Australian Water Recycling
Centre of Excellence

Green chemicals to remove biofouling and preserve reverse osmosis membranes

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About the Australian Water Recycling Centre of Excellence

The mission of the Australian Water Recycling Centre of Excellence is to enhance management and use of water recycling through industry partnerships, build capacity and capability within the recycled water industry, and promote water recycling as a socially, environmentally and economically sustainable option for future water security.

The Australian Government has provided $20 million to the Centre through its National Urban Water and Desalination Plan to support applied research and development projects which meet water recycling challenges for Australia's irrigation, urban development, food processing, heavy industry and water utility sectors. This funding has levered an additional $40 million investment from more than 80 private and public organisations, in Australia and overseas.

Acknowledgements

Executive Summary

Application of FNA and hydrogen peroxide for fouling control

Biofouling is generally regarded as a major issue in reverse osmosis (RO) membrane filtration. Chemical cleanings with alkaline and disinfection agents are typically applied to restore the treatment capacity by removing organic foulants and stopping bacterial re-growth, respectively. In this study, the feasibility of using free nitrous acid (FNA) was investigated as a novel low cost cleaning agent. The FNA cleaning solution was prepared by acidification of a sodium nitrite solution with hydrochloric acid. Seven fouled RO membranes collected from full-scale wastewater recycling and desalination plants were used to perform lab-scale cleaning trials. Membrane fouling characterisation revealed six out of the seven membranes were mainly bio-fouled, while one membrane was severely fouled by calcium carbonate. The key finding of this study is the high bactericidal efficiency of FNA. While the effect of FNA is shown to be positive for all the different matrices tested, the effectiveness appeared to be dependent on the degree of membrane fouling. CLSM analysis demonstrated the biocidal effect of FNA is higher than that of NaOH (pH 11.0). The FNA-based cleaning is also effective for the removal of calcium carbonate scaling. No significant difference was observed between the commonly used cleaning agents (HCl and citric acid) and the FNA cleaning solution *(p-values > 0.05)*, indicating that the scaling removal capacity was maintained when nitrite was added to an acid solution (to form FNA). This study showed the feasibility of using FNA at pH 3.0 for biomass removal as well as for calcium carbonate scaling removal. The results from the lab-scale cleaning tests suggested that FNA can be used as a single cleaning agent for both biofouling and scaling removal in order to reduce costs associated with two-step cleaning. In the presence of combined inorganic/organic fouling, such as calcium-organic complexes, the benefit of tackling all types of fouling in one step can be highly valuable.

The optimal FNA-based cleaning strategy selected from the lab-scale cleaning tests was tested with fouled RO membrane modules generated at pilot-scale. For this, a pilot unit with 4-inch commercially available thin-film composite RO membranes were fed with a real effluent to provide the source of bacteria needed. Due to several technical issues on the pilot-plant and lack of time, only two cleaning trials were conducted. FNA-based cleaning improves permeability by 20-29% and reduces differential pressure drop by 28-38%. Although FNA showed to be an efficient biocide based on the results collected from the lab-scale study, FNA-based cleaning appeared to be less effective than NaOH cleaning at pH 11.0 in permeability recovery during the pilot-scale cleaning trials. The higher efficiency achieved with NaOH (80-105% versus 20-29% permeability recovery) could be explained by the large proportion of organics (e.g., extracellular polymeric substances) in the biofouling.

Preliminary lab-scale trials using FNA as a biocide (off-line shock dosing) rather than cleaning agent showed high killing efficiency (data not shown). The potential of using FNA as a biocide for biofouling prevention rather than cleaning should be investigated.

Application of FNA for the preservation of RO membranes during long-term storage

Sodium bisulphite (SBS) is the current strategy to control biofilm formation during membrane storage due to its efficiency and low price. SBS is formed from sodium metabisulphite (SMBS). However SBS preservation solutions are not stable leading to additional costs of maintenance and operation especially for large RO plants. Developing new sustainable preservation solutions for RO membranes is important.

In this study, the application of FNA as novel preservation agent was investigated. Short-term (one month) and long-term (six months) preservation trials were conducted using RO membrane coupons and spiral-wound RO modules, respectively. Stability tests revealed that FNA solutions are stable at pH 5.0. Different concentration of FNA (0-10 $mgHNO_2$-N/L) at pH 5.0 were used for the short-term preservation experiments in order to determine the lowest suitable concentration for preservation without biofilm development. Very low biomass concentration were measured in the FNA-based preservation solutions compared to the control solution (DI-water only), demonstrating that no significant microbial growth occurs during the short-term storage trials. The higher the FNA

concentration, the lower biomass concentration in the preservation solution. For all the conditions tested, no dramatic changes in membrane performances were observed in terms of permeability and salt rejection. Infrared spectroscopy (ATR-FTIR analysis) revealed no structural changes of the polyamide active layer.

