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The role of cell-surface interactions in bacterial initial adhesion and consequent biofilm formation of Nanofiltration/Reverse Osmosis membranes

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ABSTRACT

Until recently, the realization that membrane biofouling during nanofiltration (NF) and reverse osmosis (RO) processes is an unavoidable occurrence, has led to a paradigm shift in which biofouling management approaches rather than biofouling prevention are now being considered. To implement this new concept, it is crucial to understand the fundamentals of cell-surface interactions during bacterial adhesion, a prerequisite to biofouling of membranes. As such, with membrane biofouling already being widely studied and documented, greater attention should be given to the factors involved in the initial bioadhesion onto membranes during NF/RO processes. This review focuses on the interactions between bacterial cells and NF/RO membranes, emphasizing the mechanisms of bacterial adhesion to NF/RO membranes with particular reference to the effects of micro-environmental conditions experienced at the membrane interface, such as feed-water composition, hydrodynamics, permeate flux and conditioning layers. This review also discusses membrane surface properties and how it relates to bacterial adhesion as well as latest advancements in antibacterial membranes, identifying areas that need further investigation.

Keywords: bacterial adhesion, membranes, nanofiltration, reverse osmosis, biofouling, fouling, operating conditions.
1. INTRODUCTION

Biofouling remains a major operating problem in nanofiltration (NF) and reverse osmosis (RO) plants and is a topic that has been extensively documented in the literature[1-6]. Biofilms are at the core of the problem and their recalcitrance leads to performance loss and the use of significant quantities of cleaning chemicals. In extreme cases the biofouling problem may reduce the operating life of the membrane module. Scientific studies in the context of NF/RO operations have predominantly focused on the mature biofilm and to a lesser extent on initial phase of bacterial adhesion. Initial colonization of a surface is the first step in biofilm formation [7]. This transition from a planktonic to a sessile lifestyle is often in response to a variety of environmental cues, such as osmolarity, pH, carbon, iron availability, oxygen tension, and temperature [8].

The first step in adhesion is the immediate attachment of bacteria to a surface which is a reversible non-specific process. It is generally accepted that initial bacterial adhesion is a key part of the biofilm development process. However there is an increasing body of evidence suggesting that the rate of bacterial adhesion is not predictive of the extent of biofilm formation [9]. Experimental studies where both initial adhesion rate and biofilm formation rate were measured under comparable conditions are rarely found in the literature, showing a need for further investigation of the relationship between initial adhesion and biofilm formation. From the few studies that exist, it is generally accepted that there is no direct correlation between the levels of initial adhesion and the amount of biofilm formed [10-12]. A low adhesion rate might delay biofilm formation, but not prevent it [13]. This conclusion has important implications for
the critical analysis of studies where biofouling resistance is claimed based on experimental data where only initial bacterial adhesion tests were undertaken.

Bacterial adhesion in membrane systems is a complex process that is affected by many factors including the environmental milieu, the characteristics of a conditioning film, bacterial properties and the material surface physical/chemical characteristics. Notwithstanding the poor relationship between initial adhesion rate and extent of subsequent biofouling, it is important to review the fundamentals of bacterial-membrane interactions, not least because of the possible important role of initial adhesion in the biofilm developmental process, but also to elucidate the role of these interactions in biofouling control strategies. The role of bacterial-membrane-solute interactions in composite fouling whereby biofilm formation occurs in tandem with other fouling processes such as organic fouling, scaling, etc., is particularly poorly understood. Some of the complexities of the environment in which biofilms are initiated on NF/RO membranes are shown schematically in Figure 1.

The purpose of this article is to provide a comprehensive review of the mechanisms of bacterial adhesion to NF/RO membranes with particular reference to the effects of micro-environmental conditions experienced at the membrane interface. Key concepts relevant to NF/RO membrane operations including feed-water composition, hydrodynamics, permeate flux and conditioning layers are all discussed in the context of bacterial-surface interactions with cognizance of the current understanding of bacterial adhesion and biofilm formation.

2. BACTERIAL ADHESION: GENERAL PATTERNS

Mechanisms by which bacteria are transported to a surface can include Brownian motion, sedimentation due to differences in specific gravity between the bacteria and the bulk liquid, or
convective mass transport, by which cells are physically transported towards the surface by the movement of the bulk fluid. When bacteria approach a surface they must overcome an energy barrier to establish direct contact with the surface. The repulsive or attractive forces consist of Lifshitz-van der Waals attractive forces, electrostatic repulsive forces and acid base forces. As an oversimplified rule of thumb, primary adhesion between bacteria and abiotic surfaces is generally mediated by nonspecific interactions [14]. Only when the cell and surface are in close proximity do short-range interactions become significant (including hydrogen bonding as well as hydrophobic interactions). The theoretical approaches for describing these interactions usually involve DLVO or XDLVO theory and are reviewed in detail elsewhere [15-18]. This theory has been applied in investigations of bacterial adhesion on membranes, in controlled environments, by taking into account the membrane contact angle, roughness and surface charge, as well as the bacteria cell wall properties [19-21]. It should be noted, however, that these theories should be applied with caution; for example, bacterial cell properties can change due to a change in EPS expression, consequently affecting their adhesion [22]. Furthermore, the presence of bacterial appendages, even negatively charged ones, can pierce the electrostatic energy barrier between the negatively charged surface of the bacteria and the negative charge of the adhering surface [23]. Finally, the presence of organic matter and other solutes in real water will foul the membrane by forming a cake layer on the membrane surface which will change with time and affect the adhesion of bacteria [24], adding substantial complexity to the system.

In the second stage of adhesion, loosely bound organisms consolidate the adhesion process by releasing extracellular polymeric substances that complex with surface materials and/or receptor-specific ligands located on pili, fimbriae, and fibrillae. [14]. At the conclusion of the second stage, adhesion becomes irreversible in the absence of physical or chemical intervention, and the
organism becomes firmly attached to the surface. In one of the earliest studies on bacterial adhesion to membranes [25] the initial adhesion onto RO cellulose acetate membranes of *Mycobacterium sp.* was studied in carbon free media. It was found that initial adhesion reached an apparent steady-state (in log scale representation) after 1 to 2 hours and that it followed a Langmuir type of isotherm, showing that the membrane had a limited amount of sites for adsorption. Attached cells were observed to be arranged singly or in pairs and well separated from other attached cells. Some regions of the membrane surface were found to be free of bacterial adhesion, the reasons for which were unclear. Subramani and Hoek [19] studied the initial deposition of several microbes on various NF and RO membranes, in particular they investigated the effect of membrane physico-chemical properties and topology. Initially, individual cells were observed to deposit and attach at discrete locations on the membrane surface. Subsequently, new cells were deposited at the leading stagnation points created by previously attached cells and form growing aggregates. Large cell aggregates were occasionally removed by cross-flow forces, tending not to redeposit downstream unless they encountered another large aggregate presenting a substantial stagnation point. In a more recent study, Myint et al [26] showed that surface roughness and hydrophobicity of NF membranes not only determined the level of bacterial initial attachment and aggregation, but were ideal hotspots for colony formation leading up to biofilm development [26].

