APPLICATION OF AN ENZYME PREPARATION FOR CLEANING REVERSE OSMOSIS MEMBRANES

Giovana P. Roma[1], Moisés A. Silva[2], Ingrid.V.F. Veleza[2], Giordana D. Arend[2], Karina Cesca[2], Alan Ambrosi[2], Elpídio C. Martins Junior[3], Haline Bachmann Pinto[3], Rodrigo S. D. Souza[3], Marco D. Luccio[2*]

[1] *Biological Sciences Center (CCB)/ Federal University of Santa Catarina (UFSC), Florianópolis, Brazil.*
[2] *Chemical and Food Engineering Department (EQA)/ Federal University of Santa Catarina (UFSC), Florianópolis-SC, Brazil.*
[3] *Petrobras R&D Center, CENPES, Av. Horácio Macedo, 950, Cidade Universitária, Ilha do Fundão, Rio de Janeiro, RJ 21941-915, Brazil.*
** Corresponding author: di.luccio@ufsc.br*

ABSTRACT

Biofouling on reverse osmosis membranes is a problem in industrial water treatment units. Some species of bacteria can adhere to surfaces and create a protective film, also known as biofilm. Operation includes maintenance measures to solve or mitigate the problem, such as physical and chemical cleanings, use of biocides, and membrane replacement, which imparts high operational costs. This study aims to evaluate two commercial enzyme preparations to remove biofilm formed on commercial reverse osmosis membranes. Total heterotrophic bacterial count (THB) before and after enzyme treatment was determined to analyze biofilm formation on the membranes quantitatively. The biofilm morphology was qualitatively analyzed using confocal laser scanning microscopy (CLSM). Enzyme preparation 2 was suitable for disintegrating the biofilm and reducing the microbial population by nearly 100%.

Keywords: Biofouling. Membrane. Enzyme preparation. Treatment. Biofilm reduction.

1 INTRODUCTION

The use of membrane filtration technologies for water purification has gained popularity with the increasing demand for clean water[1]. A critical cost factor for operating membrane-based desalination units is biological fouling, commonly called biofouling. Biofilm formation is a natural process by which groups of bacteria adhere to the surface of a membrane and mature through an encapsulation process[2]. Mature biofilms exhibit antibacterial properties and are often resistant to detachment[3]. When aggravated, biofilm formation leads to biofouling, which reduces water production efficiency and membrane degradation in desalination systems, resulting in increased costs associated with cleaning procedures and reduced membrane lifespan. Among the ways to clean biofilms on reverse osmosis (RO) filtration membranes, the application of enzymes is presented as a promising alternative.

The enzymes degrade the biofilm's extracellular matrix, formed by proteins, lipids, and carbohydrates, resulting in the exposure of microorganisms that benefit from EPS's protection[4]. Consequently, they assist in maintaining membrane permeability and enhance the effects of applying antimicrobials since bacterial cells become unprotected and susceptible[5]. This research evaluates the effect of applying two commercial enzymatic preparations to clean membranes containing biofilm grown in a laminar flow tank with water recirculation.

2 MATERIAL & METHODS

Water provided by oil refining units (A and B) was used for biofilm formation. Bacterial isolation was performed by filtering raw water samples through a sterile 0.22 µm membrane. The retained was resuspended in 20 mL of peptone water (0.1% m/v) containing glycerol (20% v/v) and stored in a freezer at -80 ºC. The inoculum was reactivated in nutrient broth and cultivated for 12 hours, 30 °C at 150 rpm.

Biofilm growth assays on seawater (SW) and brackish water (BW) polyamide membranes were performed in a laminar flow tank, which was inoculated (10^5 UFC/mL) of A and B bacteria pool in nutrient medium containing 1 mmol/L urea, pH 5.5, sodium chloride (160 µS/cm electrical conductivity), glucose and glycerol (200 mg/L) as a carbon source for B and A, respectively. The system was incubated at 30 °C, with carbon source feed every three days. At the end of 21 days, the commercial enzyme preparation was diluted in water to a concentration of 0.5%, as described by the manufacturer, and this solution was used to evaluate membrane cleaning. The membranes were immersed in the enzyme preparation and incubated at 24 °C, 50 rpm for 2 hours. An enzyme-free test was also carried out for control. THB by plate count and dry mass were determined before and after applying the enzyme blend.

