

Bacterial adhesion onto nanofiltration and reverse osmosis membranes: effect of permeate flux

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Abstract

The influence of permeate flux on bacterial adhesion to NF and RO membranes was examined using two model *Pseudomonas* species, namely *Pseudomonas fluorescens* and *Pseudomonas putida*. To better understand the initial biofouling profile during NF/RO processes, deposition experiments were conducted in cross flow under permeate flux varying from 0.5 up to 120 L/h.m², using six NF and RO membranes each having different surface properties. All experiments were performed at a Reynolds number of 579. Complementary adhesion experiments were performed using *Pseudomonas* cells grown to early-, mid- and late-exponential growth phases to evaluate the effect of bacterial cell surface properties during cell adhesion under permeate flux conditions. Results from this study show that initial bacterial adhesion is strongly dependent on the permeate flux conditions, where increased adhesion was obtained with increased permeate flux, until a maximum of 40% coverage was reached. Membrane surface properties or bacterial growth stages was further found to have little impact on bacterial adhesion to NF and RO membrane surfaces under the conditions tested. These results emphasise the importance of conducting adhesion and biofouling experiments under realistic permeate flux conditions, and raises the questions about the efficacy of the methods for the evaluation of antifouling membranes in which bacterial adhesion is commonly assessed under zero-flux or low flux conditions, unrepresentative of full-scale NF/RO processes.

1. Introduction

Nanofiltration (NF) and Reverse Osmosis (RO) are well-established processes for the production of high quality water. NF is principally used for the removal of hardness, trace contaminants, such as pesticides and organic matter (Cyna et al. (2002)), while RO is used for desalination (Greenlee et al. (2009)). NF and RO performance are however adversely affected by biofilm formation resulting in permeate flux and quality decline (Flemming (1997), Houari et al. (2009), Ivnitsky et al. (2007), Khan et al. (2013), Vrouwenvelder et al. (1998), Vrouwenvelder et al. (2008)), generally caused by the initial adhesion and subsequent colonization of bacterial cells on the surface of the membrane, amalgamating in a biomass consisting of, and not limited to, polysaccharides, proteins, and extracellular DNA (Pamp et al. (2007)).

The first stage of biofilm formation is initiated by the adhesion of bacteria to the membrane surface, a precursor of biofilm formation (Costerton et al. (1995)). Previous studies have shown that NF and RO membrane properties (Bernstein et al. (2011), Lee et al. (2010), Myint et al. (2010)), bacterial properties (Bakker et al. (2004), Bayoudh et al. (2006), Mukherjee et al. (2012)) and environmental conditions affect bacterial adhesion (Sadr Ghayeni et al. (1998)). However, most of these studies were conducted without permeate flux, which is an inherent part of NF and RO processes. The hydrodynamic and concentration polarisation effects associated with flux may alter the micro-environmental conditions at the interface thereby playing an important role in the characteristics and rate of bacterial adhesion. A recent study showed that under the same flux conditions, the biofilm formed on the surface of three different RO membranes had similar characteristics and affected the membrane performance to the same extent (Baek et al. (2011)): the percentage flux decline was identical for all the membranes studied. In a previous study (Suwarno et al. (2012)) it was shown that higher permeate flux resulted in increased biovolume on the membrane surface. Although previous studies suggest biofilm formation is independent of membrane surface properties but dependent on pressure, no systematic studies to date have

attempted to investigate the relationship between initial adhesion and membrane properties at different flux conditions.

Surprisingly, few studies have focused on bacterial deposition under permeate flux conditions (Eshed et al. (2008), Kang et al. (2004), Kang et al. (2006), Subramani and Hoek (2008), Subramani et al. (2009)). These studies focussed on developing an understanding of the fundamental mechanisms of bacterial attachment under permeate flux conditions, often combined with the DLVO or XDLVO theory. The only studies where bacterial deposition specifically to NF and RO membranes under permeate flux conditions were reported, are those from Subramani *et al.* (Subramani and Hoek (2008), Subramani et al. (2009)) where it was found that bacterial adhesion was influenced by membrane properties. However, these studies were conducted at comparatively low fluxes, of less than 20 L/h.m^2 (equivalent to 2.5 bar). In full-scale NF and RO processes for water, seawater and brackish water, treatment fluxes can reach up to 70 L/h.m^2 (Cyna et al. (2002), Greenlee et al. (2009), Houari et al. (2009), Ventresque et al. (2000)). One of the conclusions (Subramani and Hoek (2008)) was that according to the DLVO theory, permeation drag overwhelms interfacial forces at fluxes greater than 20 L/h.m^2 . However, to our knowledge, there are no reports in the literature concerning bacterial adhesion at fluxes greater than 20 L/h.m^2 for NF/RO membranes or at Reynolds numbers representative of spiral wound elements in full-scale plants where values range between 150 and 2000 (Schock and Miquel (1987)).

Furthermore conflicting results can be found in the literature. One study showed adhesion rates onto MF membranes subjected to permeate fluxes $\sim 70 \text{ L/h.m}^2$ to be considerably different between membranes with different surface properties (Kang et al. (2006)). In contrast, another study (Subramani and Hoek (2008)) observed a decrease in the differences of adhesion rates as one increased the permeate flux through several NF and RO membranes from no permeate flux up to $\sim 20 \text{ L/h.m}^2$. A clear gap in the knowledge of bacterial adhesion to NF and RO membranes was therefore identified, where the mechanisms of adhesion under common cross-flow and pressure

filtration conditions for different commercially available NF and RO membranes needed to be clarified.

This paper therefore investigates the initial adhesion of two bacterial strains, *Pseudomonas fluorescens* and *Pseudomonas putida*, to 6 different NF and RO membranes under permeate flux conditions, as well as the adhesion of *P. fluorescens* at different growth stages.

2. Materials and Methods

2.1 Model Bacteria Strains and Media

The selected model bacterial strains for this study were fluorescent mCherry-expressing *Pseudomonas fluorescens* PCL1701 (Lagendijk et al. (2010)) and *Pseudomonas putida* PCL1480 (Lagendijk et al. (2010)). *Pseudomonas* strains were stored at -80°C in King B broth (King et al. (1954)) supplemented with 20% glycerol. Cultures of both *Pseudomonas fluorescens* and *Pseudomonas putida* were obtained by inoculating 100 mL King B broth supplemented with gentamicin at a final concentration of 10 µg mL⁻¹ using respective single colonies previously grown on King B agar (Sigma Aldrich, Ireland) at 28°C. Subsequently, cultures were incubated at 28°C with shaking at 75 rpm and left to grow to early exponential, mid exponential or late exponential growth stages, corresponding to Optical Densities (OD₆₀₀) of 0.2, 0.6 and 1.0, respectively, for the study of the impact of bacteria growth stage on adhesion to NF and RO membranes. The experiments for the study of the impact of flux on the adhesion of bacteria *P. fluorescens* and *P. putida* to different NF and RO membranes were performed using cells in their late exponential growth stage (OD₆₀₀=1.0).

2.2 Microbial Adhesion to Solvents

Microbial adhesion to solvents (MATS) (BellonFontaine et al. (1996)) was used as a method to determine the hydrophobic and Lewis acid–base surface properties of *P. fluorescens* cells at

different growth stages. 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. Hexadecane (nonpolar solvent), chloroform (an electron acceptor solvent), decane (nonpolar solvent) and ethyl acetate (an electron donor solvent) were used of the highest purity grade (Sigma-Aldrich, USA). Experimentally, overnight bacterial cultures grown at different stages (early, mid and late exponential phase) were washed twice in sterile 0.1 M NaCl solution as previously described and re-suspended to a final OD₄₀₀ of 0.8. Individual bacterial suspensions (2.4 ml) were vortexed for 60 seconds with 0.4 ml of their respective MATS solvent. The mixture was allowed to stand for 15 min to insure complete separation of phases. One mL from the aqueous phase was then removed using glass Pasteur pipettes and the final OD₄₀₀ was measured. The percentage of cells residing in the solvent was calculated by the following equation:

$$\%Adherence = \frac{(OD_i - OD_f)}{OD_i} \times 100$$

where (OD_i) is the initial optical density of the bacterial suspension before mixing with the solvent, and (OD_f) the final absorbance after mixing and phase separation. Each measurement was performed in triplicate.

