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A microbiology-based assay for quantification of bacterial early stage biofilm formation on reverse-osmosis and nanofiltration membranes

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ABSTRACT

Membrane-based water treatment and purification technologies are crucial globally, and are especially essential in areas that suffer from a shortage of water. In the source water bacteria are ubiquitous and microbial attachment and growth on membranes is a major problem associated with these technologies. Here we describe an assay to quantify early stage biofilm growth on reverse-osmosis (RO) and nanofiltration (NF) polyamide membranes. The adherence and growth of *Klebsiella oxytoca* on RO and NF membranes was monitored over time under static conditions, with repeated removal of planktonic bacteria, and this assay was effective in showing especially the early stages of bacterial attachment and growth. Comparison of bacterial growth on NF270, SW30, TM820-400, and LE-400 membranes showed only slight differences, and in all cases the bacterial growth rate accelerated between 5 and 10 h of incubation time. The average surface growth rate was 1.3×10^6 CFU/($cm^2 \times h$) during 5–10 h of incubation, and was measured by counting colony-forming units (CFU) after detachment of bacteria from the membranes. Fluorescence microscopy confirmed early stage biofilm growth between 5 and 10 h of incubation time. This assay will be useful to assess the susceptibility of membranes to bacterial attachment and subsequent biofilm growth and will be especially useful in screening novel antimicrobial and anti-biofilm membrane coatings and modifications and gaining insight into the mode of action of surface-tethered antimicrobial agents. Since initial irreversible adhesion is a critical step in the development of biofilms and hence biofouling on membranes, a deeper understanding of the process may lead to novel strategies for biofilm prevention.

1. Introduction

The problem of fresh water scarcity occurs globally and is expected to worsen in the coming decades [1]. Membrane-based technologies play pivotal roles in meeting the challenge of water scarcity and they will become even more important due to their low energy demand in water treatment [2]. Key technologies in membrane-based water treatment include reverse osmosis (RO) and nanofiltration (NF); RO is the most common method for desalination of seawater and brackish water, and NF is highly important for industrial wastewater treatments, surface water purification and many other applications. Today, thin-film composite (TFC) polyamide membranes are the most commonly used membranes in RO and NF technologies. TFC polyamide membranes are cost-effective and combine a high solute rejection with high water permeability [3].

A major drawback in membrane filtration applications is membrane fouling, which causes an increase of required feed pressure and consequently higher energy consumption, and is usually accompanied by decreased solute rejection. The major fouling types of NF and RO membrane elements are scaling (inorganic deposits), particulate and colloidal matter, organic fouling and biofouling. Different types of fouling may occur simultaneously and can influence each other. The first three types of fouling can generally be controlled by reduction of foulant concentration in the feed water or by acidic solution wash (for scaling). Biofouling, however, is harder to control since microorganisms multiply on the

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membrane if nutrients are available, even if the bacterial concentration in the feed effluents is controlled [4]. Biofouling in NF or RO operations is complicated by the fact that backwash is not possible, and because disinfection with chlorine or other oxidizing agents damages the membranes.

Bacterial biofilms are the accumulation of microbial aggregates on a surface due to either deposition or growth [5]. The biofilm is the preferable environment for more than 99% of microorganisms to live. Microorganisms are embedded in a matrix of extracellular polymeric substances (EPS), which forms the scaffold for the three-dimensional architecture of the biofilm [6] and is mainly responsible for its structure and integrity. The EPS is 50–90% of the total organic matter in the biofilm structure, and contains amphiphilic compounds such as phospholipids, as well as proteins, nucleic acids, polysaccharides and other polymeric compounds. The EPS matrix is important for initial attachment and the aggregation of bacterial cells, the EPS is also a nutrient source, it provides mechanical reinforcement to the biofilm, and is a protective barrier from antimicrobial compounds or other type of stress. An accepted model for biofilm development, which was based on biofilm formation of *Pseudomonas aeruginosa* describes biofilm development in five consecutive stages (see a review [7]): (i) reversible adhesion – initial deposition of cells to the surface. These adherent cells may leave the surface to resume the planktonic lifestyle; (ii) irreversible adhesion – production of EPS results in irreversible attachment of bacteria to the substratum; (iii) maturation I – the cells produce EPS to form micro-colonies; (iv) maturation II – the biofilm reaches its maximum thickness; (v) dispersion – release of cells from the biofilm to return to planktonic mode of growth. The initial adhesion of bacterial cells to RO and NF membranes is highly important stage in biofilm formation on such surfaces, and probably has dramatic influence on extent of biofouling in these processes (see a recent review [8]).