Biofilm detachment was possible by removing the membrane and washing it in 0.85% sodium chloride solution to remove planktonic bacteria. THB on the biofilm attached to the membrane was determined after biofilm detachment using an ultrasonic bath for three cycles of 2 min interspersed with vortex agitation. The count of total heterotrophic bacteria was performed by plating a 100 µL aliquot of the liquid phase from the serial dilution detachment on a plate containing Plate Count Agar (PCA). The plates were stored in an incubator at 37 °C for 24 hours, and at the end, the number of colony-forming units was counted.

For the confocal analysis, the membrane was rinsed in 0.85% saline water, stained with the FilmTracer™ LIVE⁄DEAD Biofilm Viability kit (Thermo Fisher Scientific) for 20 min, and the images were acquired on a confocal laser scanning microscope (DMI 6000, Leica Microsystems) according to manufacturer specifications.

3 RESULTS & DISCUSSION

The cleaning efficiency related to the reduction of CFU/mL was determined using two commercial enzyme preparations. The counting of the total heterotrophic bacteria before and after applying the enzymes is presented in Figure 1, which demonstrates a significant decrease in the microbiota. Enzymatic preparation 2 reduced more than 99% of the microbiota in both membranes (SW and BW). Concerning enzyme preparation 1, a more pronounced reduction was noted for both membranes fouled with the A pool, around 95% and 92%, respectively, demonstrating that cleaning efficiency depends on the composition of the biofilm.

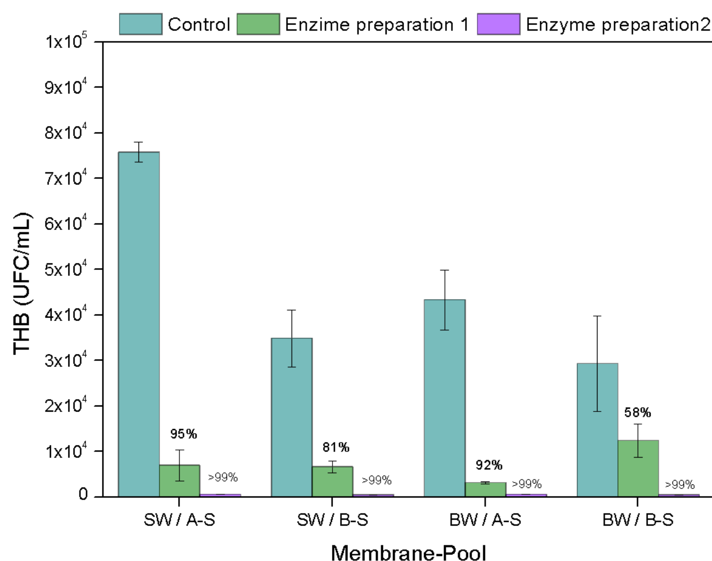

Figure 1 Total Heterotrophic Bacteria (THB) in the SW and BW membranes polymeric with the bacterial Pool (A-S and B-S) grown by 21 days. Before and after treatment with 1 and 2 enzyme preparation (0.5% in water) for 2 hours, 24 °C and 50 rpm.

Figure 2A shows the distribution of the biofilm on the surface of the SW and BW membranes before and after applying the two enzymatic preparations, 1 and 2, with both inoculum (A and B). The green cells represent live bacteria, and the red cells represent dead or non-viable bacteria.

