ENZYMATIC TREATMENT FOR BIOFILM CONTROL ON REVERSE OSMOSIS MEMBRANES

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ABSTRACT

The present work focuses on biofilm growth and enzymatic treatment on two reverse osmosis membranes, providing valuable information on the efficiency of enzymes in breaking down the exopolymeric matrix surrounding the biofilm. The study of biofilm growth was carried out by quantifying total heterotrophic bacteria and dry mass. A 0.5% of an enzymatic blend was applied to evaluate the effects on cleaning membranes with 21-day biofilm. The zeta potential of membranes dried at different temperatures was determined to understand how charge distribution and biofilm adhesion were related. Two bacterial pools tested showed biofilm-forming characteristics, with more affinity for membranes of lower zeta potential. The application of the 0.5% enzymatic blend significantly reduced the sessile heterotrophic total bacteria count and dry mass, reaching up 99% and 92%, respectively, depending on the membrane or bacterial pool. The results confirmed the efficiency of enzymatic blends in cleaning RO membranes, highlighting the potential for practical application in water treatment processes.

Keywords: Reverse Osmosis. Biofilm Formation. Enzymes. Biotechnology.

1 INTRODUCTION

The intensification of discussions concerning water access sets water scarcity as one of the leading global retrogressions. By 2025, 1.8 billion people are expected to live under absolute scarcity conditions. In this sense, agriculture's excessive water use and climate change that unsettles rainfall regimes in different regions contribute to increased freshwater demand[1]. Numerous processes have been studied to produce freshwater on a large scale. Reverse osmosis emerges as a promising alternative. In the last five decades, technological innovations have allowed energy costs to be reduced by almost five times [2], which increased the number of active plants. However, biofouling remains the main hindrance to the entire operation due to the intensive attachment of microorganisms to the membranes, forming a protective exopolymer matrix that provides resistance to gene stress from the environment. Biofouling causes a decrease in the permeate flux, and the membrane gradually loses selectivity, leading to water quality loss and a reduction in the membrane's lifespan[3].

Biofilm control has been studied by developing several pretreatments[4]. Chemical cleanings have been studied using different agents, including acids and bases combined with chlorination. Enzymatic treatment has been a recent subject of investigation, demonstrating recovery of parameters such as membrane permeability and pressure drop[2] greater than conventional cleaning-in-place (CIP) methods. Thus, this study aimed to explore how biofilm derived from two bacterial pools develops on reverse osmosis membranes, focusing on analyzing the efficiency of enzyme treatment on biofilm control. The microbiological formation dynamics were investigated, and the effects of enzyme cleaning on the biofilm matrix mitigation were determined.

2 MATERIAL & METHODS

The biofilm formation on membrane surfaces was studied using two water samples collected from reverse osmosis units of two oil refineries, A and B. Bacterial isolation was performed by filtering raw water samples through a sterile 0.22 µm membrane. The retained material was resuspended in 20 mL of peptone water (0.1% m/v) containing glycerol (20% v/v) and stored at -80 ºC. The inoculum was reactivated in nutrient broth and cultivated for 12 hours, 30 °C, at 150 rpm.

Biofilm growth assays on SW and BW polyamide membranes were performed in a 24-well plate over 21 days. The SW and BW membranes were cut, washed in distilled water, and dried at 45 °C for 24 h. Then, they were distributed in 24-well plates, and 250 µL of inoculum ($1 \cdot 10^5$ CFU/mL) was added. After five h of bacterial adhesion, 1 mL of nutrient medium containing urea (1 mmol/L), pH 5.5, sodium chloride (160 µS/cm electrical conductivity), glucose, and glycerol (200 mg/L) was added as a carbon source for B and A, respectively. The plates were incubated at 30 °C, 50 rpm, with medium replacement every three days. Samples were taken at 10, 14, and 21 days to analyze biofilm formation for total heterotrophic bacteria (THB)[5] and dry mass.

At the end of 21 days, a 0.5 % (v/v) enzyme preparation was added to evaluate the potential for membrane cleaning. The membranes were immersed in the enzyme blend and incubated at 24 °C, 50 rpm for 2 h. 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.

Zeta potential analyses were performed with raw membranes dried in an oven at 25 °C, 45 °C, and 100 °C for 24 h to characterize the charge pattern on both membranes and understand the bacterial adhesion process after drying.