Extensive research has been devoted to combat biofouling by developing anti-microbial coatings on surfaces, including RO and NF membranes. For example, anti-microbial substances such as metallic nanoparticles and enzymes were immobilized on membrane surfaces [9–11]. Complementary to novel anti-microbial membrane modification and fabrication studies, there is an increasing interest to develop reliable and accurate methods to evaluate the anti-microbial performance of modified RO or NF membranes. Direct observation under high-resolution microscopes has been widely used to assess the biofilm bacterial community. Due to its ability to capture 3-dimensional images, confocal laser scanning microscopy (CLSM) is a useful tool to characterize the thickness and viability of the biofilm. The image information is digitally processed to quantify the bacterial population [12]. CLSM provides good imagery of the biofilm, although depending on the bacterial species and stage of biofilm development may require optimization. For example, bacterial strains that tend to form a thin layer of biofilm on the surface leading to low intensity of the fluorescence signal [13] may necessitate intensive experimental development. Other measures for extent of a biofilm include protein or other biomolecule quantification. For example, biofilm growth was estimated on polysulfone membranes by measuring protein concentration on the surface [14]. Extraction of protein from cells and EPS in the biofilm on RO membranes was done by use of sodium dodecyl sulfate and sodium hydroxide, and protein was measured by Bradford reagent. A study by Vrouwenvelder and his coworkers showed biofilm determination on RO and NF membranes by measuring adenosine triphosphate (ATP), total cell count and heterotrophic plate count. Quantification of biomass in membrane autopsies was correlated with increase of normalized pressure drop to investigate biofouling [15].

The goal of this present study was to observe early biofilm growth on a series of RO/NF membranes by quantifying the bacterial population present. The information obtained in this study can be potentially applied to other microbiology-based assays for evaluating the performance of novel anti-microbial, or anti-biofilm coatings on modified RO or NF membranes in development of anti-fouling membranes for example. Other microbiology-based assays have been used to characterize biofilm population on inert anti-microbial surfaces [16,17] and included the use of high energy sonication for the detachment of bacterial cells from biofilms [18]. We used a short sonication step (<1 min) to detach adhered cells, since extended ultrasound at low frequencies has been reported to be lethal to attached bacteria in comparison to suspended cells [19,20]. Joyce et al. studied the effect of power ultrasound treatment on bacterial viability and showed that it was primarily a combination of declumping and inactivation; using TEM analyses they observed a significant effect on bacterial cell structure [21]. The biofilm growth was monitored with respect to time and early stage biofilm growth could be monitored. Thus this assay proved to be a useful tool to assess the susceptibility of a polymer membrane surface to early stage biofilm development. CLSM images confirmed that biofilm development was at an immature stage. This assay, optimized for RO and NF membranes, maximizes the recovery of attached cells on the surfaces and is potentially suitable for screening novel anti-microbial coatings or membrane-surface modifications.

2. Experimental

2.1. Materials

Flat-sheet brackish water LE-400 RO membranes, SW30HR RO membranes, and NF 270 membranes were provided as a gift from FILMTEC Membranes-Dow Water Solutions (Midland, MI); Seawater TM820-400 flat sheet RO membrane was provided as a gift from Toray Industries (Japan). RTV-186 was purchased from Polymer-G'vulot (Kibbutz G'vulot, Israel). Tryptic Soy Broth (TSB) was purchased from Acumedia Manufacturers (Lansing, MI).

2.2. ATR-FTIR analysis

Attenuated total reflection fourier transform infrared (ATR-FTIR) spectroscopy measurements were recorded on a Vertex-70 FTIR spectrometer (Bruker Optiks, Ettlingen, Germany) using a Miracle ATR element (45° single-reflection diamond-coated KRS-5 internal reflection crystal). The membranes were dried overnight under vacuum prior to the FTIR measurement. Each IR spectrum was an average of 40 scans collected within the spectral range 4000–400 cm^{-1} at 4 cm^{-1} resolution. The resulted spectra were then corrected for the wavelength-dependent penetration depth of evanescent IR wave ($\sim$1 μm) and background subtraction with the OPUS software (version 6.5, BRUKER Optiks GmbH, Ettlingen, Germany). Seven replicate spectra at different locations were obtained for every membrane or glass-membrane sample, and an average spectrum was calculated.

2.3. Water drop contact angle analysis

Static contact angles were measured using a sessile drop of water in air on the thin-film face of the membranes using an OCA-20 contact angle analyzer (DataPhysics Instruments, Filderstadt, Germany). The membranes (pristine or glued on glass slides) were washed and dried under vacuum at room temperature prior to the measurements. The analysis was carried out by placing a 0.5 μL droplet onto the surface; the contact angles were estimated using SCA-20 software (DataPhysics) by drawing the surface baseline and the drop profile, then calculating the angle at the line of