2.3 Cell preparation for adhesion assay

To evaluate bacterial adhesion under different flux conditions, cell concentration for each growth stage (i.e. early exponential, mid exponential or late exponential growth stages) was standardized by diluting the growth cultures to a final OD₆₀₀ of 0.2 in 200 mL 0.1 M NaCl (Sigma-Aldrich, Ireland). This ensured a standardized starting feed cell concentration before every adhesion assay, in which controlled experiments with different parameters (i.e. permeate flux and growth stage) could be

compared and studied. For cells grown to early exponential phase two 100 mL cultures were prepared.

Cells were then harvested by centrifugation at 5000 rpm for 10 min using a Sorval RC5C Plus centrifuge (Unitech, Ireland) and a Fiberlite™ f10-6x500y fixed angle rotor (Thermo Fisher Scientific Inc., Dublin, Ireland). The supernatant was carefully discarded and the pellet re-suspended in 200 mL 0.1 M NaCl solution, resulting in an inoculum consisting of approximately 10^8 cells/mL. This process was performed twice. A solution of 0.1 M NaCl was used as a model solution to mimic brackish water characteristics (Greenlee et al. (2009)).

2.4 Membranes and Cross-flow Test Unit

Six NF and RO membranes were used: NF90, NF270, BW30 and BW30 FR (Dow Filmtec Corp, USA) and ESNA1-LF and ESNA1-LF2 from Hydranautics (Nitto Denko Corp, USA). BW30 FR stands for Fouling Resistant membrane. The membrane properties are presented in Table 1

Table 1 Membrane Properties

	Permeability (L/h.m ² .bar) ^a	NaCl Retention ^b (%)	Contact Angle ^c (°)	Roughness R _{MS} ^d (nm)
NF90	6.8±0.5	87.8±4.0	58.4±0.6	484.0 ± 207.1
NF270	12.6±1.2	16.0±0.3	8.4±0.5	372.9 ± 246.4
BW30	2.6±0.3	93.5±2.1	25.6±0.8	209.0 ± 41.9
BW30 FR	2.8±0.5	92.9±1.3	62.2±0.6	665.7 ± 156.9
ESNA 1- LF	3.5±0.4	88.8±1.5	68.8±0.6	214.5 ± 23.4
ESNA1 – LF2	6.8±0.8	75.2±0.2	62.4±0.7	661.3 ± 97.7

^a Permeability measured with MilliQ water at 21°C

^b 0.1 M NaCl at 15 bar, 21°C and Re=579

^c Mean contact angle of a total of 20 deionized water droplets on two independent membrane samples using a goniometer (OCA 20 from Dataphysics Instruments)

^d 45×59 μm of area measured using a Wyko NT1100 optical profilometer operating in vertical scanning interferometry (VSI) mode

As can be seen from Table 1 membrane surface properties varied substantially, with contact angles, membrane surface roughness, and salt retention parameters ranging from 8.5° to 68.8°, 214.5 up to 665.7 nm and 16.0 to 93.5%, respectively. These results clearly show the variability in surface hydrophobicity as well as topographic profile of the selected membranes.

The cross-flow test unit used was a modified version of the unit found in a previous study (Semião et al. (2013)) and the schematic and operational details can be found in the Supporting Information SI. No feed spacers were used in this study.

2.5 Cleaning Protocol

The protocol used to clean the cross-flow system consisted of two antibacterial treatments involving 30 min recirculation steps of 70% Industrial Methylated Spirit (IMS, Lennox, Dublin, Ireland), followed by 0.1 M NaOH. The system was rinsed in between treatments with 18.2 mΩ.cm⁻¹ grade 1 pure water (Elgastat B124, Veolia, Ireland). Since pure water is ineffective in completely removing NaOH, an added step of recirculating pure water with a pH adjusted to 7 using 5 M HCl and a buffer solution of 10 mM NaHCO₃ was adopted. The pH of the recirculating solution was systematically checked to ensure there was no vestige of NaOH in the system. The system was then thoroughly rinsed with pure water. No adhesion of fluorescent cells on a membrane compacted for 18 hours with pure water occurred, showing the efficiency of the washing method.

2.6 Adhesion Protocol

Three different membranes were cut, thoroughly rinsed with pure water and left soaking overnight in the fridge at 4°C. The membranes were then inserted in the cross-flow system and compacted for a minimum of 18 hours at 21°C with pure water. The membrane pure water flux was measured at 15 bar and at the pressure subsequently used during the adhesion experiment. The cross-flow system was operated in total recirculation mode (i.e. recirculation of the retentate and permeate), ensuring the feed concentration and volume during the experimental runs were constant.

A 4 L volume of 0.1 M NaCl solution was then inserted in each feed tank (tank 1 and tank 2) and recirculated in the system to remove any air bubbles. Then feed tank 2 was blocked with the ball valve system and only feed tank 1 was used. Prior to inserting the bacterial cells in feed tank 1, the cross-flow system was left to equilibrate at a constant selected pressure and cross-flow of $0.66 \text{ L} \cdot \text{min}^{-1}$ ($Re=579$) for 15 minutes with the 0.1 M NaCl solution in tank 1. Selected experimental conditions consisted of monitoring bacterial adhesion at pressures ranging from 3.1 to 15.5 bar, with corresponding membrane fluxes ranging up to $70 \text{ L} \cdot \text{h} \cdot \text{m}^{-2}$ at a constant temperature of 21°C. This range of fluxes was chosen to ensure coverage of the range used in typical full-scale applications of NF and RO processes (Cyna et al. (2002), Greenlee et al. (2009), Houari et al. (2009), Ventresque et al. (2000)). In the specific case of the NF 270 membrane this range was extended to $120 \text{ L} \cdot \text{h} \cdot \text{m}^{-2}$ purely for scientific reasons, for example in the case where novel membranes can operate at higher fluxes than the ones commonly applied in today's water treatment plants. A bacterial inoculum containing approximately 10^8 cells/mL was then added to feed tank 1 and recirculated in the system for 30 minutes at the constant filtration conditions of pressure and cross-flow as the ones used during equilibration. Permeate flux, feed and permeate conductivity were measured for each membrane cell before (i.e. during equilibration with 0.1M NaCl) and after bacterial inoculation (i.e. during bacterial adhesion). After 30 minutes of adhesion, feed tank 2 outlet with 0.1 M NaCl solution was opened and feed tank 1 outlet was closed in order to rinse any non-adhered bacterial cells from

the system under the filtration conditions used prior to *ex-situ* analysis of the bacterial adhesion. Every experiment was repeated at least twice. The effect of rinsing and the effect of opening the MFS for *ex-situ* analysis of bacterial surface coverage was investigated by comparison with a control study performed with an MFS fitted with a sapphire glass window for *in-situ* measurements. The results of these control studies are described in the Supplemental Information (S2).