Bereschenko et al. [27] used flow cells connected in parallel to a full-scale RO system to monitor microbial biofilm formation. This approach allowed investigation of microbial biofilms under conditions similar to those in the full-scale RO system. Analysis of the membranes over defined time-points in the early stages of the biofouling showed two patterns of development, i.e. cells
that mainly adhered in clumps and grew in the form of large micro colonies and cells that mainly adhered as single cells and colonized the surface almost as a monolayer.

In terms of microbial species, the work of Bereschenko et al. found that members of the genus *Sphingomonas* played a very important role in the initial formation and subsequent maturation of biofilms on RO membrane [27, 28]. Because they are facultative oligotrophs, they are metabolically well adapted to a low-carbon environment and can proliferate under conditions of limited substrates. Moreover they are also able to survive at high nutrient concentrations that occur close to the membrane surface due to the concentration polarization effect. Of perhaps upmost importance is their ability to produce several different kinds of extracellular polysaccharides [29]. Pang et al [30] showed that an isolate of *Sphingomonas* sp. strain RO2, colonized several different types of RO membranes regardless of membrane surface properties: this was attributed to its ability to produce extracellular polysaccharides to initiate biofilm formation. However there is clearly a noticeable lack of studies that identify the species that contribute to early colonization depending on feed characteristics and how these affect subsequent biofilm development in membrane systems. Several other important studies have been performed on biofilm microbial species diversity in RO systems [31-36]. These studies show inclusion of *Betaproteobacteria*, which are in general not commonly recovered using conventional isolation methods, and a number of phylotypes related to yet-uncultured organisms [37]. This study also found an abundance of *Rhizobiales* organisms. These are of significance because they are able to adapt to the environment by switching substrate types to avoid direct competition with other biofilm populations. These studies highlight the differences between feedwater microbial composition and the evolution of the biofilm community over time in NF and RO systems. They further highlight that biofilm formation in highly complex and prevention
is difficult, if not impossible, as the lack of adhesion of a colonizer at specific conditions does not translate into an absence of biofilm formation, as other species are involved. It is also apparent that bacterial attachment to membrane surfaces is a very complex process involving many variables: bacteria and membrane surface properties, feed conditions, operational parameters, among others. The impact of these different parameters on bacterial adhesion is reviewed in the following sections.

3. FACTORS INFLUENCING BACTERIAL ADHESION

3.1. Bacterial characteristics
Among the bacterial characteristics involved in adhesion, cell wall hydrophobicity, cell surface charge, cell surface structure, as well as the type of synthesized exopolymeric substances determined by the cell’s life cycle stage and nutrient availability, are very much determining factors. For any given material surface, different bacterial species and strains adhere to different extents [38, 39]; this is because physicochemical characteristics of bacteria are different between species and strains [40]. These variations are linked to differences in cell wall architecture and the presence and attributes of biomolecules found on the cell wall. Cell wall architecture distinguishes bacteria as either Gram-positive or Gram negative [41]. The bacterial cell wall of Gram-positive cells is primarily made up of thick peptidoglycan layer (~30nm), consisting of a network of crosslinking carbohydrates and peptides, which acts as a tough and flexible barrier capable of withstanding significant levels of external stress. The outer surface of Gram positive cells is usually covered with appendages covalently attached to either the peptidoglycan layer (i.e. cell wall protein, S-layer, teichoic acid, polysaccharides) or the inner plasma membrane (i.e. Lipotechoic acids) [42]. Unlike Gram positives, the cell wall of Gram negative bacteria consists
of a thinner peptidoglycan layer (≈10nm), which is topped by an outer membrane consisting of proteins (i.e. pilus, adhesins), lipopolysaccharides and phospholipids [43]. The gap between the outer membrane and the peptidoglycan layer is called the periplasmic space, which fulfills a physiological role such as allowing the passage of proteins to move from the cytoplasm where they are synthesized, to be anchored to the outer membrane [44]. Differences to either the ratios of the various cell wall appendages or their chemical buildup are the factors determining the variations in the physicochemical properties of different species and even different strains of the same species having been exposed to different environmental or growth conditions.

### 3.1.1. Hydrophobicity

Bacterial attachment in aquatic environments usually involves cell wall hydrophobic groups, especially those composed of nonpolar groups surface proteins, allowing cells to approach the substratum, followed by conformational changes in surface polymers leading for other functional groups to approach the surface for the formation of short-range attractive polymeric interactions [45].

Generally bacteria with hydrophobic properties prefer hydrophobic surfaces, hydrophilic bacteria prefer hydrophilic surfaces and hydrophobic bacteria adhere to a greater extent than hydrophilic bacteria [46-48]. In the particular case of NF and RO membrane surfaces, increase in levels of bacterial adhesion is correlated with increased bacterial cell wall hydrophobicity [30]. Herzberg et al. [22] showed that hydrophobicity of the cells varies with the growth stage and with the amount of alginate they produce by comparing a mucoid strain with a wild strain of *P. aeruginosa* in their exponential and stationary phase. The mucoid strain with higher alginate expression in the stationary phase was found to be more hydrophilic, and deposited less onto a quartz surface. It further caused a delayed permeate flux decline of an RO membrane compared
to the wild strain, where it took an extra five hours to achieve 30% decline of the permeate flux, and it produced a biofilm with a 5-fold amount of exopolysaccharides (EPS) compared to the wild strain. However, one could argue that the reduced adhesion properties of the mucoid strain could in part be attributed to the viscoelastic properties of the mucoid layer, susceptible to shear stress. In a similar study, Habimana et al [49] showed that a EPS-producing mutant strain of a *Lactococcus lactis* strain was found to be not only highly hydrophilic, but also unable to properly adhere on glass. It was suggested that the produced EPS substances masked the real physicochemical properties of the cell, but also was susceptible to shear stress causing a delayed early colonization and slow biofilm formation. The EPS composition of a developing initial colonizer on a surface could nonetheless facilitate the recruitment of other bacterial cells to the substratum, especially when the composition of the EPS matrix changes during biofilm development. In one recent study, it was shown that *Vibrio cholerae* biofilm formation is characterized by changes in matrix composition during early stages biofilm growth, in which an envelope made up of different types of polysaccharides and proteins enclose cell clusters and was found to be capable of stretching and expanding to accommodate cell growth. [50]. These observations have not been addressed in the context of membrane biofouling, but could be important considering the composition of feedwater with respect to potential early colonisers.