3 RESULTS & DISCUSSION

Biofilm formation depends on the composition of the species in the water and the properties of filtration membranes. This work tested the adhesion and growth process of two bacterial pools from two different reffineries (A and B) on two membranes (BW and SW). THB analysis showed significant biofilm growth over time for the SW membrane (Fig. 1A), reaching 10^7 CFU/mL and 10^6 CFU/mL at the end of 21 days for the B and A bacterial pools, respectively. The biofilm mass corroborated this finding since the SW-B membranes contained more than 1 mg biofilm/membrane and SW-A close to 0.6 mg/membrane (Fig. 1B).

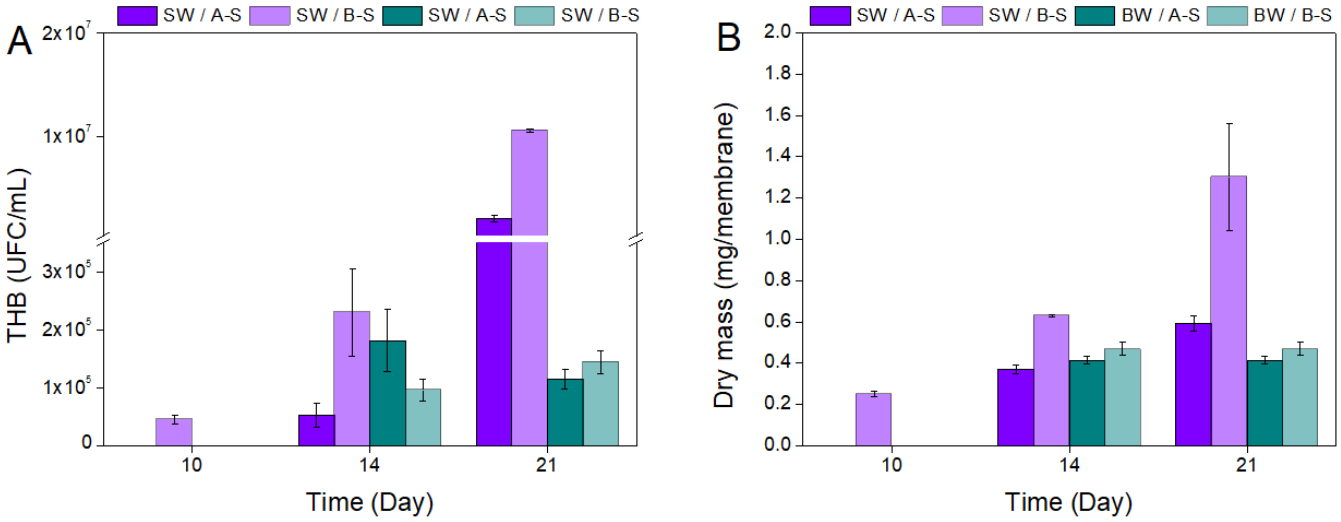

Figure 1 Biofilm growth analyzed by THB (A) and dry mass (B) for SW and BW membranes by the A and B bacterial pools.

After analyzing biofilm formation on the membrane for 21 days (Fig. 1), a commercial enzyme preparation was used to assess the membrane cleaning process. The application of the enzymatic preparation after biofilm growth was effective in reducing biofilm (Fig. 2). That is noticeable in the THB and dry mass values before (Fig. 2A and 2C) and after treatment (Fig. 2B and 2D), respectively. Favorable high reduction, reaching 99% and 92% in THB and dry mass, respectively, on SW / A-S, were observed. In addition to lower bacterial adhesion observed for the membrane-pool BW / A-S, a lower decrease was observed for the THB and dry mass after the enzymatic cleaning (reduction of 46.78% and 84.58%, respectively).

