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Effect of N-acetylcysteine against biofouling of reverse osmosis membrane

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ABSTRACT

The influence of (1.5 mg/mL) of N-acetylcysteine (NAC), a non-antibiotic, mucolytic agent, on the biofouling of a reverse osmosis (RO) membrane by a multi-species culture (four environmental strains) of biofilm forming bacteria was studied. NAC was found to considerably suppress the formation of the biofilm on the RO membrane. The inhibitory effect of NAC on biofilm formation was verified by image based studies. There was over a 70% reduction in biofilm surface coverage when grown in the presence of NAC. Similarly, the average thickness and total biomass content of the biofilm formed in presence of NAC were significantly less than those of the control. These results suggest that NAC could be a potential agent for the control of biofouling of a RO membrane. However, the chemical stability, potential toxicity and consistent performance of NAC in the field will have to be further investigated for optimization of its use on a field scale.

1. Introduction

Fouling is an inherent problem within the membrane based water purification industry; even a small amount of fouling can cause significant loss of permeate flux [1], considerably reducing the plants efficiency. Several types of fouling can occur in the membrane system, e.g. inorganic, organic, particulate, colloidal and biofouling [2]. Of these, biofouling is recognized as the most difficult to control [3], as the causative microorganisms are found to survive pretreatment of the feed water (a first aid to prevent fouling) and; therefore, form biofilm over the membrane. Chlorine is commonly used during the pretreatment process to control the bacterial count in the feed water, which would be expected to reduce biofouling. However, the use of chlorine is reported to intensify biofouling, as micro-organisms subjected to low levels of chlorine are found to exude large amounts of extracellular polysaccharides (EPS) for protection. The EPS support biofilm formation [3]. Chlorine based biocides have also been known to deteriorate membranes [4], adding to its low preference as an antifoulant.

Chemical agents, such as salicylic acid and NAC [5], have been reported to prevent the formation of a biofilm by microorganisms on medical polymers. Extracellular slime produced by bacteria strengthens the bacterial adhesion to produce biofilm, which makes its removal from any surface more difficult. It has been estimated that the bacteria embedded in biofilms are ca. 1000 times more resistant to many antibiotics than when in a free floating planktonic form [6]. NAC is a non-antibiotic chemical, which acts as a mucolytic agent by disrupting the disulphide bonds in mucus to reduce its viscosity and allow for their easy detachment. Hence, NAC is expected to have inhibitory effect on bacterial biofouling. N-acetylcysteine is globally the most widely used mucolytic agent; hence, is a molecule with an extremely fast medicinal career and wide therapeutic profile [7]. The purpose of this study was to determine the suitability of NAC as an agent to prevent bacterial fouling of a RO membrane. The effect of NAC was tested against biofilm development by a multi-species bacterial community originally isolated from the natural environment, i.e. from a bio-fouled membrane.

2. Materials and methods

2.1. Environmental strains from fouled membrane, laboratory media & RO membrane

Aeromonas hydrophila KU1, *Pseudomonas putida* KU22, *Stenotrophomonas* sp. KU34. and *Serratia marcescens* KU16 isolated from a fouled RO membrane (supplied by a local water purification plant in Deasan, Chungbuk, Korea) (strains were identified using 16S rRNA gene sequencing) were used for this study. The strains were stored short term on LB Agar slants at 4 °C and in 20% glycerol at −70 °C for long term preservation. The same full strength LB medium was used to revive the culture, for preliminary studies in polystyrene plates and for growing biofilm. The pH of all media was set at seven. The culture medium was purchased from Difco (Franklin Lakes, NJ, USA). A FILMTECTM SW30HR-380, polyamide, thin film composite seawater reverse osmosis membrane (Dow Chemical Company, Midland, MI, USA) was used for this study.

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2.2. Experimental set up with CDC Reactor, sampling, & CLSM imaging

Preliminary studies, with individual strains (monocultures), were carried out in polystyrene microtitre plates to gain an understanding of the minimum inhibitory concentration (MIC) of NAC and the effect of NAC on biofilm formation on a polystyrene surface. This MIC was used as the standard concentration in the CDC (Centers for Disease Control) reactor (BioSurface Technologies Corp, MT) against a multi-species culture consisting of four different environmental strains *viz. Aeromonas hydrophila*, *Pseudomonas putida*, *Stenotrophomonas* sp. and *Serratia marcescens*.

The reactor, medium storage tank, tank for spent medium and tubing were autoclaved and connected aseptically. RO membrane segments (1.5 cm × 1.5 cm) were UV sterilized before use. NAC stock solution was prepared in double distilled water and then filter sterilized. The least effective concentration of NAC (1.5 mg/mL) was used for the CDC reactor based studies, as decided from MIC determination test.

To each of the eight removable rods in the CDC reactor, a sterile glass slide was fixed in such a way that it faced the baffle when placed into the reactor. Two pieces of RO-membrane were fixed (feed side facing out) onto each glass slide using sterile double sided cellophane tape. Starter cultures for each of the four strains were prepared, with 350 μl of each culture then used to inoculate 348.6 mL of LB in the CDC reactor (1 L) to initiate biofilm formation. The reactor was run with 350 ml of LB in the batch mode for one day and afterwards with fresh medium continuously supplied at a flow rate of 35 mL/h. The volume of the culture medium within the reactor was maintained at 350 mL. All other parameters were as per Kappachery et al. (2010) [8]. Another reactor was run in parallel under similar conditions, with the exception that the medium contained 1.5 mg/ml of NAC. After each specific incubation period of 1 and 2 days (as separate experiments), membrane coupons were removed and the biofilm quantified by analysis of images (8 samples per day & 8 images per sample) taken using a confocal laser scanning microscope (CLSM). For all experiments, sampling, processing, CLSM observations and image analysis were carried out as per Kappachery et al. (2010) [8].