The optimal FNA-based preservation strategy selected from the lab-scale tests (10 mgHNO$_2$-N/L, pH 5.0) was tested using unused and used (fouled and cleaned) RO membranes. For this, long-term preservation tests were conducted using spiral-wound RO modules in order to work more closely to real industrial conditions. FNA performance was compared with SMBS as the reference chemical since it is already used in industrial practice. While the unused membranes were stored in vacuum sealed bags to simulate membrane preservation out of the plant, the used membranes were stored in PVC tubes to simulate the membrane preservation within pressure vessels. The results showed that biofouling occurred more readily when vacuum sealed bags were used, irrespective of the preservation solutions applied. For long-term preservation within pressure vessels, FNA can be used as a preservation solution equivalent to SMBS for short and long-term storage up to 4 months. Very low biomass and cell concentrations were measured in the preservation solutions, demonstrating that no significant microbial growth occurs, whatever the preservation solution applied. The filtration trials revealed no significant impact of different preservation strategies on the performance of the membrane in terms of permeability and salt rejection. After 5 months, FNA-based preservation solution showed to be slightly less efficient compared with SMBS due to increase of bacteria activity. However, no negative impact was observed in terms of membrane performances and the benefit of using FNA versus SMBS (e.g., cost benefit related to the stability of FNA resulting in decrease of solution replacement frequency) should be investigated more in details.

Impact of FNA on membrane integrity

Biofouling is often controlled with the application of disinfectants (e.g., chlorine, monochloramine, chlorine dioxide). However, most commercial RO membranes, consisting of thin polyamide active layer on top of a thicker polysulphone support layer, can be damaged by these chemical agents. Studying the impact of new chemical agent on RO membrane performance is critical for the development and implementation of new treatment processes.

In this study, the compatibility of FNA with membrane materials was investigated. Accelerated ageing tests conducted in both passive (soaking) and active (cross-flow) mode with an unused polyamide thin-film composite RO membrane (ESPA-2, Hydranautics). Different exposure time at a high concentration of FNA (100 mgHNO$_2$-N/L) and pH of 2.0 and 4.0 were used for the ageing experiment using the ppm.hour concept. Exposure times and concentrations were selected to simulate 21600 mgN.h/L total exposures. The ppm.hour concept assumes similar chemical impact as long as the same ppm.hour value is applied (high dose and short exposure time versus low dose and long exposure time). This concept has been applied in many studies but has not been always verified. Therefore, an additional accelerating ageing test was conducted with a lower FNA dose (10 mgHNO$_2$-N/L) for a long exposure time (6 months), resulting in 43200 mgN.h/L total exposure. In this case, pH 5.0 was used as recommended for membrane preservation applications. For all the ageing experiments, hydraulic performance (permeability and salt rejection) were monitored as a function of FNA exposure. The results did not show dramatic changes in membrane performances in terms of permeability and salt rejection. ATR-FTIR analysis revealed no additional structural changes of the polyamide active layer after 21600 mgN.h/L total exposure, compare to the control at pH 2.0.

This study showed the feasibility of using FNA at pH 3.0 and pH 5.0 for biomass removal and long-term preservation, respectively. No negative effect on polyamide RO membranes was observed when FNA was applied.

Economic and environmental impacts

The environmental and economic benefits of the new cleaning chemicals were determined. Based on the optimum conditions for RO membrane cleaning and preservation defined in the previous chapters and in comparison to the current practice, quantification of the cost savings and environmental benefits achievable was carried out. Based on a life cycle analysis approach, the economic and environmental impact of the new cleaning agents was determined by considering chemical

production, transport, use and disposal, and compared to the current costs of commonly used cleaning chemicals.

Cost analysis showed that FNA is a cost-effective solution for biofouling removal and long-term preservation in RO filtration applications. One of the key benefits would be the compatibility of FNA with polyamide membrane, which can allow a more frequent usage and at an earlier stage of fouling compared to other chemicals, without membrane damage. Decreased cost related to membrane replacement would is another crucial aspect of the cost benefit of FNA application.

No significant changes would be required for the chemicals handling, storage and discharge when using FNA compared to the commonly used chemicals. Sodium nitrite used to generate FNA is classified as Dangerous Goods by the criteria of the Australian Dangerous Goods Code (ADG Code) for transport by Road and Rail because it is toxic if swallowed. However similar specific transport is also required for sodium hydroxide (50%) and Hydrochloric acid (33%), commonly used for membrane cleanings. Although lower concentration requires a larger volume to be dosed to achieve the same concentration and pH reduction, it would be recommended to use lower concentrations of chemicals in order to increase safe handling and storage conditions. After use, the FNA-based solution can be discharged to a sewer or the head of a treatment plant for wastewater reuse. Due to the dilution effect, it is likely that the proportion of nitrogen added will be negligible, and the nitrite could be easily removed through the denitrification process. For desalination application, the spent cleaning solutions would have to go to a downstream treatment operation.

