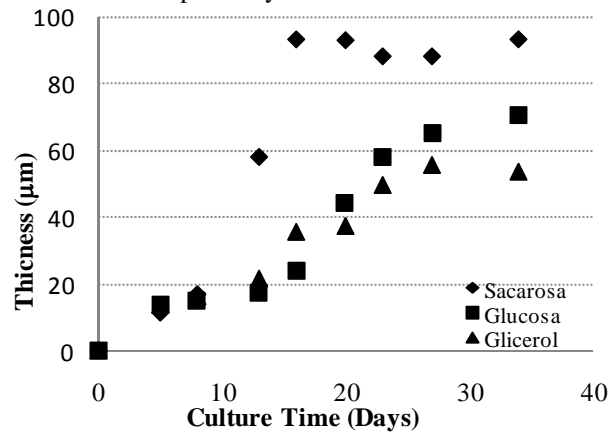


Figure 1. Membrane thickness with culture time.

The purification treatment showed that when only distilled water is used, some components of the culture medium remain, and it can allow the microbiological attack of the membranes.

#### 3.2 SEM

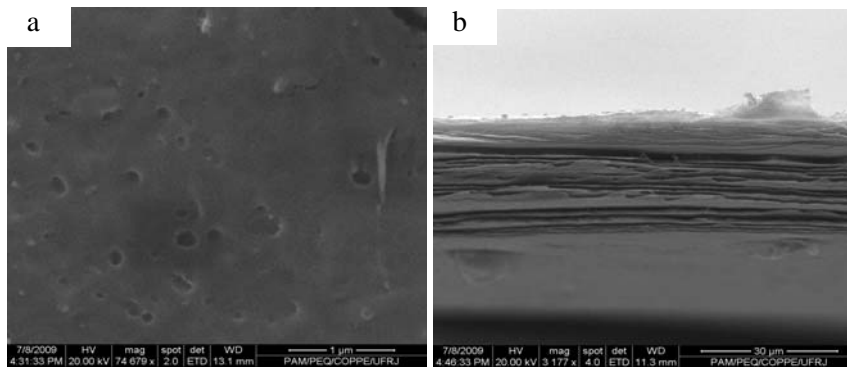


Figure 2. Micrographs of one bacterial cellulose membrane. a) Surface. b) Cross Section

Membranes do not have a porous morphology. Surface shows a homogeneous structure with a few crevasses probably originated from the culture stage. Cross sections

confirmed what was reported earlier about the layer formation of the membrane (ROSS et al, 1991.,KLEMM et al, 2001), Pores were not also found through the cross section.

### 3.3 DSC

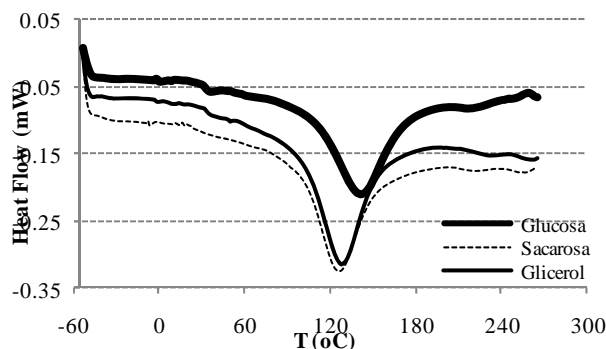


Figure 3. DSC thermograms of bacterial cellulose membranes obtained using three different carbon sources at 30°C. Purification treatment: Distilled water.

Figure 3 shows thermal behavior of the membranes. It can be seen a fusion peak after 125°C. The transition temperature is around 30°C. As shows table 2, no significant change in  $T_g$  was observed with the chemical treatment. It can be rationalized in terms of treatments procedures, involving temperatures above the culture temperature. Temperature in treatment can affect the mobility of cellulose fibrils more than the presence of a chemical agent. However  $T_m$  has significant variations, showing an increase with the intensity of the treatment.

Table 2. Glass transition temperatures for bacterial cellulose membranes

Treatment	Glucose		Sucrose		Glycerol	
	$T_g$	$T_m$	$T_g$	$T_m$	$T_g$	$T_m$
Distilled Water	33	142	24	127	34	128
1N 60°C 10 min	34	146	26	130	28	152
5N 90°C 30 min	31	164	25	155	27	163

### 3.4 Hydraulic Permeability

Membranes obtained at 25°C are less permeable than the obtained at 30°C (see figure 4). This may be due to culture temperature as it changes the metabolism of the microorganism which affects rate of cellulose production and then, the rate of fibril formation. On the other hand, the effect of treatment can be seen clearly in membranes from glycerol, when an increase in NaOH concentration increases permeability, may be because the agent solubilizes the thinnest fibrils, leaving space that bigger fibrils can use for their segmental mobility, which increases the flux of water through the membrane. All membranes have low hydraulic permeability, so, maybe these membranes have a dense structure.