3. Results and discussion

The investigation involved growing biofilm on the RO membrane in a CDC reactor in presence and absence of NAC. The biofilm formed by the multispecies community of bacteria was quantified and measured in terms of its structural parameters, including surface coverage, average thickness and total biomass.

In terms of surface colonization in the NAC amended reactor, it was found that the biofilm formation was significantly suppressed within 24 h to the tune of 70.3%, which was even more significant after 48 h (90%) compared to the untreated control (Fig. 1). Similar trends were observed for the measurements of the total biomass and average thickness. The reductions in the biofilm development (in the presence of NAC) after incubation for 24 and 48 h were 73.6 and 87.3%, respectively, in terms of total biomass, which was similarly observed with the average thickness (Figs. 2 & 3). In the untreated control reactors, the biofilm development in terms of increased surface colonization from 0 h to 48 h was 229%, while in the NAC amended reactor this was negligible (14.9%). Measurements of the biofilm-thickness and biofilm-biomass indicated the biofilm development from 0 h to 48 h in the untreated control was 179%, but only 35% in the NAC amended reactor, showing that NAC had a profound influence on the growth of the multispecies bacterial community on the RO membrane. These results showed that the presence of NAC changed the texture of the biofilm formed, making NAC an interesting candidate for use as a general inhibitor of bacterial biofilm formation (Fig. 4). It would be expected that in real systems, the presence of NAC in the feed water could prolong the time period for biofilm formation and reduce the frequency of membrane cleaning, as currently the most common practice of sanitization is every 3–5 days during peak biological activity (summer) and about every seven days during low biological activity (winter).

The planktonic cell concentration in terms of optical density at 600 nm (OD_{600}) and viable cell count per milliliter of the culture broth were consistent after 24 h in the batch mode, indicating the stability of the chemostat system used. Throughout the experimental period, no significant difference in abundance of planktonic cells was observed between the control and treatment batches, indicating that NAC at concentration tested did not hinder the growth and proliferation of the multispecies culture. This is highly attractive as it underlines the role of NAC as a biofilm inhibitor via different mechanisms, which may include reduction of extracellular polysaccharides (EPS) production and weakening of the slime layer formation.

While forming biofilms, bacteria produce large amounts of EPS, which bind a biofilm together as a matrix, anchoring it to the surface [9, 10]. Therefore, anything that targets the EPS would be expected to be an appropriate method for dealing with the control of biofilm formation. NAC is a thiol containing antioxidant, which disrupts the disulfide bonds in mucus and competitively inhibits amino acid utilization [11]. The effect of NAC against bacterial biofilm formation on a polystyrene surface has previously been reported [5]. Oloffson (2003) reported that NAC not only reduced adhesion, but also detached adhered cells from a steel surface and reduced the production of EPS in most bacteria (multispecies and monoculture), even at concentrations where the

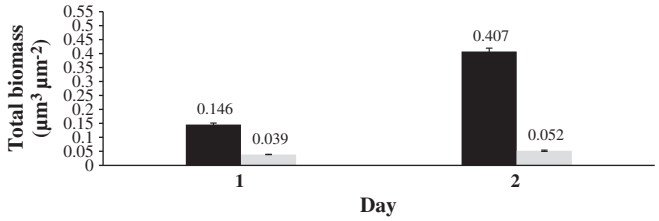

Fig. 2. Total biomass of biofilm formed by the multispecies, environmental strains on to the RO membrane (actual value given with the bar). Values are means ± SE. ■ = control; ■ = treated from 0 h incubation.

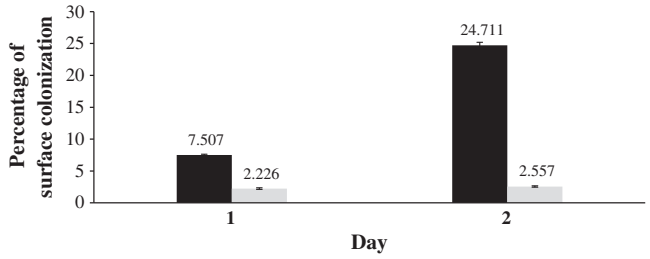

Fig. 1. Percent surface coverage of biofilm formed by the multispecies, environmental strains on to the RO membrane (actual value given with the bar). Values are means ± SE. ■ = control; ■ = treated from 0 h incubation.

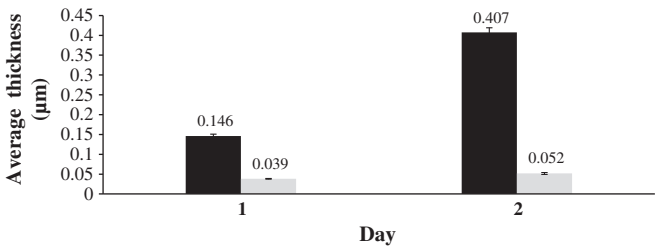

Fig. 3. Average thickness of biofilm formed by the multispecies, environmental strains on to the RO membrane (actual value given with the bar). Values are means ± SE. ■ = control; ■ = treated from 0 h incubation.