2.7 Adhesion quantification

Membrane Fouling Simulator (MFS) cells were separated from the system at the end of adhesion experiments, and carefully opened whilst submerged in 0.1 M NaCl solution. The fouled membranes were removed, 3 pieces cut from different locations of the membrane and each sample was placed at the bottom of small petri dishes submerged with 0.1 M NaCl solution. The submerged fouled membranes were then observed under an epi-fluorescence microscope (Olympus BX51) using a 10X objective. Fluorescent mCherry-tagged *Pseudomonas* cells were observed using a 550 nm filter cube. Ten micrographs were obtained at random points from each membrane sample. Cell surface coverage (%) was then determined for each membrane using ImageJ® software, a Java-based image processing program (<http://rsbweb.nih.gov/ij/>). The emission intensity of the mCherry tagged *Pseudomonas* cells was found to be perfectly distinguishable from the autofluorescent background of the tested membranes. In some instances, the mCherry to background fluorescence signal was further improved by controlling the level of excitation light through samples using fluorescence excitation balancers, attached in parallel to the light path, and by adjusting the field iris diaphragm (Supporting information: S4). Acquired images were subsequently grayscaled and thresholded. Bacterial deposition on membranes was then estimated as the percentage of solid surface covered by bacteria, based on the number of black and white pixels of thresholded images.

3. Results and Discussion

3.1 Effect of flux on *Pseudomonas fluorescens* adhesion

The effect of permeate flux on the initial adhesion of *P. fluorescens* for different NF and RO membranes is presented in Figure 1. The surface coverage of all 6 membranes was found to increase from $1.6 \pm 0.2\%$ for a permeate flux of $0.5 \pm 0.1 \text{ L/h.m}^2$ ($0.14 \text{ }\mu\text{m.s}^{-1}$) for the BW30 FR up to $39.4 \pm 3.3\%$ for a permeate flux of $35.47 \pm 0.01 \text{ L/h.m}^2$ ($9.9 \text{ }\mu\text{m.s}^{-1}$) for the ESNA 1-LF2. The range of permeate fluxes was extended for the particular case of the NF270 membrane, as stated in the Materials and Methods section. It was found that an increase of the permeate flux from 35.47 L/h.m^2 to 116 L/h.m^2 did not significantly increase the surface coverage which was constant at around 40%. Similarly, a previous study involving yeast on microfiltration membranes also correlated increased cell deposition with increased permeate flux (Kang et al. (2004)). Nonetheless, this present study shows that bacterial adhesion reached a maximum surface coverage of around 40% for permeate fluxes higher than 36 L/h.m^2 as shown for membranes NF270 and ESNA1-LF2. Ridgway et al. (Ridgway et al. (1984)) also observed a similar plateau of adhered bacteria to a RO membrane. The authors explained the adhesion plateau effect to be the direct result of a limiting number of adhesion sites available, independent of the increased bacterial concentration during the course of the fouling experiment. Differences between a “nearly linear” adhesion (Kang et al. (2004)) with increased permeate flux and an adhesion that reaches a plateau as observed in this study, could be explained by the differences in cell feed concentration. As shown in an earlier study (Kang et al. (2004)), differences in cell feed concentration led to significant differences in the amount of bacteria adhered when subjected to identical filtration conditions; the degree of membrane fouling on a membrane will be directly proportional to the bacterial concentration used, where the lower the bacterial concentration, the lower the number of adhered bacterial cells.

NF and RO membranes have been shown to vary substantially in their surface properties. For example, surface contact angle have been previously reported to range between 38.6° and 73.2° , the root mean square (RMS) roughness to range between 5.9 and 130 nm and the zeta potential

measurement to range between -4.0 and -19.7 mV for several commercial NF and RO membranes (Norberg et al. (2007)). Moreover, previous studies investigating bacterial adhesion onto NF and RO membranes clearly demonstrate the role of membrane surface properties on bacterial adhesion, in which attributes such as membrane hydrophobicity, surface charge and roughness have shown to significantly influence bacterial adhesion (Bernstein et al. (2011), Kang et al. (2006), Lee et al. (2010), Myint et al. (2010), Subramani and Hoek (2008)). The quantitative differences in adhesion between the studied membranes were large, with bacteria adhering to some membranes up to 21 times more than others. However, as previously mentioned, these studies were carried out under the absence of or under very low pressure conditions (<2.5 bar), and/or at very low Reynolds numbers ($Re < 80$). One of the objectives of this study was to investigate bacterial adhesion using realistic hydrodynamic conditions in order to mimic NF and RO spiral-wound modules. It was observed that NF and RO membrane surface properties had a small effect on bacterial adhesion under the wide range of permeate flux conditions tested. The highest significant differences were obtained in the region of permeate fluxes of 20 L/h.m^2 , where surface coverage varied from $17.1 \pm 2.8\%$ for the NF270 with a flux of $19.0 \pm 1.3 \text{ L/h.m}^2$ up to $32.5 \pm 0.7\%$ for the ESNA1-LF with a flux of $18.8 \pm 0.1 \text{ L/h.m}^2$. This translates to the ESNA1-LF adhering only 1.8 times more than the NF270, which comparatively to the previous mentioned studies (Kang et al. (2004), Lee et al. (2010), Suwarno et al. (2012)) is a small difference. The small differences obtained in surface coverage for these two membranes is probably due to the fact that the NF270 membrane is more hydrophilic with a contact angle of 8.4° compared to the ESNA1-LF which has a more hydrophobic nature, with a contact angle of 68.8° , as can be seen in Table 1. Hence the more hydrophobic membrane ESNA1-LF shows greater adhesion compared to the more hydrophilic membrane NF270.

When comparing the other membranes for a permeate flux in the region of 20 L/h.m^2 , it can be seen from Figure 1 that surface coverage does not vary substantially: BW30 FR with a flux of $21.2 \pm 5.3 \text{ L/h.m}^2$ has a surface coverage of $27.6 \pm 5.9\%$, the BW30 with a flux of $21.3 \pm 0.3 \text{ L/h.m}^2$ has a surface coverage of $28.5 \pm 1.3\%$ and the ESNA1-LF2 with a flux of $18.1 \pm 3.5 \text{ L/h.m}^2$ has a surface coverage of

29.6±0.2%. The properties of the membranes tested are however very different, as can be seen in Table 1: the contact angle measurements varied from 25.6° for the BW30 to 62.4° for the ESNA1-LF2 and the roughness varied from 209 nm for the BW30 to 665.7 nm for the BW30-FR. Despite the significant differences of the membrane surface properties surface coverage did not vary substantially for the same permeate flux conditions, showing that under pressure membrane surface properties have a small effect on *P. fluorescens* adhesion (Figure S3.1 in the Supporting Information). This suggests that membranes with anti-bacterial or anti-biofouling properties should be tested under representative pressures in order to fully assess their true performance. In contrast, adhesion rates onto microfiltration membranes subjected to a permeate flux similar to the ones tested in the present paper (20 $\mu\text{m.s}^{-1}$) were considerably different depending on the membrane surface properties (Kang et al. (2006)). These differences might be due to the tested species characteristics, to different filtration conditions or to solution characteristics.

It was further noticed that the 30 min adhesion of bacterial cells to the membrane surface did not cause a decrease in the measured permeate flux as this did not vary by more than 3% compared to the flux measured before the introduction of bacterial cells into the system (i.e. during equilibration with 0.1 M NaCl). Despite the adhesion of bacterial cells to the membrane surface covering up to 40% of the surface, this did not cause enhanced concentration polarisation that has been identified in previous studies in the case of cake and biofilm formation (Herzberg and Elimelech (2007), Hoek and Elimelech (2003)).

Two main conclusions can be drawn from this study at the experimental conditions studied: (1) *P. fluorescens* adhesion is dependent on the permeate flux and does not substantially vary for different membrane properties; (2) *P. fluorescens* adhesion reached a maximum of surface coverage of 40% for permeate flux higher than 35.5 $\text{L.m}^{-2}.\text{h}^{-1}$.