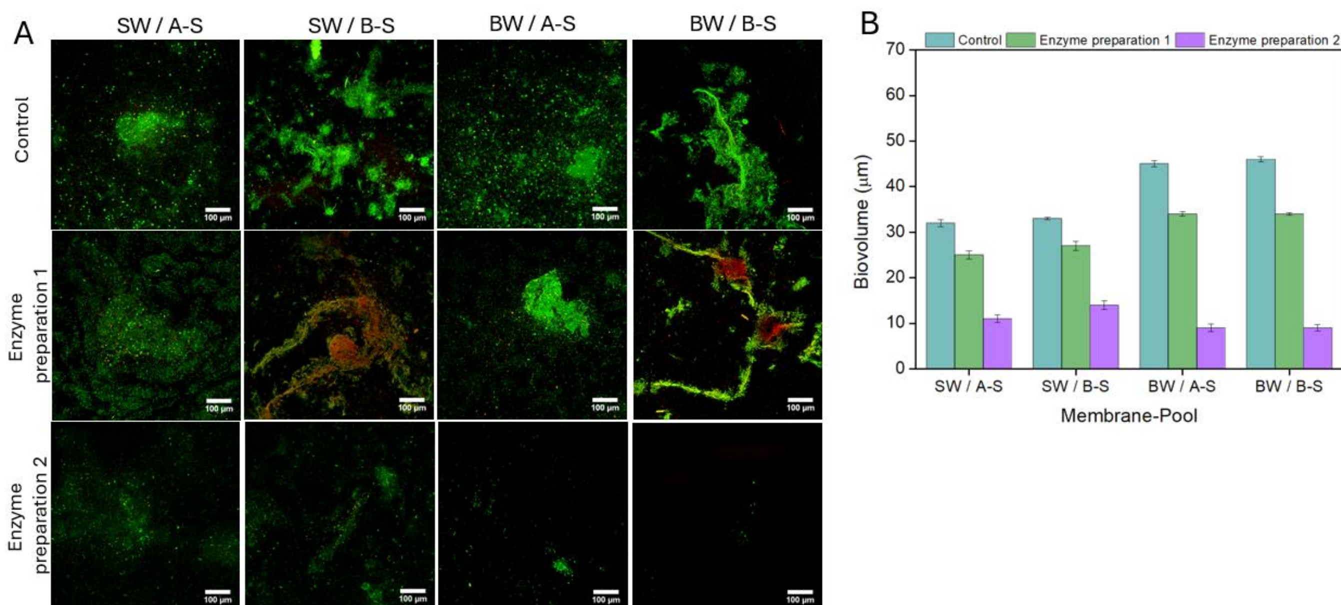

Figure 2 Confocal microscopy (A) and (B) Biovolume of SW and BW membranes with the bacterial pool (A and B) after 21 days. Before and after treatment with enzyme preparation from 1 and 2 (0.5%) for 2 hours, 24 °C and 50 rpm.

Biofilm formation is present more frequently in membranes cultured with the B bacterial pool on the surface of the BW membrane (Fig 2B). Confocal microscopy measured the biofilm's thickness before and after the cleaning process (Fig. 2B). We observed that the thickness of the biofilm changed little when we applied enzymatic preparation 1, despite demonstrating a significant change in CFU/mL. Enzymatic preparation 1 was effective for the control of both biofilms, as shown by the presence of red cells. When the enzymatic preparation 2 was applied, a reduction in the distribution of live and dead cells was observed both in the morphology (Fig. 2A) and in the thickness of the biofilm (Fig. 2B). The reduction of the microbiota to almost zero was observed when applying enzymatic preparation 2, corroborating the data of CFU/mL (Fig. 1).

Studies that reported using enzyme preparations containing α-amylase, alginate lyase and trypsin-EDTA for cleaning membranes contaminated with biofilm indicated even further reduction (71%)[5]. In the present work, both enzymatic preparations have amylase enzyme as one of the components and were efficient in microbial reduction of membranes. The efficiency of biofilm inactivation depends significantly on the microbial community present and on the enzymes that make up the enzyme preparation.

4 CONCLUSION

The cleaning efficiency of membranes containing biofilms depends significantly on the microbial community and the specific enzymes in the formulation. This research shows that the two enzymatic preparations effectively disintegrate the biofilm from the membranes in the time and concentration tested. Furthermore, the enzymes in the first enzyme preparation showed a more significant reduction in biofilms produced by the microbial community present in the A pool, showing an effectiveness of almost 90%. Future work must be carried out to identify the strains present in these water samples and understand the mechanism of action of these enzymes.

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