### 3.1.2. Surface Charge

Bacteria acquire a surface charge due to the ionization of their acid-base cell wall functional groups [51]. In aqueous suspension, bacterial cells generally have a net negative charge on their cell wall at neutral pH. However, the magnitude of the charge varies from species to species and is influenced by such factors as the age of the culture, ionic strength, growth medium, pH and bacterial surface structure. According to previous studies conducted on membrane surfaces,
increased bacterial cell wall electronegativity generally led to reduced bacterial adhesion onto the substratum [19]. In another study, van Merode et al [52] showed that one culture of an Enterococcus faecalis strain may contain cell subpopulations having different surface charges. This study concluded that the heterogeneity in cell surface charge significantly improved cell adhesion and early initial stages of biofilm formation; however culture heterogeneity disappeared in later stages of biofilm development. In a parallel study, van Merode and colleagues explained that the heterogeneous strains offered two possible surface charges to its environments, allowing them to adhere to surfaces with different surface properties, thus increasing their chances of successful colonization on surfaces [53]. This interesting finding could in part explain the successful colonization and subsequent biofouling of NF and RO membranes which are exposed to a host of different organisms found in the bulk liquid, having different cell surface properties. With the outer membrane interface of bacterial cells being complex and charged with macromolecules, different authors [54, 55] proposed a theory in which bacterial cells are soft particles having an ion-permeable polyelectrolyte layer controlling both surface charge distribution as well their interaction with inert surfaces during adhesion. The authors went on to claim that most electrokinetic theories are biased in the sense that they were originally developed for non-impermeable inert particles, which can’t be applied for soft particles. However this theory assumes that the surface potential surrounding the bacterial cell is homogeneous, whereas surface charge distribution is highly heterogeneous due to the heterogeneous spatial location of certain macromolecules on the bacterial surface. In one recent experimental study, de Kerchove et al [56] showed that the bacterial outer surface potential or soft particle theory, failed to predict bacterial adhesion to quartz surface, which was found to be linked to the non-uniform distribution of charged groups on the surface of lipopolysaccharide surface molecule as well as
the combined random patch like distribution of these outer-membrane surface molecules on bacterial surface.

The ionic strength and pH of the bulk liquid usually determines the degree of electrostatic repulsion or attraction between suspended cells. As a rule-of-thumb, cells suspended in solutions of high ionic strength tend to have a reduced electrical double layer causing cell aggregation and enhanced adhesion, whereas in low ionic strength solutions, the size of the electrical double layer surrounding suspended cells increases to such an extent that it causes electrostatic repulsion[57]. Since RO and to a lesser extent NF systems generally encounter water with high salt concentrations, this has significant implications for the enhancement of bacterial adhesion. Several studies have found that the presence of ions in the bulk liquid, namely NaCl and CaCl₂, affects the electrostatic interactions between the surface and the cell by shielding the negatively charged surface and enhance the adhesion of negatively charged cells. Chen et al. [58] further elaborated the role of NaCl and CaCl₂ on bacterial adhesion onto quartz. It was shown that the selected bacteria became less negatively charged with increased ionic strength, with charge neutralization being more effective with calcium than with sodium. Consequently, adhesion increased with increased ionic strength until a maximum was reached, at which point the bacterial rate stabilized. It was also found there was a minimum ionic strength needed to obtain adhesion. These minimum and maximum ionic strengths were, however, different for the bacteria species studied. The difference in cell deposition between both cells is thought to be caused by the masking of cell surface molecules by Ca²⁺ ions, consequently reducing the cell’s overall negative charge as well as arbitrarily affecting their hydrophobicity when suspended in CaCl₂ solution. Similarly, Subramani and Hoek [19] also demonstrated that the higher the ionic strength in the bulk solution, the less repulsion occurred between the cells in suspension and the
cells with the NF and RO membrane surface, resulting in higher bacterial adhesion. In van Hoogmoed et al. [59] adhesion to stainless steel by 3 different strains of the same bacteria were affected by the presence of calcium in the solution. For one strain adhesion increased and then decreased with CaCl$_2$ concentration increase, whilst for the other 2 strains the opposite happened. It was concluded that electrostatic interactions played a minimum role in the adhesion of bacteria on the surface because the bacteria zeta potential did not vary with increased CaCl$_2$ concentration. The same happened with hydrophobicity. However, when analysing the zeta potential data it can be seen that the bacteria surface charge generally decreases, although slightly, with increased CaCl$_2$. The contact angle of the bacteria was measured by depositing bacteria in a filter, air-dried and then measuring the contact angle with the sessile droplet method. However, no analysis on the integrity of the cells was done.

3.1.3. **Bacterial surface structure**

Bacterial surface structures are not only heterogeneous but the surface properties can change dramatically in response to changes in their environment [47, 60]. The presence of EPS on the cell surface plays an important role in initial cell adhesion. Long et al. [61] used a cation exchange treatment to remove EPS from the cell wall of several strains of bacteria and their deposition on silica surfaces at several ionic strengths was studied. The zeta potential and the size of the bacteria was the same for treated (EPS removal) and untreated bacteria: the treatment did not impact on the electrokinetic properties of the cell surface (zeta potential and mobility). The deposition rates for the untreated bacteria (with EPS) were consistently higher than for the treated ones (without EPS), demonstrating that the absence of EPS decreases bacteria cell deposition onto surfaces, regardless of cell types and motility. Recent studies using genetic approaches have shown that specific interactions are triggered by the surface chemistry and the
fluid conditions. For example, a study using *S. epidermis* showed that the combination of interactions between the bacteria and the substrata including the chemical functionality of the surface and the presence of shear stress, significantly affected the expression of genes implicated in the regulation of biofilm formation which in turn regulated the production of a key polysaccharide [62]. Likewise, the presence of external elements present in the environment, such as salts, was also shown to influence bioadhesion and biofilm formation. Recent studies demonstrated that the presence of inorganic phosphate played a key role in the biofilm formation of *Pseudomonas fluorescens* and depending of the level found in the environment, determined the adhesive action of LapA, an adhesin localized outside the bacterial cell membrane [44, 63]. In the presence of low levels of inorganic phosphate, cell detachment is induced through a cascade of internal molecular mechanisms leading up to the autolytic action on LapA, promoting cell detachment and the return to a planktonic mode of life of *P. fluorescens*. Interestingly, when trying to limit the levels of phosphate to control biofouling of RO membranes, Vrouwenvelder and colleagues observed postponed biofouling at low phosphate concentrations, which restricted biomass growth [64].

The substratum is also accepted to influence the response of the bacterium, capable of altering its gene-expression profile, resulting in the production of essential components for biofilm formation. This was particularly demonstrated in a recent study performed on four *S. epidermidis* strains, where levels of bacterial adhesion, EPS synthesis and biofilm formation were much higher on CH$_3$-terminated glass substratum compared to OH-terminated glass [62].