3.2 Effect of flux on *Pseudomonas putida* adhesion

P. putida was employed as an alternative species in a similar series of experiments to those conducted with *P. fluorescens*. The results shown in Figure 2 can be seen to follow the same trend as observed with *P. fluorescens* with surface coverage increasing with permeate flux. It is clear that the membrane surface properties do not have a substantial impact on the rate of bacterial adhesion for the conditions tested. For a flux of 13.8 ± 0.9 L/h.m² NF90 has a surface coverage of $15.5 \pm 0.9\%$, the BW30 FR with a flux of 19.6 ± 1.7 L/h.m² has a surface coverage of $16.9 \pm 3.0\%$ and the NF270 with a flux of 19.0 ± 0.3 L/h.m² has a surface coverage of $15.0 \pm 1.2\%$. The properties of the surfaces of the membranes tested are however very different with respect to contact angle and roughness, as can be seen in Table 1, showing that as for *P. fluorescens*, membrane surface properties have an insubstantial effect on *P. putida* adhesion under permeate flux conditions.

The only difference noticed between the two bacterial species tested, *P. fluorescens* and *P. putida*, was in the rate of adhesion as a function of the permeate flux: *P. fluorescens* reaches a maximum coverage of about 40% at a permeate flux between 40 and 60 L/h.m². *P. putida* in comparison only reaches a surface coverage of >40% for permeate fluxes higher than 100 L/h.m². These differences could be associated to outer membrane heterogeneities between *P. fluorescens* and *P. putida* (Bodilis and Barray (2006), Bodilis et al. (2004), Vermeiren et al. (1999), Williams and Fletcher (1996)).

The study by Subramani and Hoek (Subramani and Hoek (2008)) showed that during filtration at low pressures, the difference in adhesion rates between species studied was significant, but as the pressure increased, corresponding to fluxes up to 20 L/h.m², the difference in adhesion rates between species diminished resulting in similar adhesion rates at higher pressures/permeate fluxes regardless of species studied. Furthermore, the same study (Subramani and Hoek (2008)) showed that the differences in adhesion rate of *Saccharomyces cerevisiae* on different tested membranes became smaller with increasing permeate flux conditions, hence showing an overwhelming effect of

the convective flux compared to membrane surface properties. Although this present study differs from the previous studies by focusing primarily on “end-points” following 30 minutes adhesion, a common conclusion can be drawn in which higher permeate flux will lead to higher bacterial surface coverage but membrane and cell surface properties have very little impact on the surface coverage. The design of this present study therefore allowed a comparison of multiple membranes at different flux conditions in regards to bacterial adhesion, which was especially necessary when evaluating the claimed anti-fouling properties of specialized commercial membranes.

3.3 Effect of bacterial growth stage deposition under flux conditions

During bacterial adhesion the outer cell membrane is usually the first point of contact when interacting with abiotic surfaces. The bacterial outer membrane functions as a permeability barrier regulating the passage of solutes between the cell and the surrounding environment, determining the physicochemical properties of the cell (Caroff and Karibian (2003), Gargiulo et al. (2007), Makin and Beveridge (1996)). Surface macromolecules such as lipopolysacchides and surface proteins that constitute the outer membrane have been shown to significantly influence the physicochemical properties of bacterial cells (van Loosdrecht et al. (1987)). Moreover, the composition of macromolecules on the outer membrane is known to be influenced by the bacterial growth phase (Hong and Brown (2006)). In one recent study (Walker et al. (2005)) it was shown that the adhesion profile of *Escherichia coli* was dependent on its growth phase, which was determined by the charge distribution resulting from electrostatic repulsion forces. Differences in biofouling of RO membranes have also been showed to depend on the growth stage of the bacterial species studied (Herzberg et al. (2009)). Differences were caused by the bacterial cell properties such as zeta potential. It is however unclear how the growth stage impacts on the initial adhesion of bacteria onto NF and RO membranes at high flux conditions. Hence the initial biofouling onto different NF and RO membranes was investigated in the present study at a fixed but representative pressure (11.3 bar)

using bacteria at different growth phases to determine whether the effect of cell surface physico-chemistry was significant. The physicochemical surface properties of *P. fluorescens* cells grown at different exponential growth stages based on their affinities to different polar and apolar solvents were studied and are presented in Table 2. Considerable variations in the affinity of *P. fluorescens* cells to apolar solvents hexadecane and decane revealed changes in surface hydrophobicities as cells enter into different exponential growth stages. Affinity to hexadecane decreased from 67.2 % to 27.0%, as cells enter early exponential ($OD_{600}=0.2$) to late exponential ($OD_{600}=1.0$) growth stages. Likewise affinities to decane decreased from 47.6% to 28.9%.

A high affinity to chloroform (>94%) was observed for all tested *P. fluorescens* cells, irrespective of their growth stage. The high affinity to chloroform compared to affinities to hexadecane is an indication that the tested *P. fluorescens* cells possess a dominating electron donor character. Although lower, the affinities to ethyl acetate were on average $\approx 50\%$, irrespective of *P. fluorescens* growth state. When comparing affinities to decane and ethyl acetate, *P. fluorescens* cells grown to mid exponential ($OD_{600}=0.6$) and to late exponential phases ($OD_{600}=1.0$) possess a secondary electron acceptor character, based on their higher affinity to ethyl acetate than decane. This Lewis acid surface property is negligible for *P. fluorescens* cells entering early exponential growth stage ($OD_{600}=0.2$) as seen by their similar affinities to both decane and ethyl acetate. These results clearly indicate the subtle surface physicochemical differences between *P. fluorescens* grown at different exponential stages. Surface hydrophobicity has been shown to affect cell adhesion to surfaces (Bos et al. (1999), Habimana et al. (2007), Vanloosdrecht et al. (1987)).

Table 2: Mean affinities of *P. fluorescens* at different growth stages to solvents hexadecane, chloroform, decane, and ethyl acetate. Error represents standard deviation of three replicates.

Growth stage OD ₆₀₀	Solvents			
	Hexadecane	Chloroform	Decane	Ethyl Acetate
0.2	67.2 ± 0.6	96.0 ± 0.2	47.6 ± 0.5	44.6 ± 5.0
0.6	41.4 ± 7.4	94.4 ± 0.9	24.1 ± 2.3	53.7 ± 3.3
1	27.0 ± 1.1	94.4 ± 1.2	28.9 ± 0.8	52.8 ± 1.1

In the particular case of *P. fluorescens*, there is no significant effect of the growth stage on the adhesion onto different NF and RO membranes, as shown in Figure 3. It seems that the convective flux towards the membrane surface overcomes the effect of the membrane surface properties, as suggested in a previous study (Subramani and Hoek (2008)).

The work presented in this paper clearly shows that for Reynolds numbers and permeate fluxes representative of full-scale spiral wound modules, membrane properties or bacterial growth phases do not substantially affect the rates of initial bacterial adhesion to NF and RO membranes. This has very important implications, particularly for studies where anti-biofouling membranes are under evaluation: the true efficiency of these membranes can only be fully evaluated when tested under realistic permeate flux conditions. Furthermore, membranes labelled as Fouling Resistant such as the BW30 FR have been shown to have the same bacterial adhesion outcome as the other membranes when subjected to typical flux conditions of NF and RO membranes: the surface modifications carried out on this membrane were not sufficient to avoid bacterial adhesion and consequently biofouling. The convective flux has been found to overcome any short range interactions that occur between a polymeric membrane and the bacteria surface. This poses an important question: will an efficient anti-biofouling membrane ever be developed? Should future research focus on anti-adhesion surfaces or should it focus on more efficient cleaning strategies?