Formation potential tests showed the generation of carcinogenic nitrosamines due to the reaction between nitrite and secondary amines under acidic conditions. However, nitrosamines were not formed in excessive amounts during membrane cleaning.

Finally life cycle assessment (LCA) showed that the comparison between FNA vs. conventional is fairly ambivalent. The implications would depend strongly on operational circumstances; therefore depend on site specifics for any particular plant. The biggest implications (in a GHG sense) would be if the FNA strategy reduced/increased average fouling rates over the long term.

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1. Introduction/Background

Due to unequal water distribution across Australia, climate change, chronicle drought and growing population, Australia needs alternatives for water supply. To meet the growing demand for water, multi-billion dollar schemes have been set in motion to augment the water supply by a combination of: i) wastewater recycling plant; and ii) desalination plant, both using RO technology.

Reverse osmosis

The number of reverse osmosis plants in Australia has rapidly increased in the past decade. Six seawater desalination plants have been commissioned across Australia. Also, several plants for wastewater recycling have been built including the Western Corridor Recycled Water Project in South East Queensland with a total capacity of 85 GL/year, the Wollongong (7.3 GL/year) and St Mary's (18 GL/year) recycling plants in New South Wales and the Kwinana water reclamation plant (6 GL/year) and the Beenyup ground water replenishment project in Western Australia [1] (Figure 1).

Figure 1. Map of water recycling schemes in Australia. Source: Australian Water Recycling Centre of Excellence, Presentation 'Future Directions for Water Recycling in Australia: Where will be in 2030?', Greg Oliver [2]

Biofouling issue

Reverse osmosis membranes have been shown to consistently produce very high quality water independent of source water quality and can be used for a wide range of applications, including potable use. However, membrane fouling and more specifically biofouling, remains one of the major operating challenges [3, 4]. Biofouling is defined as the adhesion, growth and multiplication of bacteria present in the water on membrane surfaces, and was shown to have a negative impact on operation. The main consequences observed are decreased membrane flux, increased pollutants passage through the membranes and increased loss of pressure across the membranes train. This can eventually result in biodegradation of the membrane polymer and other components of the modules [5]. These effects ultimately result in increased energy and chemical costs, loss of water production and quality and reduced membrane life. Overall, membrane biofouling critically reduces the process efficiency and cost-effectiveness. The preventative measures to alleviate biofouling in the desalination industry is estimated to cost approximately 15 billion $US yearly worldwide [6].

Biofouling is usually described to the accumulation of microorganisms such as bacteria, algae and fungi on the membrane surfaces forming the biofilms, via multi-step and complex formation process. Microorganisms are the source of biofilm; however they account for less than 10% of the dry mass. The matrix of extracellular materials, so called extra polymeric substances (EPS) account for over 50-90% [7, 8]. EPS are excreted from the organisms themselves and mainly composed with polysaccharides, proteins, glycoproteins, lipoproteins and other macromolecules of microbial origin [8]. Amy (2008) described biofouling as a biotic form of organic fouling while organic matter derived from microbial-derived cellular debris are described as an abiotic form of biofouling [9]. EPS facilitate the growth of biofilm by attaching microbes, accumulate dissolved ions and store nutrients from feed water. Although, membrane biofouling is an issue in operation of both wastewater recycling plant and seawater desalination plant, the RO plants operate under different conditions and the propensity for biofouling is greater in the wastewater recycling application due to the nature of the feedstock. Wastewaters contain a larger abundance of organic nutrients that bacteria can utilize, resulting in increased biofilm formation rate [10]. EPS contribute to the formation of cross-linking network with mineral ions and the entrapments of biopolymers, which protect embedded microbes against shear force and cleaning attacks, and substantially limit the diffusion of biocides and other disinfectants into the biofilm.