It is clear that the initial adhesion of bacteria on membranes is not solely dependent on the bacterial characteristics, but also on the membrane characteristics, as well as the conditions during filtration processes.
3.2. Membrane Characteristics

Although NF membranes are distinguished by their higher water permeability than RO membranes, their surface properties can be characterized in terms of their physicochemistry (i.e. surface hydrophobicity, charge and chemical composition) as well as their physical attributes (i.e. surface topology and morphology). Membrane surface properties can vary remarkably from one manufacturer to another. In one example, data from 20 different NF and RO membranes resulted in extreme variation for surface contact angle (38.6° to 73.2°), root mean square (RMS) roughness (5.9 to 130 nm), and zeta streaming potential measurement values (-4.0 to -19.7 mV) [65]. All of these membrane properties have been shown to be involved in bacterial adhesion and biofilm formation [66]. In general it has been previously shown that the more hydrophobic, less negative and rougher a membrane is, the greater the likelihood of bacterial adhesion on the membrane [19, 20, 26, 67]. However this cannot be generalized since some exceptions are found, as described in the following sections.

3.2.1. Surface Hydrophobicity

In general, hydrophilic materials are more resistant to bacterial adhesion than hydrophobic ones [68, 69]. Surface contact angle is mainly used to indicate the membrane's hydrophilicity or hydrophobicity, based on how water droplets form on the surface on which they are deposited. High contact angle is an indicator of hydrophobicity, whereas low contact angle is an indicator for hydrophilicity. In the specific case of NF and RO membranes, the higher the membrane contact angle, the more cells will adhere (Figure 2). Lee et al. [67] used several membranes with different characteristics to investigate initial cell adhesion of *Pseudomonas aeruginosa* in a flow channel up to 180 min. This study showed a clear increase of cell adhesion with increase of membrane contact angle or hydrophobicity. Myint et al. [26] undertook experiments with
different membranes without permeate flux. It was shown that the more hydrophobic a membrane is the greater the number of cells adhered to the surface. However, 3 out of 4 membranes used had very similar contact angles (black lozenges in Figure 2) so a clear correlation between contact angle and adhesion was difficult to obtain. In a different study a higher attachment to RO membranes was obtained compared to NF membranes, which were less hydrophobic [19]. It was hypothesized by Knoell et al. [70] that bacteria attachment is avoided in or near water saturated pores and channels since these structures represent unstable hydrophilic regions, which might explain why attachment is higher in more dense RO membranes compared to more porous and opened NF active layer structure. When dealing with porous materials such as NF and RO membranes care must therefore be taken when comparing the hydrophobic/hydrophilic properties of the active layer, since contact angle is affected by porosity.

3.2.2. Membrane Surface charge

When brought into contact with an electrolytic solution polymeric membrane surfaces acquire an electrical surface charge through several mechanisms, such as dissociation of surface functional groups, adsorption of ions from solution and adsorption of polyelectrolytes, ionic surfactants and charged macromolecules [71, 72]. The surface charge is dependent on the degree of dissociation and hence the pH of solution. The surface charge is compensated by counter-ions in solution creating an electrical double layer at the surface[72]. Given the effect of operating conditions on membrane surface charge and on bacterial surface charge, conclusions from published studies must be placed in context of the very specific condition in which the studies were undertaken. Furthermore it is important to highlight that the effect of electrostatic interactions between bacteria and a charged surface diminish as the ionic strength increases [73].
In a study by Terada et al. [74], cell adhesion rate had no relation with surface charge, when the polymer surface charge was negative, but once the surface charge became positive due to different degrees of grafting, adhesion rate increased with increased surface charge. In the specific case of NF and RO membranes there does not seem to be a clear correlation between the membrane surface charge and the amount of cells adhered on the membrane surface for identical experimental conditions (Figure 3). This might be linked to the fact that most commercial NF and RO membranes are negatively charged and hence, other surface properties such as roughness might be the dominant property that determines the degree of adhesion.

Surprisingly however, polymer surface charge can affect cell viability and biofilm formation. Terada et al. [75] showed that the surface charge is very important not only during initial cell adhesion but also in the long term biofouling formation of E. coli cells onto polymeric surfaces. A positive surface charge resulted in higher cell adhesion but also a lower cell viability of the adsorbed cells. Negative surfaces resulted in less cell adhesion and higher viability. In the latter case the biofilms were heterogeneous and less shear-resistant whilst in the former they were homogeneous and exhibited greater resistance to shear-induced biofilm detachment. In fact it seems that although a positive charged surface compromises the cell integrity providing a high bactericidal effect in a short period, the damaged cells can act as a scaffold to initiate and promote biofilm accumulation. It remains unclear the effect that a less negative NF and RO membrane surface has on cell viability during adhesion.

3.2.3. Membrane Chemical composition

Bacterial attachment for some microorganisms may be correlated to surface chemistry [76]. In an important study by Cunliffe et al. [77] glass surfaces were modified with different and precisely defined functional groups, such as amine and amides of different chain lengths. Hydrophilic
uncharged surfaces showed greater resistance to protein and cell attachment. Adsorption of proteins and *L. monocytogenes* on amine was very high and decreased with decrease of chain length of the amide functional group. However, different results were obtained for other types of bacteria, where for example the hydrophilic acetamide which adsorbed very low amounts of *L. monocytogenes* and *E. coli*, adsorbed high amounts of *S. aureus* and *S. Typhimurium*. This shows that cell properties also play a role in the adsorption onto different surfaces. Polyamide, similar to the active layer of NF/RO membranes, was shown to provide greater adherence of spores than other polymers such as Teflon and polyvinyl chloride (PVC), whilst less *E. coli* adhered in 2 hours compared to PVC [78].

In general, cells adhere more onto NF and RO membranes with an active layer made of polyamide, compared with ones made of cellulose acetate (CA) [30, 79] and CA ultrafiltration (UF) membranes adhere more than polysulphone and polyethersulphone membranes [80]. This can be linked to either the membrane chemical composition or the different roughness and other characteristics of the membranes. In fact, according to a study by Lee et al. [81] some RO membranes with a polyamide active layer gave higher adhesion compared to a CA membrane whilst others gave less adhesion, showing that chemical composition is not the only factor governing bacterial adhesion onto membranes. CA membranes are known to be damaged by hydrolysis from microbial products which is not known to happen with polyamide and polysulphone membranes [3], suggesting that bacteria-CA interactions might differ from bacteria-polyamide ones.

In spite of the numerous reports on the susceptibility to bacterial adhesion of NF and RO membranes, it is still difficult to draw well-defined conclusions on how specific membrane surface properties affects their initial interaction with bacterial cells, taking into account the
disparate nature of membrane characteristics [19, 26]. In fact, membranes and fouled membranes with antagonistic properties, such as hydrophobic smooth membranes against hydrophilic rough membranes have been compared based on their susceptibility to bacterial adhesion [24]. Consequently, relating specific membrane surface properties to cell adhesion and subsequent biofilm formation would be valuable and should be performed based on varying one factor at a time, when comparing membranes, whether it be surface roughness or surface physico-chemical properties.

