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Nanofiltration and reverse osmosis surface topographical heterogeneities: Do they matter for initial bacterial adhesion?

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ABSTRACT

The role of the physicochemical and surface properties of NF/RO membranes influencing bacterial adhesion has been widely studied. However, there exists a poor understanding of the potential role membrane topographical heterogeneities can have on bacterial adhesion. Heterogeneities on material surfaces have been shown to influence bacterial adhesion and biofilm development. The purpose of this study was therefore to investigate whether the presence of membrane topographical heterogeneities had a significant role during bacterial adhesion as this could significantly impact on how biofouling develops on membranes during NF/RO operation. An extensive study was devised in which surface topographical heterogeneities from two commercial membranes, NF270 and BW30, were assessed for their role in the adhesion of two model organisms of different geometrical shapes, *Pseudomonas fluorescens* and *Staphylococcus epidermidis*. The influence of cross-flow velocity and permeate flux was also tested, as well as the angle to which bacteria adhered was compared to the flow direction. Bacterial adhesion onto the membranes and in their surface topographical heterogeneities was assessed using Scanning Electron Microscopy (SEM), Atomic Force Microscopy (AFM), fluorescence microscopy and image analysis. Results showed that up to 30% of total adhered cells were found in membrane defect areas when defect areas only covered up to 13% of the membrane surface area. This suggests that topographical heterogeneities may play a significant role in establishing environmental niches during the early stages of biofilm development. Furthermore, no noticeable difference between the angle of cell attachment in defect areas compared to the rest of the membrane surface was found.

1. Introduction

The removal of trace contaminants and organic matter by nanofiltration (NF) and reverse osmosis (RO) processes from wastewater and surface water has become an important step in providing clean potable water [1–3]. However, bacteria adhere to the membrane surface eventually forming a biofouling layer [2,4,5]. Biofilm formation on membranes has a significant negative effect on process performance through permeate flux decline, loss of retention and increased pressure loss over the membrane elements [6–8]. Biofilm removal requires extensive chemical cleaning which is disruptive to the process, may cause damage to the membrane and prevent a full recovery of membrane flux and retention [9]. This in turn can lead to a financial burden, usually in the form of processing costs associated with greater energy consumption, the replacement of defective filtration units and costs pertaining to halting processes for non-routine cleaning procedures.

Similarly to other substrata, biofilm formation is prompted by the initial adhesion and subsequent consolidation of microorganisms onto membrane surfaces [10,11]. It is therefore important to identify the different factors involved in the initial bacterial adhesion onto NF and RO membranes as this would help develop novel antifouling membrane surfaces and cleaning strategies for sustaining membrane performance. Bacterial adhesion has been found to be influenced by the surface properties of membranes such as surface charge [12–14], hydrophobicity and surface roughness [15,16], as well as bacterial cell wall physico-chemical properties and structure [17]. Surface roughness is a parameter used to evaluate the surface topography of membranes indicating heterogeneous nano-scale peak protuberances and depressions on the membrane surface [15,18,19]. Analyses suggest that these nanoscale heterogeneities provide favourable binding sites for bacteria to deposit and accumulate [10,20].

The use of surface roughness as a parameter is usually quantified as the average roughness and root mean squared roughness. However, quantifying membrane topography in the presence of

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surface topographical heterogeneities (redefined as surface defects throughout this study) can be challenging [21], since these are usually in the order of several micrometres in width and depth. As such, defects can be easily overlooked and excluded during AFM studies, in which scanning raster areas are usually performed at random small areas at a time [15,18]. The presence of large surface defects on NF and RO membranes are areas larger than those featured in the minimum value of surface roughness presenting areas with lower shear rate. Microscale surface defects on other types of surfaces such as stainless steel [22,23] have been found to influence bacterial adhesion [24–27]. Moreover, a previous study has demonstrated preferential bacterial adhesion to substrates comprised of surface topographical heterogeneities compared to flat surfaces [28]. The analysis of bacterial adhesive behaviour to various structured surfaces has provided insights into preferential sites with high likelihood of cell adhesion and proliferation as previously demonstrated by Hou et al. [29]: the presence of micro-topographic confining features larger than $20\,\mu m \times 20\,\mu m$ on structured PDMS was shown to promote the adhesion and subsequent enhanced biofilm formation of *Escherichia coli* cells.

While surface defects can promote preferential surface colonization, the size and shape of bacterial cells also need to be considered. One previous study conducted by Medilanski et al. [22] demonstrated that cell morphology influenced cell's proficiency to adhere within surface topographical heterogeneities in the form of scratches on stainless steel created at the width of the the bacterial cells. More specifically larger *Rhodococcus* sp. showed a maximum percentage cell adhesion alignment with topographical heterogeneities of up to 7% while smaller *Pseudomonas aeruginosa* cells showed an alignment of up to 44%.

Although, the surfaces of NF and RO membranes are composed of micrometre scale surface defects, it is still unclear whether these influence the initial bacterial adhesion under full scale filtration processes. The consequence of these pronounced surface defects on membranes should not be neglected since it is unclear how these might contribute to the rate of bacterial adhesion and potentially the characteristics of the subsequent biofilm. This work provides a framework by which novel membranes with deliberate micro-topographical modification [25,27,28,30–32] can be assessed from the point of view of early stage biofouling.