Several studies have shown that bacteria adhere on the membrane surface and form a biofilm, with the first layers constituting of dead bacteria (Herzberg and Elimelech (2007), Khan et al. (2011)). The reason why they die is still unclear. Although the bacteria viability of first layer is affected, these

dead bacteria could be used as a surface for further adhesion by other bacteria. A possible answer might therefore be in developing surfaces that allow for an easy detachment of the biofilm during cleaning steps.

4. CONCLUSION

This study offers an increased understanding of bacterial adhesion on NF/RO membranes under conditions typically found on full-scale processes. Under the permeate flux and hydrodynamic conditions tested, the effect of membrane properties and bacterial cell surface characteristics were found to play an insignificant role compared to permeate flux in controlling bacterial adhesion rate. It is hoped that these findings will lead to an enhanced understanding of the effect of permeate flux on the development of microbial population development in the early stage of NF/RO biofouling. Future work will also need to examine biological factors involved during the early stage of membrane fouling such as EPS synthesis. An understanding these factors would help better devise or select optimal processing strategies for controlling the level of fouling during NF/RO processes.

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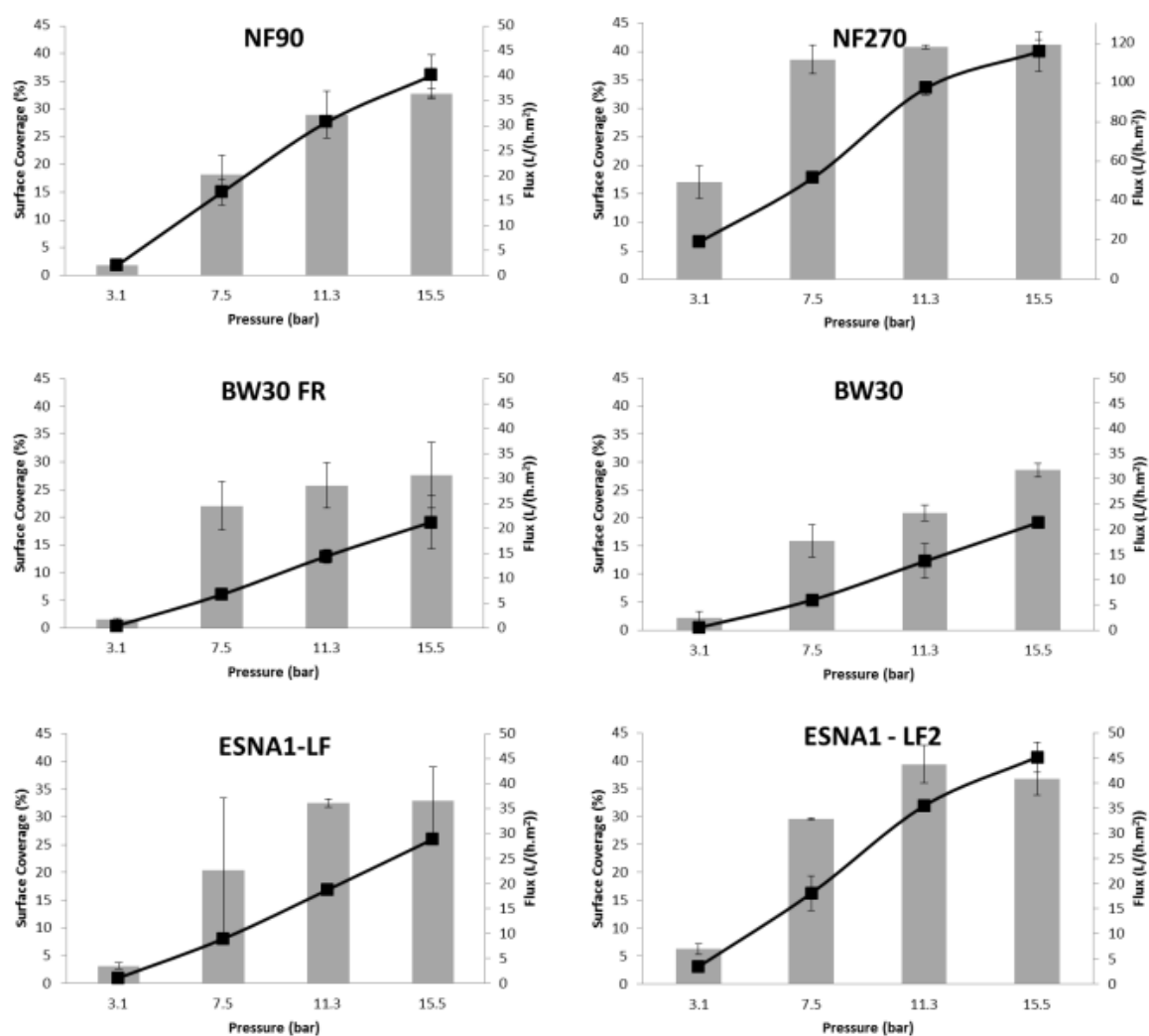


Figure 1: Effect of flux on bacterial adhesion to NF and RO membranes: NF90, NF270, BW30, BW30-FR, ESNA1-LF and ESNA1-LF2, 10^7 cells/mL of *P. fluorescens* in 0.1 M NaCl. Cross-flow conditions: 21°C, pH~7, 0.66 L.min^{-1} or $Re=579$ in each cell with each adhesion experiment repeated at least twice. The columns represent surface coverage. Permeate flux for each membrane is represented by the black square. Error bars show standard deviation in flux and surface coverage of repeated experiments.

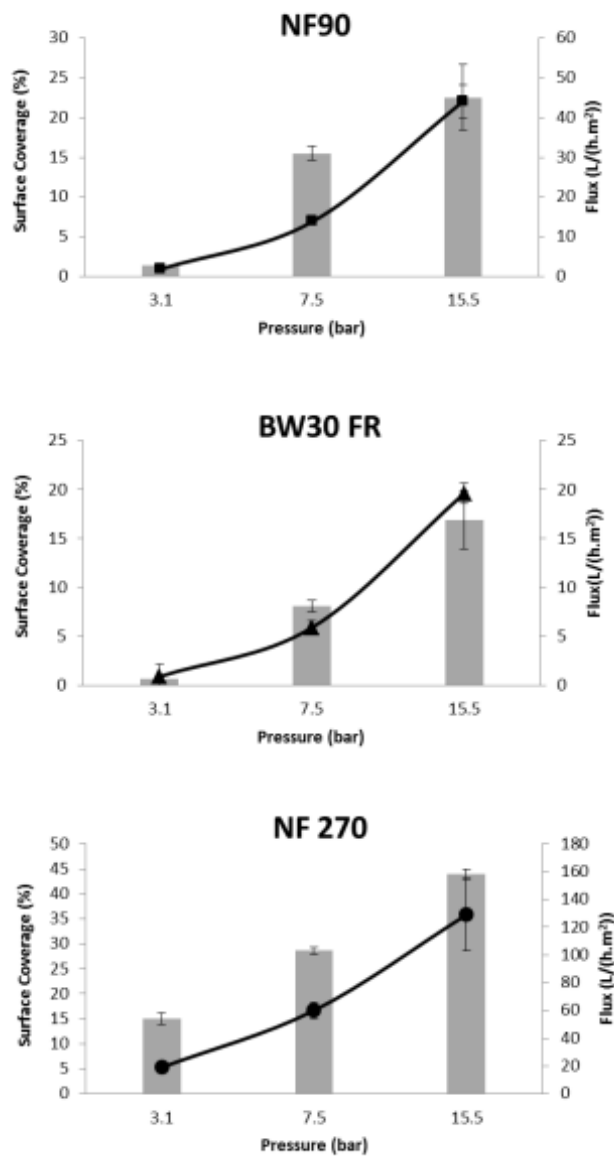


Figure 2: Effect of flux on bacterial adhesion to NF and RO membranes: NF90, NF270, BW30 FR, 10^7 cells/mL of *P. putida* in 0.1 M NaCl. Cross-flow conditions: 21°C, pH~7, $0.66 \text{ L} \cdot \text{min}^{-1}$ or $\text{Re}=579$ in each cell, with each adhesion experiment repeated at least twice. The columns represent surface coverage. Permeate flux for each membrane is represented by the black symbols. Error bars show standard deviation in flux and surface coverage of repeated experiments. (Note: the permeate flux is apparently not seen as a linear relationship with pressure because the columns are not equally spaced in pressure. The linear correlation coefficient of permeate flux vs pressure is in fact $r^2 > 0.995$ for these experiments).