3.2.4. Roughness

It is generally accepted that surface roughness enhances bacterial adhesion: increased surface area and depressions in the surface are both responsible for enhanced colonization [74]. However, there are conflicting reports in the literature concerning the effects of roughness for NF and RO membranes. Lee et al. [67] found no clear correlation between adhesion and surface roughness. In fact, the rougher the membrane, the less cells adhered to its surface [82]. In contrast, other studies showed that the rougher the membrane is, the greater the number of cells adhered on the surface [19, 26]. However, despite some studies concluding that a correlation exists between roughness and initial adhesion [26, 67], in reality the roughness values for the chosen membranes had a small variation, between 8 and 20 nm [26]. As can be seen in Figure 4, there does not seem to be a correlation between the membrane roughness and the amount of cells adhered on the membrane surface. In fact, in some cases such as *P. aeruginosa* and *P. fluorescens*, adhesion seems to decrease with increase of surface roughness.

Membrane structure seems however to impact on long term biofouling [26]. Poly-piperazine membranes (e.g. NF270) with smooth surfaces revealed layers of cells and EPS stacks, where sparse and hill-like features were found in the cell clusters, whilst polyamide based membranes
revealed evenly distributed live cells with scattered EPS lumps, where dense cell clusters continuously accumulated and became entrapped within the surface crevices due to their dimensional compatibility. In the study by Pang et al. [30] the amount of biovolume growing on the membrane depended on three things: the characteristics of the bacterial cell under investigation, the membrane roughness and the time scale of the experiment. For P. putida, the rougher the membrane, the more biovolume grew for the first 6 days. However, after 8 days, the resultant biofilms on the different membranes were very similar. In comparison, no difference in biovolume was obtained between the different membranes for Sphingomonas sp.

### 3.2.5. Surface morphology and microtopography

Surface morphology, as distinct from roughness encompasses features of the surface that are generally large in scale compared to those of roughness and could include, for examples ridges and depressions in the membrane surface associated with the manufacturing process. This aspect of membrane characteristics has been generally overlooked in the literature, particularly in the context of its role in fouling and biofouling. Subramani and Hoek [19], for example, noted that deposition in NF membranes seems to occur in discrete points caused by microscopic heterogeneities inherent to interfacially polymerized polyamide thin film membranes. These heterogeneities might be associated with the surface defects and ridges that can be seen in NF and RO membranes [83].

When looking at AFM images of NF membranes the differences in roughness vary considerably depending on the scan size [84, 85] and the area where the topography is measured. In some areas, the membrane is very smooth [86] but in others, membrane defects from the manufacturing process show deep ridges and valleys that could accommodate bacteria and protect them from cross-flow, as can be seen in Figure 5 for the NF 270 and the NF 90.
membranes. The NF 90 shows a variation in roughness between 50 and 70 nm for a scan size of 10 µm×10 µm and a variation between 60 and 147 nm for a scan size of 25 µm×25 µm, consistent with the results reported in the literature [87]. The NF270 membrane, which is generally considered a smooth membrane shows a variation in roughness between 6 nm up to 68 nm for a scan size of 10 µm×10 µm and a variation between 14 and 341 nm for a scan size of 25 µm×25 µm. These roughness results can be very different from the ones reported in the literature [65, 87, 88], which are dependent on whether surface heterogeneities and defects are measured using AFM or not: care should therefore be taken when reporting a roughness value for a membrane.

NF and RO membranes have surface properties that vary considerably. It is difficult to pinpoint exactly why certain membranes are more susceptible to bacterial adhesion than others based on their differences in surface roughness and hydrophobicity properties, which may translate into antagonistic effects in bacterial adhesion. More systematic studies with distinct membrane surface properties that allow for a clear comparison between them would add more conclusive results on the impact of the different membrane surface properties in bacterial adhesion and consequent biofilm formation. Furthermore, the duration of the experiment and the bacterial concentration used will impact on adhesion and biofilm formation translating into greater difficulty when comparing results and drawing general patterns.

Moreover, a separate issue is that membrane characteristics may vary with time over long term operation due to necessary cleaning operations [89, 90]. Simon et al. [91] showed that prolonged exposure of an NF membrane to acid and sodium dodecyl sulphate (SDS) rendered the membrane slightly less negatively charged. Caustic and acidic cleaning resulted in a marked increase in the membrane surface hydrophobicity. It is therefore possible that membrane with an
apparently low fouling propensity might undergo surface modification, due to cleaning, that subsequently enhances biofilm formation. Little attention has however been given to the effect of membrane cleaning on subsequent bacterial adhesion and biofilm formation.

3.2.6. Antibacterial membranes

There has been a significant increase in the number of studies describing membranes that have been modified in order to minimize or even prevent biofouling. Different surface modifications or treatment techniques have recently emerged for the fabrication of antibacterial membranes. These modifications include surface polymerization [82, 92], functionalization [93-95], derivatization [96], involving the use of chemicals for altering membrane surface properties. Likewise, the surface modification of spacers used in NF/RO processes have also been targeted for surface functionalization as a means for controlling biofouling [92, 97, 98]. Detailed descriptions of existing surface modification techniques for the creation of antibacterial surfaces are well described in a recent review [99].

Among the different strategies used for functionalizing NF/RO membranes, immobilizing antibacterial enzymes through covalent binding described by Saeki et al. [93] showed sufficient biocidal activity against Gram-positive with lingering bactericidal activity after a storage period for 5 months at 5°C. Although covalently bonded lysozyme enzymes effectively prevented the formation of biofilms, as evidenced by its significant lower flux decline compared to untreated membranes, these membrane still suffered from fouling. Moreover, no indication of the temperature conditions during biofouling experiments were described, which is of key importance, given that enzymatic activity is temperature dependent. Antibacterial membranes involving immobilised enzymes is not on its own a viable solution since the layer of dead cells at the membranes active interface may serve as a buffer zone on which cells might be shielded.
from enzymatic activity. Furthermore, the temperatures used for maximal enzymatic activity (in this case 30°C for lysozyme) would not only favour the growth of surviving adhered cells, but would likely to be of limited feasibility in water treatment plants due to the costs involved.

Alternatively, by grafting a hydantoin derivative (MDMH groups) onto a polyamide RO membrane surface, Wei et al [96] showed that N-halamine groups could be obtained following MDMH chlorination. These novel chlorine resistant membranes not only possessed anti-biofouling properties, but could be regenerated to maintain its antibacterial function following chlorination procedures. This type of novel membrane would in principal be a cost effective alternative, however, more research is needed to understand the potential fouling on this type of membrane in the long run from chorine resistant organisms that could become detrimental in RO processes.

