

# Application of non-thermal Plasma as an Alternative for Purification of Bacterial Cellulose Membranes

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**Abstract** Innovation to mitigate environmental impact and alignment with the Sustainable Development Goals, encourages the cosmetic industry to explore new methodologies and materials. Biopolymers become attractive, such as bacterial cellulose that has biocompatibility, high crystallinity and wettability. During the production of cellulose, there is the purification process, which is essential for safety in the applicability, as it removes metabolites and bacterial cell debris. Currently, the most adopted methodology is the immersion of the membranes in NaOH solution, however, there is no concise methodology, causing, besides the generation of chemical residues, discrepancies between authors. In this sense, the proposed work aims to use cold plasma technology (NTP) to verify the action in the BC purification process. With the association of gases, after 15 minutes of treatment with NTP in membranes, no new membranes appeared when they were placed back in the culture medium. Attesting the potential of NTP in the BC purification process, optimizing time and avoiding the generation of aggressive chemical residues.

**Keywords:** bacterial cellulose; cold plasma; non-thermal plasma; sodium hydroxide; purification

## 1. Introduction

Nowadays science faces a huge challenge, in terms of developing a “green chemistry”, therefore, they seek the development of ecologically correct processes and technologies (Matouk et al., 2020). In view of numerous researches on the use of bacterial cellulose (CB), several methods are being explored to enhance it. As in the purification stage, it is carried out with NaOH, occurring through the eradication by cell lysis of all bacteria, to then be applied in the health area. After being used for purification, NaOH becomes a chemical residue, becoming less environmentally sustainable, time consuming and does not fit into a green chemical (Panget al., 2019; Kim, Kim, & Sohn, 2020).

A highly explored technology in sterilization processes is non-thermal plasma (PNT), which can be an efficient and green alternative for this process, as it does not use chemicals and does not generate aggressive waste. The

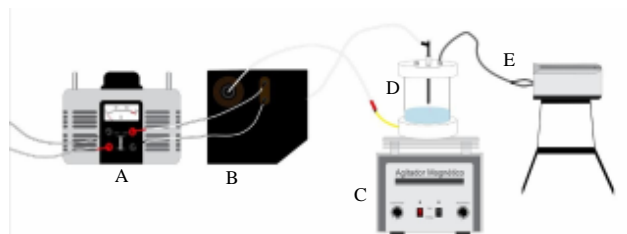
PNT is formed by an ionized gas consisting of multicomponent such as electrons, highly excited atoms, ions, radicals and neutral particles (Afshari & Hosseini, 2014).

Therefore, the main objective of this work was to evaluate the applicability of non-thermal plasma in CB membranes as a purifying process.

## 2. Experimental conditions

The experiments were carried out using a non-thermal plasma reactor (figure 1) high voltage AC source ( $\pm 17$  kV) and current of 30 mA for maximum output power of 510 W and flow of Argon gas and air mixture of 3, 0 L min<sup>-1</sup>. The reactor operating at atmospheric pressure with 40 mL of sterile distilled water and CB membranes, kept under electromagnetic agitation. The treatment time used was 15 min, afterwards, the membranes were relocated in a new culture medium to check if there would be the appearance of new membranes, all experiments were carried out in duplicate. The plasma reactors were cleaned and sterilized with 70% ethanol and dried in a laminar flow hood under ultra violet radiation for two hours.

One of the BC controls was through conventional purification with complete immersion of the membranes in 1 N NaOH solution for 1 h at 90 °C, and then they were washed with distilled water until the pH reached 7.4. Another control was the removal of the membrane from the medium and inserted directly into a new culture medium.



**Figure 1.** Schematic system of non-thermal plasma (A - energy source; B - variac; C - magnetic stirrer; D - reactor; E - gas outlet).

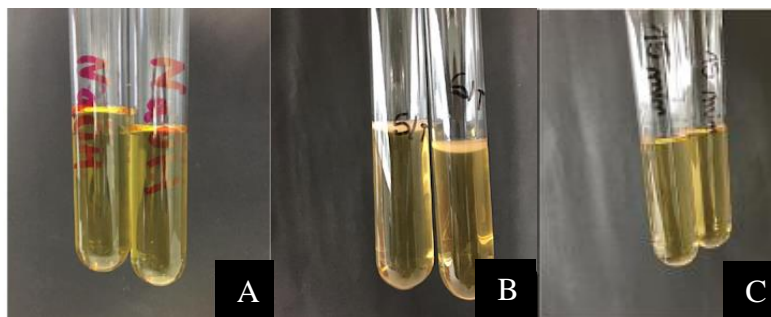
## 3. Results

Through Figure 2, it is possible to observe how each membrane behaved after being inserted in a new culture medium.

Figure 2A demonstrates that the HS culture medium that received the control membranes treated with sodium hydroxide, did not show new membranes. Demonstrating that there are no traces of bacteria in them. Proving total purification of the membranes.

The culture medium that received the membrane without any treatment (just removed from the culture and inserted in a new one), presented in its upper part, the appearance of new membranes (Figure 2B). Therefore, this results from the presence of bacteria carried through the BC, as it has not undergone any treatment aimed at purifying it.

**Figure 2.** Growth analysis of new membranes in culture medium. A: NaOH treated membranes; B: without any treatment; C: treated with 15 minutes of NTP.



#### 4. Conclusions

The results demonstrate that it is possible to use non-thermal plasma as a membrane purification process, since no other one has appeared in the new culture medium, proving the effectiveness of cell death without the need for the use of aggressive chemical agents such as NaOH. In addition to be a faster process, being 15 minutes. This becomes attractive and feasible when aiming at applicability on an industrial scale, when dealing with the health area, such as biomedicine, dentistry, pharmacy and in the production of biocosmetics. Further studies are needed to analyze the residual water from this post-plasma process, just to ensure that there are no bacterial remains and other possible changes. Studies suggest that the PNT also generates changes in BC, as in its hydrophilicity. Finally, this clean technology is aligned with the Sustainable Development Goals and green chemistry, as it aims at not generating harmful chemical residues.

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In Figure 2C through the analysis of bacterial cellulose production capacity, with 15 minutes of PNT there was no formation of a new membrane, it can be deduced that the bacteria were lysed, as with the treatment in NaOH.

There are still no concrete explanations about the action of PNT on bacterial lysis. It is believed that it arises due to the formation of reactive species (ROS) such as atomic oxygen in the fundamental state, hydroxyl radical (OH), simple oxygen molecules, superoxide anions and ozone (O<sub>3</sub>). Among EROs, O<sub>3</sub> has a high oxidizing power, (Leclaire et al., 2008; Matouk et al., 2020) reaching the bacterial cell structure (Lu et al., 2014).

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