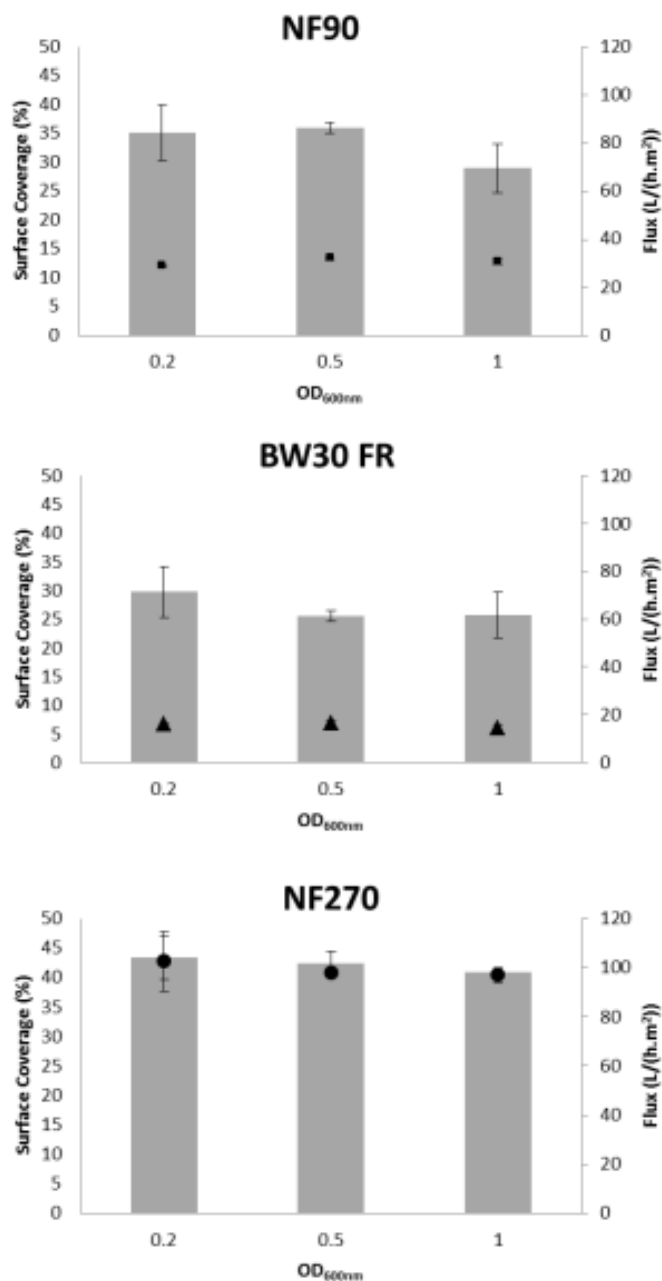


Figure 3: Effect of bacterial growth stage on adhesion to NF and RO membranes under 11.3 bar pressure: NF90, NF270, BW30FR, 10^7 cells/mL of *P. fluorescens* in 0.1 M NaCl. Cross-flow conditions: 21°C, pH~7, 0.66 L.min⁻¹ or Re=579 in each cell, 11.3 bar, with each adhesion experiment repeated at least twice. The columns represent surface coverage. Permeate flux for each membrane is represented by the black symbols. Error bars show standard deviation in flux and surface coverage of repeated experiments.

Supporting Information:

Bacterial adhesion onto nanofiltration and reverse osmosis membranes: effect of permeate flux

Andrea J.C. Semião, Olivier Habimana, Eoin Casey*

Supporting Information: S1

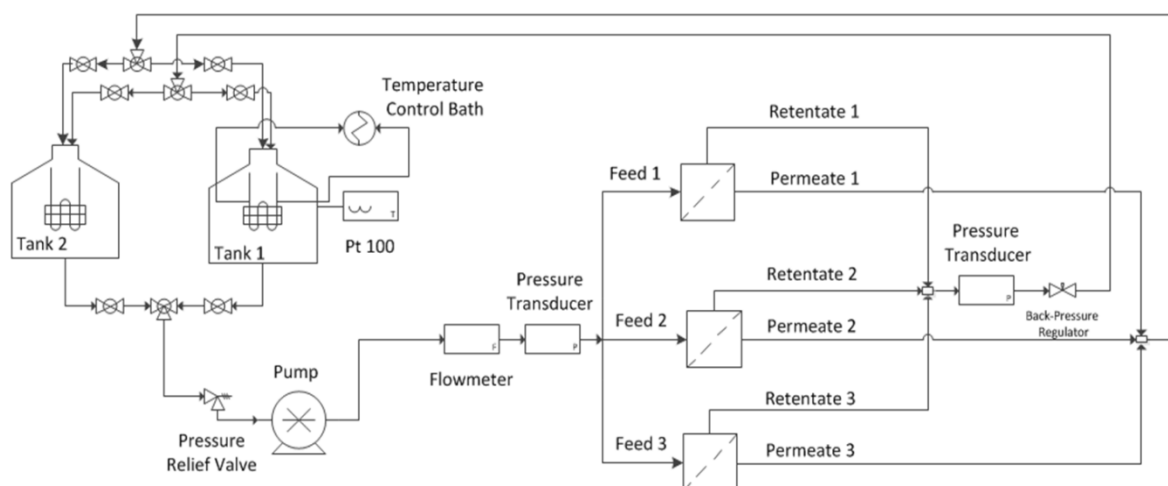


Figure S1 – Cross-flow system used for the bacterial adhesion experiments under permeate flux conditions

The experimental system was designed as a loop arrangement comprising; two parallel autoclavable 10L feed tanks (Nalgene, VWR Ireland), a high pressure pump (P200 Hydra Cell, UK) and an array of three Membrane Fouling Simulator (MFS) devices (Internal channel dimensions: 0.8mm in height, 40 mm width and 255 mm length) positioned in parallel. Each MFS holds a membrane of approximately 102 cm². The loop arrangement allowed the recirculation of both retentate and permeates of each MFS. The P&ID of the crossflow filtration system is depicted in Figure S1.

A ball valve system at the inlet and outlet of each tank allowed the interchanging of flow between tanks during filtration and rinsing operations. Temperature was monitored during filtration in one of the feed tanks with a temperature probe (Pt 100, Radionics, Ireland) and maintained at 20°C ± 1°C with a heating/cooling coil inside the tank, which was connected to a temperature controlled MultiTemp III water bath (Pharmacia Biotech, Ireland). A back pressure regulator (KPB1L0A415P20000, Swagelok, UK) allowed the pressurization of the system. The pressure was monitored in both feed and retentate side of the membrane cells

with two pressure transducers (PTX 7500, Druck, Radionics, Ireland). The feed flow was measured using a flow meter (OG2, Nixon Flowmeters, UK). Datalogging was set-up allowing the collection data from membrane cells inlet and outlet pressures, feed flow rate and temperature (PicoLog 1000, PicoTechnology, Radionics, Ireland). The permeate flux was determined by measuring a volume of permeate with a balance HCB123 balance (Adams, Astech Ireland) at different time intervals using a stopwatch.