Although antibacterial membranes seem an attractive strategy for partly solving the fouling problem facing NF/RO processes, the ideal functionalized membranes should prevent the settlement of bacteria during NF/RO processes or possess both antibacterial and anti-adhesive properties. One recent study clearly demonstrated the possibility of creating smart polymers possessing two reversibly switchable equilibrium states by coating the surface with a cationic N-dimethyl-2-morpholinone (CB-Ring) and a zwitterionic carboxy betaine (CB-OH ring), to inactivate the incoming bacteria upon contact with the surface, while at the same time preventing their adhesion to the surface [100]. This type of strategy has been successfully implemented for membranes as demonstrated by Bernstein et al. [82] who demonstrated a substantial reduction in bacterial deposition rates by grafting RO-membranes with zwitterionic monomers (molecules carrying both a positive and a negative charges). In a similar study in which NF membranes were fabricated by interfacial polymerization of trimesoyl chloride and diethylenetriamine, Chiang et
al. [92] also showed promising antifouling behavior of both Gram-negative and Gram-positive bacteria, as well as reducing the fouling of humic acid, bovine serum albumin (BSA) and egg-white lysozymes. Interestingly, the interfacial zwitterionization of NF-membranes showed signs of being bactericidal only towards tested Gram-positive bacteria.

Despite promising results, future novel NF/RO membranes combining antibacterial and antifouling properties should aim for inactivation of both Gram positive and Gram negatives as well as other organisms, whilst preventing surface fouling. Some of these approaches have also been tried for biomedical surfaces. However it should be noted that any modification to RO/NF membranes must be able to maintain or improve permeate flux and membrane solute retention capability, as well as withstand the effects of chemical and/or physical cleaning, and convective forces across and through the membrane (i.e. mechanical properties). Special emphasis should be placed on the duration of the experimental runs when testing such novel membranes, since this would avoid any biases and would provide realistic perspectives on the feasibility of implementing such technologies for optimising RO/NF processes.

3.3. Operating/Environmental conditions

3.3.1. Conditioning layers

Every surface, regardless of chemical or physical properties will absorb proteins, polysaccharides and other macromolecules. For example, in the presence of humic acid (HA), bacterial attachment to sand decreased due to competition for attachment sites between the bacteria and HA and due to the HA changing the properties of the sand [101]. In contrast, the presence of HA had a small effect on adhesion of \( E. \ coli \) to silica or glass surfaces [102]. Besides
competition for adhesion sites, an adsorptive layer may provide a metabolically favourable environment for bacterial cells, due for example to enhanced nutrient availability at that surface [103]. However, the presence of this conditioning layer has been overlooked [104], especially in a membrane filtration context. NF and RO membranes are mainly used for the treatment of surface water, groundwater, wastewater effluent and seawater. Table 1 represents the different water characteristics used during NF and RO water treatment from different pilot scale and full scale plants in several different geographical locations. These water sources have different characteristics such as pH, salinity (i.e. conductivity), organic carbon concentration and characteristics, as well as different bacterial strains and bacterial concentrations [30, 105, 106]. As the conditions at the membrane interface are generally different from the bulk fluid, the process conditions create a local microenvironment at the interface thereby influencing the fouling characteristics and the adhesion rates. This is due to the convective flux towards the membrane surface which causes concentration polarization, i.e. a higher concentration of these molecules compared to their concentration in the bulk feed. Furthermore, in most cases, membrane fouling by natural organic matter, polysaccharides and inorganic material will occur, which can change substantially the membrane surface properties such as hydrophobicity [107], roughness [108] and surface charge [109]. Several membrane autopsies carried out on NF and RO membranes have showed the fouling layer to be composed of different materials [110-113], caused by the different water quality treated. The characteristics of the fouling layer can therefore be expected to influence the nature of subsequent bacterial adhesion and possibly biofilm formation.

Subramani et al. [24] studied the adhesion of bacteria onto organic fouled NF and RO membranes. The fouling layer caused the membrane to change in roughness and hydrophilicity
which seemed to have antagonistic effects, and conclusions were difficult to extract. Baek et al. [114] undertook a comprehensive study of biofouling on RO membranes with and without pre-conditioning of the membrane with medium. It was shown that conditioning the membranes changes its surface properties. The concentration of bacteria attached to the conditioned membrane was 2 orders of magnitude higher compared to the non-conditioned membranes, resulting in more severe flux decline. The concentration of polysaccharides was 6 times higher in the preconditioned membranes compared to the non-conditioned ones and did not vary from membrane to membrane. Confocal laser scanning microscopy (CLSM) images showed an active community on the preconditioned membranes whereas a few scattered colonies were found on the non-conditioned membranes. They consisted mostly of live bacterial cells. Semião et al. [115] showed that a conditioning cake layer deposited on the NF membrane surface due to compaction with different grades of laboratory water substantially affected bacterial adhesion on the membrane. It is however unclear from these studies what actually “comes first” and what the synergies are between bacterial adhesion and the conditioning and fouling layer formation on the membrane surface. In the context of composite fouling the key questions relate to what type of fouling develops at the highest rate; does a conditioning layer enhance bacterial adhesion? Are there particular conditioning layer characteristics that enhance or minimise bacterial adhesion? if so, what are they? What is the rate of biofilm formation on a membrane which has already been fouled by organic matter or other type of fouling? Should cleaning strategies focus on removing the biofilm or the non-biological fouling layer or both? In full scale NF/RO plants, membranes suffer different degrees of biofilm formation, possibly linked to the water characteristics [4, 110, 111], and hence linked to the fouling layer that forms on the membranes surface. Moreover, if this fouling layer forms at a higher rate than bacterial adhesion and biofilm formation, it is
critically important to assess the methodologies for quantifying the performance of novel anti-biofouling membranes in the context of full scale operation as opposed to laboratory testing.

In addition to organic material, bacterial cells themselves can act as a primary layer whereby subsequent organisms attach onto pioneer organisms and their excreted EPS [116]. Different membrane materials will have different affinities for different bacterial species translating into different amounts of bacteria adhered [67, 70, 117]. However it is notable that several biofouling studies show that flux reduction and feed pressure drop increase, is generally independent of membrane surface properties [9, 30, 114, 118], and hence possibly independent of differences in the pioneer bacteria. In contrast, a recent study on biofouling in cooling towers showed that the microbial community composition can be greatly affected by the characteristics of initial adhesion of bacterial cells [119]. As with fouling, it is necessary to critically assess the methodologies for assessing the performance of novel anti-biofouling membranes, when the membrane properties will be masked by the pioneer bacteria and the EPS they excrete. It is also important to consider the fact that several studies have shown that the layers closer to the membrane surface consist mainly of dead bacteria [6, 120].