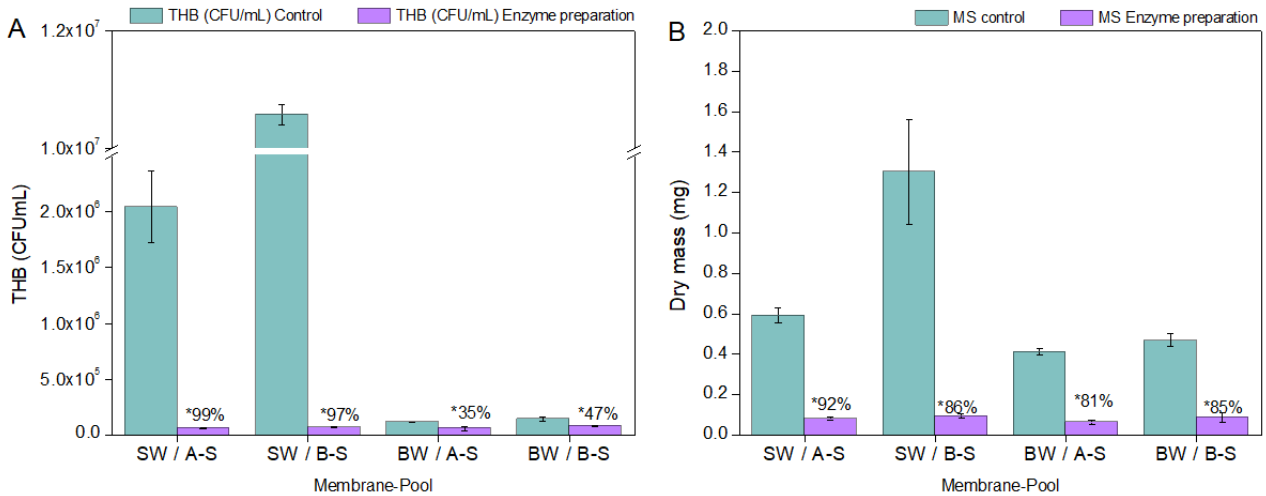

Figure 2 (A) THB (CFU/mL) and (B) dry mass (mg) before and after enzymatic treatment with 0.5% for 2 hours at 50 rpm.

Studies on the use of enzymatic preparations containing proteases and mannosidases showed biofilm reduction assessed by the existing biovolume and achieved reduction values of 43%, reaching 71% with the addition of amylases and alginate lyases[8].

Zeta potential data (Fig. 3) demonstrated that SW and BW membranes have similar isoelectric points (around 3.5). However, the latter has a lower zeta potential at pHs higher than 5.5 at the drying temperature 45 °C. At the cultivation medium pH (around 5.5 and 6.5), the SW (Fig. 2A) membrane presented a zeta potential more prone to biofilm deposition than BW (Fig. 2B). These data corroborate with other studies in the literature[7], which associated more outstanding cell adhesion in membranes with higher zeta potential.

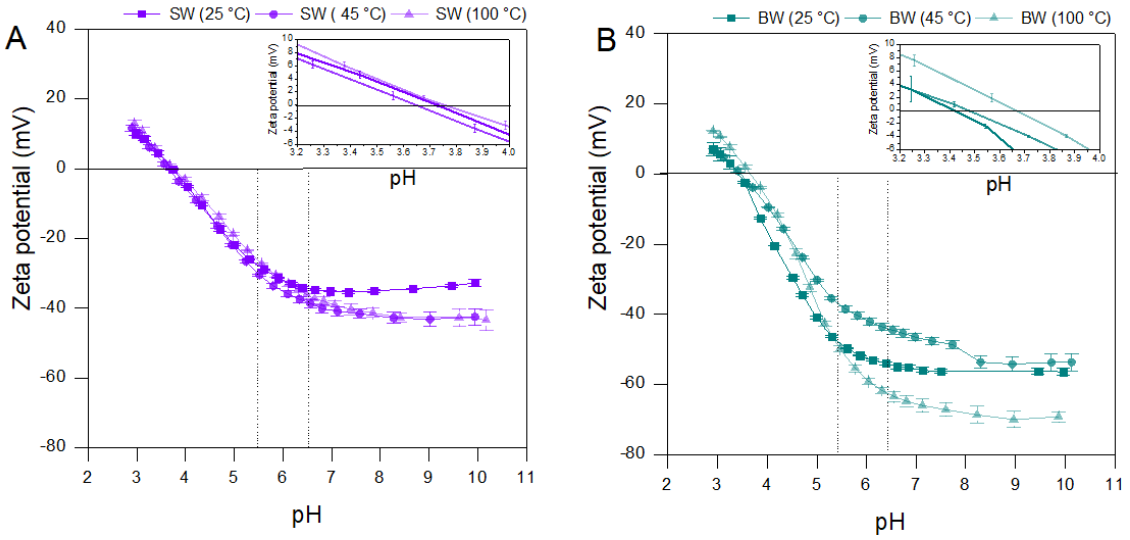

Figure 3 Zeta potential (mV) of SW (A) and BW (B) membranes dried at 25 °C, 45 °C, and 100 °C.

4 CONCLUSION

Promising results were obtained for the biofouling formation and polysaccharide matrix reduction after enzymatic treatment. Both bacterial pools showed greater affinity for the SW membrane, which has a higher zeta potential in the pH range of the bacterial pool than the BW membrane. We assume biofilm-forming bacteria are present in greater diversity in the B pool, while the BW membrane showed a better resistance to biofouling formation. Based on these conclusions, a promising path emerges in the cleaning technology scenario. In addition, treatments using enzymatic preparations could be tested in future pilot plants to assess costs and biofilm removal efficiency in full-scale plants.

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