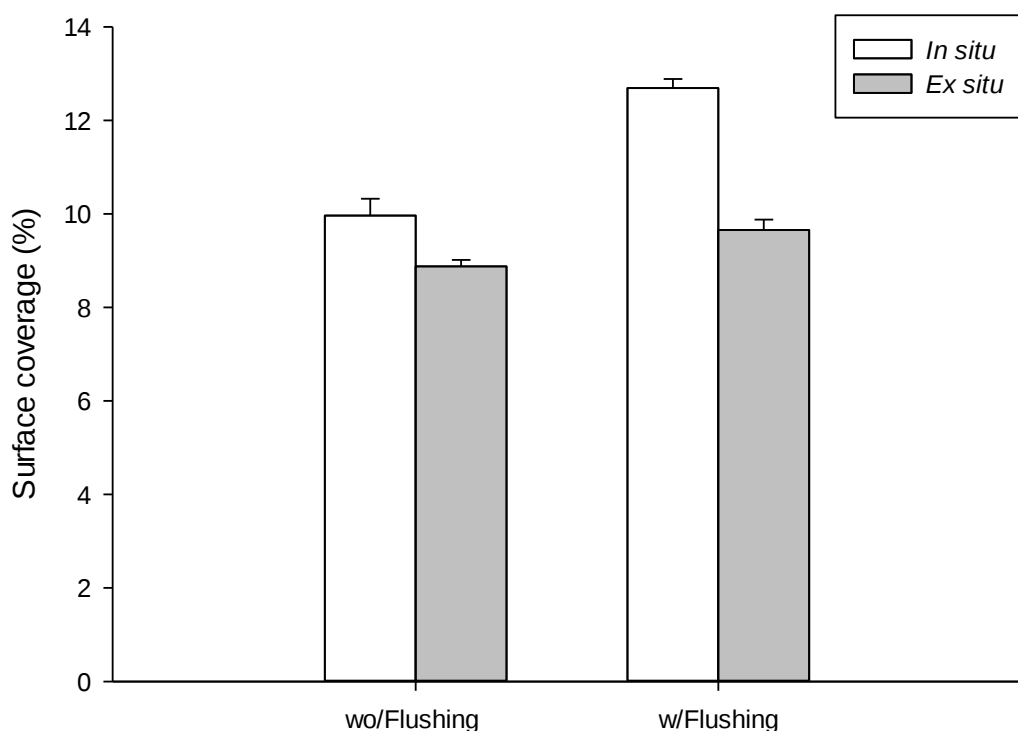


Figure S2 – Two sets of adhesion experiments were performed on compacted NF 90 membranes using approximately 10^8 *Pseudomonas fluorescens* cells suspended in 4 L of 0.02M NaCl feed solution. The adhesion was carried out in recirculation mode at 15 bar for 30 minutes. The first experimental set was performed without (wo/) flushing out the planktonic cells in the system; the second set was performed with (w/) flushing of the system using 0.02M NaCl from a second tank without stopping the pump. In each experimental set, the adhesion was observed using an MFS with fitted Sapphire glass window (*In situ* analysis), followed by a membrane autopsy directly under the Sapphire glass region (*Ex situ* analysis). Ten random fields were acquired for each analysis type during epi-fluorescence microscopy, and were used to generate means as well as standard error bars.

Results show that *In-situ* analysis lead to higher bacterial mean surface coverage on NF 90 compared to *ex situ* analysis regardless of whether the system was flushed or not (i.e. 10% loss following *ex situ* analysis). Interestingly, the loss in mean surface coverage was higher in samples from flushed system compared to non-flushed ones.

No significant differences in mean surface coverage were observed when comparing *ex situ* analyses from flushed and non-flushed systems. When comparing *in situ* analyses, the differences in mean surface coverage could be simply explained by experimental variances (i.e. second repetition). From these results, *ex situ* analyses lead to higher degree of repeatability.

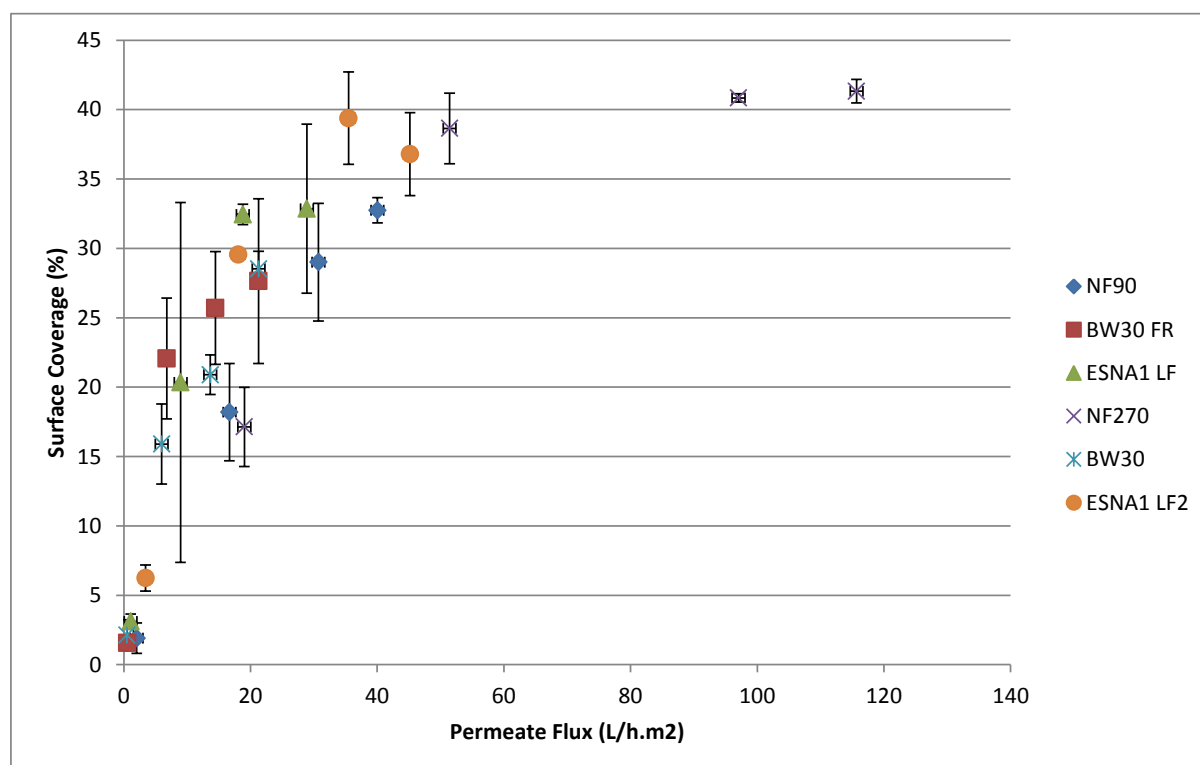


Figure S3.1 – Mean surface coverage (%) profiles of $10^8/\text{ml}$ *P. fluorescens* cells in 0.1 M NaCl, cross-flow conditions (21°C, pH~7, 0.66 L.min⁻¹ or Re=579) onto different NF and RO membranes (NF90, NF270, BW30, BW30-FR, ESNA1-LF and ESNA1-LF2) at different set pressure conditions. Each adhesion experiment was repeated at least twice. Error bar represent standard error of the mean of the repeated experiments.