3.3.2. Permeate flux

Subramani and Hoek [19] used a non-invasive technique to study bacterial deposition onto different membranes under filtration conditions. Less deposition was observed for NF membranes compared to the RO ones, for the same initial permeate flux. This was correlated to the fact that RO membranes are rougher, more hydrophobic and suffer more from concentration polarization than NF membranes for the same permeate flux. As expected, under pressure (i.e.
permeate flux) a higher deposition of cells on the membrane surface occurred compared to deposition without permeate flux due to the convective flux towards the membrane. A subsequent study from the same group [121], showed that bacterial deposition on an RO membrane was higher at a permeate velocity of 7.17 µm/s compared to 4.9 µm/s with all other conditions identical.

It is surprising that very few studies have been undertaken on bacterial adhesion and biofilm formation in NF and RO membranes under pressure. In reality, NF and RO membranes operate under pressure, which causes the hydrodynamic conditions at the membrane surface to be markedly different from those in the absence of pressure. Although it is important to study adhesion onto NF and RO membranes under zero-flux conditions, it is crucial to carry out the same studies under pressure conditions in order to compare the results and understand the fundamental mechanisms involved in adhesion and biofilm formation of NF and RO membranes under normal operational conditions. Permeate flux will affect concentration polarization, which in turn will possibly affect bacteria attachment and biofilm formation.

3.3.3. Hydrodynamics and mass transport

In the study by Subramani and Hoek [19] the higher the Reynolds number used in the cross-flow cell, the lower deposition occurred, showing that Brownian deposition is only significant at lower Reynolds numbers.

In studies without permeate flux the initial cell adhesion onto NF and RO membranes is influenced by the different membrane properties [19, 26, 67]. However, these studies were generally undertaken at very low Reynolds numbers (Re<30), not representative of membrane processes, where it would be expected for the membrane surface properties to have a higher
impact on the initial cell adhesion. Furthermore, the only studies found in the literature of bacterial adhesion under permeate flux conditions are the studies from Subramani et al. [19, 24]. It was found that membrane properties affected bacterial adhesion under the studied flux condition. However, these studies were carried out at very low pressure conditions of less than 2.5 bar, when realistically the pressures used for NF and RO membranes in water treatment can go up to at least 17 bar [112]. Another study showed that under the same flux conditions the biofilm formed on the surface of three different RO membranes had similar characteristics. Furthermore, the biofilm affected the membrane performance (i.e. flux decline) to the same extent [114], suggesting little impact of the membrane surface properties. However, no information on the synergy between initial adhesion and biofilm impact on membrane performance was assessed. The question that arises is: would membrane properties still have an impact on initial adhesion and biofilm formation at higher Reynolds numbers and pressures, representative of spiral-wound elements?

In a direct measurement of bacterial deposition rate, Huang et al [121] investigated the effect of the presence of a standard plastic mesh spacer. Bacterial deposition was enhanced directly on the membrane between spacer filaments. A computational fluid dynamics (CFD) analysis showed that the presence of the feed spacer hindered cross flow near the membrane, suggesting the role of feed spacer on creating distinct hydrodynamic conditions that could impact on bacterial deposition rate. Bacteria accumulated more readily on the downstream side of spacer filaments in a stagnation zone. Significantly less deposition was observed on the membrane areas in front of spacer filaments. In the same way, several studies have shown experimentally and through modelling development that the stagnation zones created by the feed spacers, such as behind the spacer filament crossings on NF and RO membrane modules [122, 123], enhance biofilm
formation and the creation of regions of low and high liquid flow velocity [124], also called channelling. Vrouwenvelder et al. [125] obtained a higher pressure drop caused by biomass accumulation with a spacer in the feed channel compared to without one, showing the importance of hydrodynamics on biofilm formation, and possibly on bacteria adhesion. These studies suggest that the presence of a feed spacer may play a role in enhancing biofilm development and consequently new module and/or spacer designs and materials may play an important role in mitigating biofilm development in NF and RO membranes.

4. DISCUSSION
This review is focussed on bacterial adhesion which is generally associated with the earliest stages of biofouling. It is a valid question to ask if this area of research has any significant relevance to practical biofouling control. There are several emerging areas of research that show promise in this regard. For example there is some evidence to suggest that there may be a link between the mechanisms that dictate initial adhesion, as covered in this review, and the role of adhesive failure in the detachment of mature biofilms. The rate of biofilm detachment is dictated by the balance between shear forces and the counteracting adhesive forces and cohesive forces of the biofilm [126]. The link between initial adhesion and biofilm detachment is due to the adhesive bond between the mature biofilm and the surface, where this adhesive force is governed by the same physicochemical forces that cause initial adhesion [127]. A study by Bos et al. [128] concluded that substratum hydrophobicity is a major determinant of bacterial detachment under high shear forces. It is therefore clear that an understanding of bacterial-surface interactions may play an important role in biofouling detachment, and hence control. Pasmore et al. [129] attempted to relate bacterial adhesion characteristics to ease of cleaning, although experiments were not performed under flux conditions. However it was interesting to note that
there was some evidence to suggest a relationship between ease of biofilm removal from surfaces on which initial adhesion rates were poor. Moreover, conditioning layers might not only have a critical role to play in initial adhesion, they may also play a role in biofilm detachment depending on how they mediate the adhesive force between the biofilm and the surface [127].

Another aspect where biofouling control relates to initial adhesion events has been described by Bereschenko et al [27], in which they showed that the production of EPS by Sphingomonas-like bacteria enhanced their adhesion rate onto RO membranes. This in turn leads to a relatively fast spreading of the cells over the membrane and spacer surfaces possibly enhanced by the flow conditions. They might not be the dominant organism in the fouling layer, but their almost unicellular layer and high level of EPS production likely gives them a more substantial contribution to membrane biofouling than aggregate-forming bacteria. This behaviour makes them a potential target for potential biofouling control approaches.

It is generally accepted that operation of NF/RO membranes without biofilm formation is not achievable. Fleming [130] suggested replacing the prevalent concept of “biofilm prevention” with the concept of “biofilm management”. This can be achieved by managing feedwater, operational conditions (flux, hydrodynamics) [13], cleaning strategies [131] and membrane selection. However, the costs associated with membrane cleaning and the costs associated with the increased energy expenditure in NF/RO operations under moderate biofouling necessitate further basic research into fundamental mechanisms governing biofilm development in NF/RO modules [112]. As part of this strategy is the need for a better understanding of bacterial-membrane interactions, an area of research that has not received priority but is nevertheless critical in order to fully understand several important aspects of NF/RO biofouling. These areas include:
(a) further systematic studies on how the full range of membrane surface properties affect bacterial adhesion;

(b) studies on the relationship between initial adhesion and ease of biofilm detachment in order to develop more effective cleaning strategies;

(c) investigations on the relationship between initial adhesion and the properties of subsequent biofilms;

(d) elucidation of the mechanisms involved in composite fouling, more specifically the synergies between biological and non-biological fouling in order to develop more effective cleaning procedures;