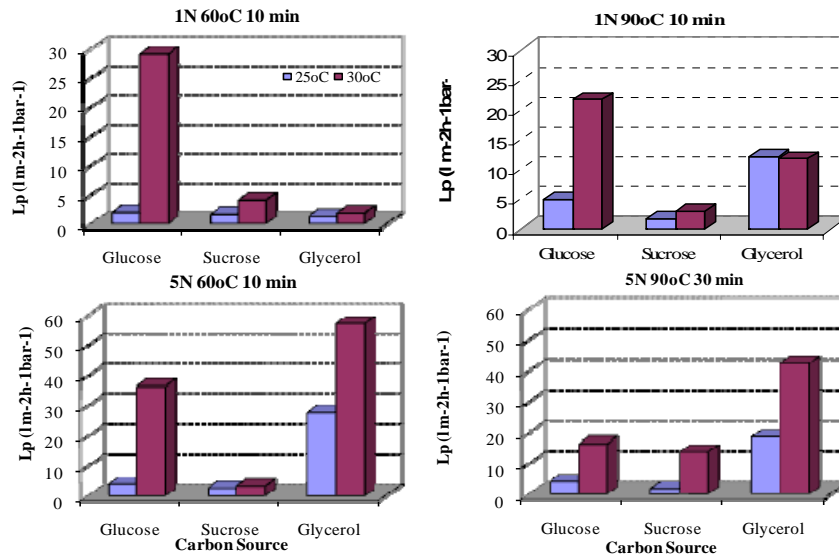


Figure 4. Hydraulic permeability of bacterial cellulose membranes, with different culture and purification conditions.

### 3.5 Solute Rejection

For this test membranes which have the highest and the lowest hydraulic permeability were selected for characterization. They were respectively obtained from Sacarose at 25°C (1N 60°C 10 min) treatment, and Glycerol at 30°C (5N 60°C 10min) treatment.

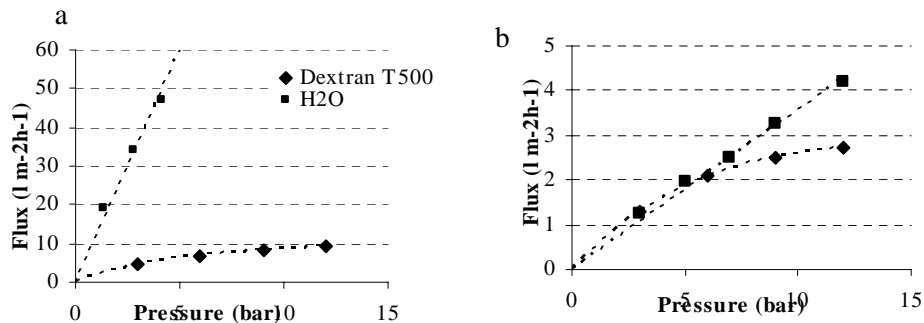


Figure 5. Dextran T500 permeation through membranes. a) Glycerol b) Sucrose.

Figure 5 shows both membranes decreases permeate flux compared with water. Membrane from glycerol present a significant reduction compared with the membrane from sucrose. This last one shows the best rejection compared with de membrane from glycerol (see figure 6), may be because its higher segmental mobility allows the transport of the solute.

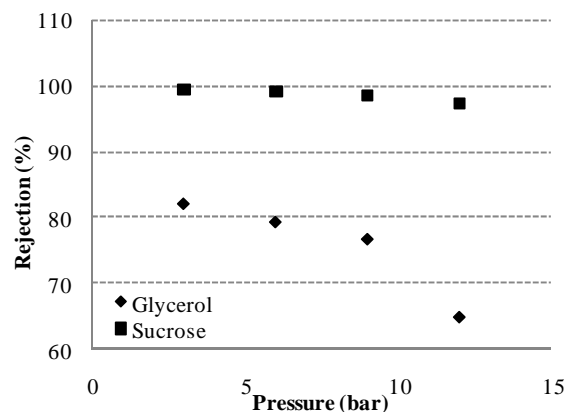


Figure 6. Dextran T500 Rejection

#### 4 Conclusions

Membranes of bacterial cellulose were obtained from three carbon sources: glucose, sucrose, glycerol, and were purified changing chemical agent, time and temperature of exposition, and it was found the effect of this parameters on the structure and performance of the membranes. Also, it was found is important to use a chemical agent to avoid microbiological degradation of the membrane.

DSC tests showed  $T_g$  has no changes with chemical treatment, but influences significantly the  $T_m$ .

Culture temperature and carbon source have impact on hydraulic permeability of the membranes as well as the solute rejection.

#### 5 References

- Czaja, W., Krystynowicz, A., Bielecki, S., Brown, R. *Biomaterials* 2006. 27: 145-151.
- Duvey, V., Pandey, L., Saxena, C. *Journal of Membrane Science* 2005. 251: 131-136
- George, J., Ramana, K., Sabapathy, S., Jagannath, J., Bawa, A. et al. *International Journal of Biological Macromolecules*, 2005. 37: 189-194
- Hirai, A., Horii, F., KITAMARU, R. *Macromolecules* 1987. 20:1440-1442.
- Klemm, D., Schumann, D., Udhardt, U., Marsch, S.. *Progress in Polymer Science* 2001. 6:1561-1603.
- Shibasaki, H., Kuga, S., Onabe, F., Usuda, M. *Journal of applied Polymer Science*, 1993. 50:965-969.
- Pandey, L., Saxena, C., Duvey, V. *Separation and Purification Technology*, 2005. 42:213-218.
- Sokolnicki, A., Fischer, R., Harrat, T., Kaplan, D. *Journal of Membrane Science* 2006. 272:15-27.
- Ross, P., Mayer, R., Benziman, M. *Microbiological Reviews* 1991. 55:35-58.
- Wanichapichart, P., Kaewnopparat, S., Buaking, K., Puthai, W. *Songklanakarin J Sci Technol* 2002. 24: 855-862.