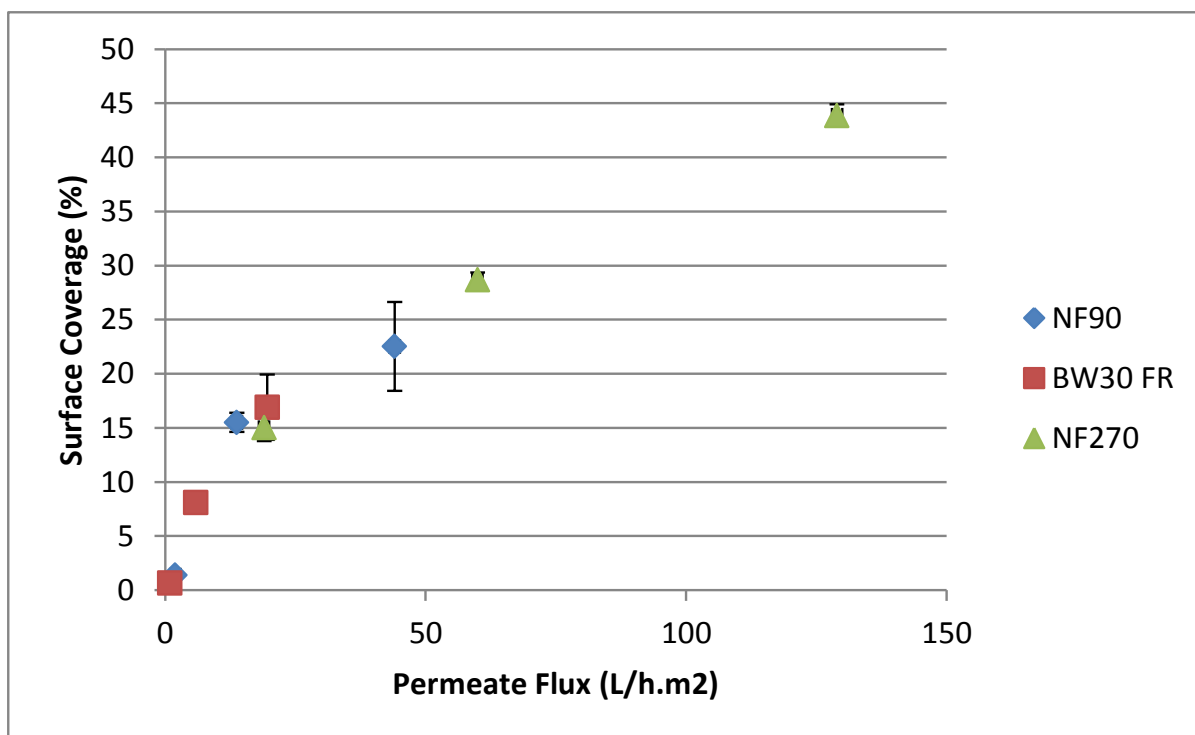


Figure S3.2 – Mean surface coverage (%) profiles of $10^8/\text{ml}$ *P. putida* cells in 0.1 M NaCl, cross-flow conditions (21°C , $\text{pH}\sim 7$, $0.66 \text{ L}\cdot\text{min}^{-1}$ or $\text{Re}=579$) onto different NF and RO membranes (NF90, NF270, BW30, BW30-FR, ESNA1-LF and ESNA1-LF2) at different set pressure conditions. Each adhesion experiment was repeated at least twice. Error bar represent standard error of the mean of the repeated experiments.

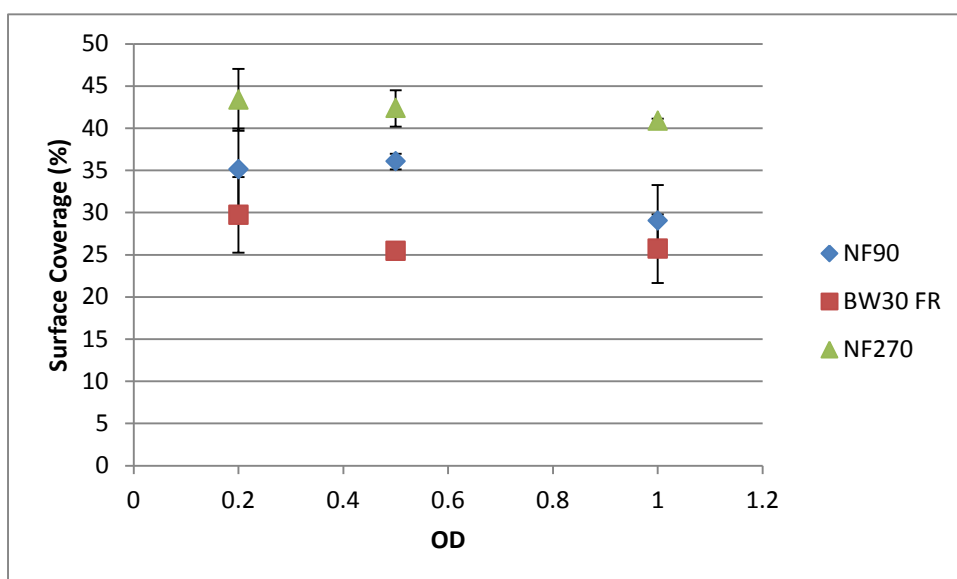
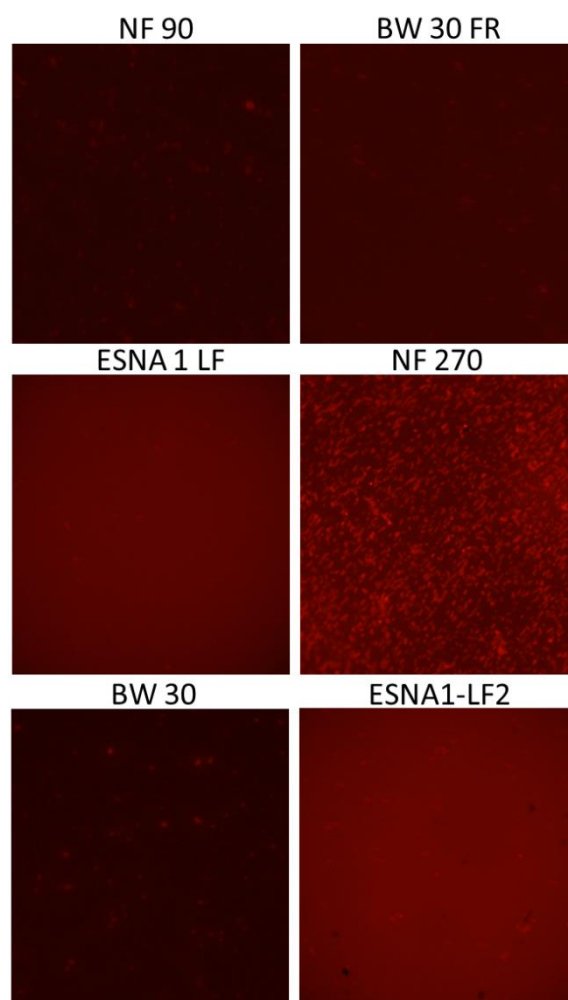


Figure S3.3– Mean surface coverage (%) profiles of $10^8/\text{ml}$ *P. fluorescens* cells in 0.1 M NaCl, cross-flow conditions for different growth stages corresponding to different optical densities OD onto the NF90, NF270 and BW30-FR (21°C , $\text{pH}\sim 7$, $0.66 \text{ L}\cdot\text{min}^{-1}$ or $\text{Re}=579$, 11.3 bar). Each adhesion experiment was repeated at least twice. Error bar represent standard error of the mean of the repeated experiments.



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24 **Figure S4** – Representative epi-fluorescence micrographs of *P. fluorescens* adhered cells onto
 25 different NF and RO membranes (NF90, NF270, BW30, BW30-FR, ESNA1-LF and ESNA1-LF2)
 26 following 30 minutes adhesion assays at 3 bar, in 0.1 M NaCl, 21°C, pH~7, with cross flow
 27 conditions set at 0.66 L.min⁻¹(or Re=579).

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