(e) development of and understanding of how established cleaning strategies affect the bacteria-membrane interactions and how that relates to consequent biofilm re-development and removal;

(f) further studies on the role of feed-water composition, hydrodynamics and permeate flux on bacterial adhesion and biofilm development under the unique environmental conditions experienced at the NF/RO membrane interfaces, i.e. permeate flux and concentration polarisation;

(g) assessment of new and emerging anti-biofouling membranes under realistic operational conditions covering the time scale from adhesion to mature biofilm development;

(h) development of new membranes that facilitate easier detachment of the biofilm, possibly by reducing the adhesive forces between bacteria and membrane surface;

(i) investigation of the role of the feed spacer on the development of biofilm in membrane modules.
(j) the choice of micro-organisms used when studying the fundamentals of biofouling on RO/NF systems in research laboratories should ideally use micro-organisms isolated from aquatic systems and isolates from membrane autopsies. For years several key studies adding to significant contributions on membrane biofouling in RO/NF systems employed model organisms that have little relevance to water environments, such as *Pseudomonas aeruginosa* [22, 67, 73, 129], a known clinical strain responsible for nosocomial and chronic wound types of infection;

(k) an understanding of the succession and dynamics of surface colonization, which may partly depend on the effects of transient attachment periods and/or competition between species, should be further developed. Siboni et al [132] analyzed the community dynamics in early stage biofilm formation in the marine environment. It was found that some early colonizers disappeared; others appeared later while others were still stable and present throughout the study. Separately, it has been shown in oral biofilms that initial bacterial adhesion is a highly selective process in which initial colonizers first bind to secondary- and late- stage colonizers to form multispecies communities [133, 134]. This has important implications in the development of the microbial community on the NF/RO membranes and highlights the need to understand the processes occurring during initial adhesion;

(l) an in-depth understanding of the bacterial surface components and the mechanisms that regulate their production and activity is needed for a better understanding of membrane biofouling. Bacterial surfaces are heterogeneous, and, importantly the characteristics change dramatically in response to changes in their environment [60].
5. Conclusions

The factors affecting bacterial adhesion on NF and RO membranes have been thoroughly reviewed and the implications for the development of an improved understanding of biofouling have been discussed. Figure 6 summarises schematically many of the key factors influencing bacterial adhesion on filtration membranes discussed in this review. In view of the importance of the biofouling problem to NF/RO operations there is a clear need to develop a mechanistic understanding of the biofouling development process. This review has highlighted, in particular, aspects of initial bacterial adhesion on biofouling development and has elucidated areas of research that require further investigation. These new areas of investigation will be facilitated by new experimental approaches, analytical techniques and insights including, but not limited to, in-situ visualisation of biofilm development under flux conditions [24], advanced simulation approaches [124], force spectroscopy [135], analysis of the role of Transparent Exopolymer Particles (TEP) [136] and investigation of biological methods for biofilm prevention and control [137]

Acknowledgments

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**Figure 1** Schematic outline of nanofiltration and reverse osmosis process operation, including fouling components and salts, the direction of cross-flow and permeate flow, the concentration polarisation effect and the presence of microbes.
Figure 2 Number of adhered bacterial cells (cells/cm$^2$) onto NF and RO membranes as a function of the membrane contact angle; adapted from [19, 26, 67, 82, 138] where WW are wastewater bacteria.
Figure 3 Number of adhered bacterial cells (cells/cm²) onto NF and RO membranes as a function of the membrane zeta potential measured between pH 6 to 7; adapted from [19, 26, 67, 82, 138]
Figure 4 Number of adhered bacterial cells (cells/cm²) onto NF and RO membranes as a function of the membrane surface roughness RMS; adapted from [19, 26, 67, 82]
Figure 5 AFM images of the NF 90 and the NF270 membranes (Dow Filmtec) obtained in contact mode with a MPP-31123-10 cantilever and a CPII Nano (Veeco, now Bruker, UK)
Figure 6. Hypothesized mechanisms of initial adhesion between cells and membrane during NF/RO filtration processes. (A) As feed water passes though the membrane, divalent cations, organic matter as well as microorganisms are concentrated onto the membrane surface during NF/RO filtration processes which involves permeation flux at high pressures. During the early stages of filtration, salt concentration at the surface of the membrane is increased by concentration polarization, which in turn increases the osmotic pressure of the feed thereby reducing the water flux. As filtration is upheld, a rapid and gradual flux decline arises from the build-up of inorganic and organic elements and thriving microorganisms, covering the entire membrane surface coated in a thick fouled layer. (B) Membrane material properties are relevant
to the initial interaction between bacterial cell and the surface of the membrane. Membrane roughness enhances bacterial adhesion through its increased surface area by favouring the likelihood of initial contact but most importantly, by protecting adhered cells from detachment. The physicochemical properties of the membrane are known to influence bacterial initial adhesion. Properties such as low electronegative surface charge and high surface hydrophobicity have been shown to be correlated to high bacterial adhesion although this cannot be generalised, since the physicochemical properties of the microorganisms can also influence adhesion. (C) The bacterial cell wall properties can influence bacterial adhesion by the presence of an enveloping polysaccharide capsule, whose chemical attributes, may enhance irreversible adhesion. Once attached the capsule producing bacteria may also recruit other “late-stage” colonizers onto the membrane surface. Specific adhesion between bacterial cells and the surface of the membrane through adhesins, cell-surface components of bacterial cell wall, can occur in the event of the recurrence of irreversibly bound organic or inorganic elements on the surface of the membrane. (D) Environmental factors such as temperature, pH, salt concentration, the presence of signal molecules are known to induce a number of different mechanisms at the cell level that might induce adhesion. For example high salt concentration is known for reducing both cell and membrane electric double layer leading to cell-cell aggregation and increased adhesion with the inert surface. The presence of elements such as inorganic phosphates, are also known to trigger a cascade of intracellular molecular reactions, allowing the cell to adhere to inert surfaces.
Table 1 Water quality (surface water, groundwater, wastewater and seawater) used in full scale or pilot scale NF and RO plants

<table>
<thead>
<tr>
<th>Water Source</th>
<th>pH</th>
<th>TDS (mg/L)</th>
<th>Conductivity (µS/cm)</th>
<th>DOC/TOC/COD (mgC/L)</th>
<th>ATP (ng/L)</th>
<th>AOC (µg AOC C/L)</th>
<th>Total coliforms/bacteria/algae</th>
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<td>-</td>
<td>1-10 (TOC)</td>
<td>4-370</td>
<td>4-90</td>
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<td>France [139]</td>
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<td>-</td>
<td>4.65 (DOC)</td>
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<td>7.7-10 (DOC)</td>
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<td>TOC Unit</td>
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<td>15-66 (algae cells/L)</td>
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<td>2-255 (algae/mL)</td>
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