The aim of this study was to determine how surface defects, present on the surface of NF and RO membranes, influence bacterial adhesion, in its most basic form. In this study, two bacterial species of different morphologies *Pseudomonas fluorescens* and *Staphylococcus epidermidis* commonly found in NF and RO biofilms during water treatment [26,33–36] were used to test their proficiency to adhere onto micrometre scale surface defects areas of two commercial NF and RO membranes, NF270 and BW30, respectively, under flux and no-flux conditions. In addition, the angular orientation of adhered cells in relation to flow direction was assessed to determine whether the orientation of bacteria during adhesion was influenced by flow hydrodynamics or whether it follows a stochastic process.

2. Materials and methods

2.1. Bacterial strains, culture conditions and preparation

One Gram-negative *P. fluorescens* PLC1701 and one Gram-positive *S. epidermidis* ATCC 12228 model strains were selected for bacterial adhesion assays in this study. *P. fluorescens* is a rod-shaped bacterium with approximately 1 μm in width and 2 μm in length and *S. epidermidis* is a cocci bacterium with approximately 1 μm of diameter. An mCherry-expressing *P. fluorescens* [11] was stored at −80 °C in King B broth [37] supplemented with 20% glycerol. Independent *P. fluorescens* cultures were obtained by inoculating 100 mL King B broth supplemented with gentamicin (Sigma Aldrich, Ireland) at a final concentration of 10 μg mL^{-1}, using a single colony of a previously grown culture on King B agar (Sigma Aldrich, Ireland) at 28 °C. Independent *S. epidermidis* cultures were obtained by inoculating 100 mL Tryptic Soy Broth (TSB) using a single colony of a previously grown culture on King B agar (Sigma Aldrich, Ireland) at 28 °C. Both inoculated media were then incubated at 30 °C with shaking at 75 RPM for 16 h until the cell culture reached an optical density (OD) between 0.8 and 1.2 at OD_{600}. Cultures were centrifuged (Eppendorf Centrifuge 5415C) at 7000 RPM for 10 min, after which the supernatant was discarded and the bacterial pellet was re-suspended in Raw Water Medium without carbon (RW^{-C}), as previously described by Semião et al. [16]. Water used in preparation of the RW^{-C} was Grade 1 pure water, referred to as MilliQ water (Biopure 15 and Purelab flex 2, Veolia, Ireland). This water was used throughout the project. Prior to adhesion assays, *S. epidermidis* cells were stained by adding 2 μL of 3.34 mM SYTO 9, followed by a 15 min incubation period at room temperature in the dark. Staining was not required for *P. fluorescens* due to the mCherry fluorescence protein marker. Bacterial suspensions were then diluted in RW^{-C} to an OD of 0.2 for dynamic adhesion essays with and without flux constituting a feed concentration of approximately 10^7 CFU mL^{-1}.

2.2. Microbial Adhesion to Solvents

The Microbial Adhesion to Solvents (MATS) assays were performed to assess the hydrophobic character and Lewis acid–base properties of the bacterial organisms used in this study. This method is based on the comparison between microbial cell surface affinity to a monopolar solvent and an apolar solvent which both exhibit similar Lifshitz–van der Waals surface tension components. The MATS solvents used in this study were chloroform (an electron acceptor solvent), hexadecane (nonpolar solvent), ethyl acetate (an electron donor solvent), and decane (non-polar solvent) and were of the highest purity grade (Sigma-Aldrich, Ireland). The experimental procedure was performed as described by Bellon-Fontaine et al. [38] with minor modifications. Briefly, bacterial cells were incubated to an OD_{600} of 1.0 and washed twice in RW^{-C} by centrifugation (Hettich, Germany) at 5000 RPM for 10 min. collected bacterial pellets were then suspended and diluted in RW^{-C} to an OD_{400} of 0.8. Individual bacterial suspensions (2.4 mL) were first mixed with 0.4 mL of the respective solvent and then mixed for 60 s using a Vortex mixer (Stuart, UK). The mixture was allowed to stand for 15 min to ensure complete separation of phases, after which 1 mL from the aqueous phase was carefully removed and its final optical density was measured at $OD_{400\ nm}$. The percentage of adhesion of bacterial cells in the solvent phase is calculated using the following equation:

$$\%\ \text{adherence} = \left(\frac{A_0 - A}{A_0}\right) \times 100 \quad (1)$$

where A_0 is the optical density of the bacterial suspension before mixing at OD_{400} and A is final optical density after mixing.

2.3. Bacterial electrophoretic mobility

Overnight bacterial cultures were harvested by centrifugation (5000 RPM, 10 min) and washed twice with 0.001 M NaCl before diluting to an OD_{600} of 0.2. Separately, the pH of individual 0.001 M NaCl solutions was adjusted to pH 3, 7 and 9 by adding nitric acid or potassium hydroxide. Prior to electrophoretic mobility readings, the bacterial suspension was diluted to a hundredth in the pre-prepared pH solutions suspension (2 mL final volume) which was then placed in a capillary cuvette that was placed in a Zetasizer instrument (Malvern Instruments, UK) for electrophoretic mobility measurements. Each experiment was performed in triplicate using three independent cultures.