Biofouling control

Current strategies to control biofouling include feedwater pre-treatment to remove bacteria before they reach the RO membranes and nutrients to limit their development, and dosing of biocides such as chlorine and monochloramine [11, 12]. Chlorine is a strong biocide, and has been widely used for biofouling control in membrane systems. However, its application to RO membrane is restricted as it can damage the polyamide active layer of RO membranes [13-15]. Nevertheless, chlorine is commonly used in seawater desalination plants but a de-chlorination stage (usually with sodium metabisulphite) is needed before the feedwater reaches the membranes to avoid structural damages. It has been shown that abundant bacteria regrowth is possible after the dechlorination stage [16]. In comparison, monochloramine is a weaker oxidant. While it was found to be less detrimental to the membranes, it has also been shown to have a limited impact on bacteria removal. Indeed, even with continuous dosing, biofouling formation has been observed [3]. In recent years, research studies investigating membrane biofouling control have focused on optimisation of pre-treatment for the limitation of nutrients in feed water [12, 17], development of novel membrane materials (chlorine resistant [18] or anti-fouling [19]), determination of novel biocides such as 2,2-dibromo-3-nitrilopropionamide (DBNPA) [20] or nitric oxide [21] and development of novel biological methods such as inhibition of biofilm growth by quorum sensing, biomass dispersion by cell wall hydrolase or bacteriophage and enzymatic disruption [22, 23]. Although some of these novel techniques are promising none of them have proved to dramatically improve biofouling control, and none can be implemented for full-scale plant operation in the medium term. In general, these methods do not allow complete/satisfactory removal of the microorganisms present in the feedwater and even if a process is very efficient, there is still enough cells remaining which can grow in the system [3, 24]. Over time, biofouling will develop on the RO membranes, and chemical cleaning of the RO membranes is regularly required to restore their treatment capacity.

Cleaning strategies/Biofouling removal

When membrane biofouling occurs to the extent that the performance of the system drops to unacceptable levels, cleaning of the membranes is required. For this, the system is stopped and chemical cleaning agents are recirculated to target and remove fouling. Typically, chemical cleanings are a sequence of cleanings with alkaline (e.g. sodium hydroxide) and acidic (e.g. citric acid, hydrochloric acid) agents (Table 1). Alkali cleaning is used to remove organics and biofilm present on the membranes, while acid cleaning is generally used to target scaling. It has been shown that organic fouling removal can be optimised when using chelating agents (e.g. EDTA) or surfactants (e.g. SDS) in combination with sodium hydroxide. Biocide can also be used to stop bacterial re-growth. However, biofilm removal using the current strategies was never found to be complete [3, 17, 25]. In addition, the commonly used cleaning agents, used in large quantities, contribute significantly to operational costs and environmental issues for their disposal.

Table 1. Usual cleaning agents according to the type of fouling [26, 27]

Type of fouling	Chemical agent
Colloidal	NaOH solution, chelating agents and surfactants
Organic	NaOH solution, chelating agents and surfactants
Metal oxides	Citric acid (low pH) or $Na_2S_2O_4$ and sequestering agent (to target metal cations)
Silica	NaOH solution (high pH)
Carbonate scales	Citric acid or HCl (low pH)
Sulfate scales	HCl solutions or sequestering agents (EDTA)

Membrane preservation

While on the west coast of Australia, RO plants are running, on the east coast, RO plants are mainly shutting down due to high level storage in reservoirs (Table 2). Biofouling control is a well-known issue when the RO plant is under operation, however biofouling risk is real even when the plant is shut down [28]. Reverse osmosis plants are occasionally shut down for either short-term periods due to maintenance work or long-term periods due to low season production. RO membrane surfaces are particularly vulnerable to microbial colonization and biofilm development, resulting membrane performance decline or membrane degradation. When RO plants are shut down, the biofouling risk is real and biological growth in the RO system should be controlled [28].

Sodium bisulphite (SBS) solution is the current strategy to control microorganism growth during membrane storage due to its efficiency and low price (Table 3). However SBS preservation solutions are not stable. SBS is easily oxidized into sulphate with oxygen resulting in continuous pH dropping [29-31]. Therefore, pH needs to be regularly measured and solutions replaced frequently (e.g., the pH should be monitored monthly and fresh preservation solution added if the pH drops to pH 3 or lower, in order to safeguard the membranes), leading to additional costs of maintenance and operation especially for large RO plants [30]. Furthermore, cracks might occur in the plastic of the pressure vessels when using SBS for long-term storage. Considering all the above mentioned reasons it becomes obvious that developing more sustainable preservation solutions for RO membranes is important.

Table 2. List of desalination plants in Australia. Source: Australian Water Association [32]

Plant	Capacity (per day)	Percent of water supply	Location	Completion	Shutdown/Operation
Gold Coast Desalination Plant	125 megalitres	27% of South-Eastern Queensland	South East Queensland	2009	May 2009 (5 weeks) and June 2010 (3 months) to repair previously identified defects. Running under low regime.
Perth Seawater Desalination Plant	130 megalitres	17% of Perth	Kwinana, Western Australia	2006	Early 2008 (two times) due to reduced dissolved oxygen levels in Cockburn Sound
Kurnell Desalination Plant	250 megalitres	15% of Sydney	New South Wales	2010	July 2012 due to high dam storage level (90% capacity)*
Wonthaggi Desalination Plant	410 megalitres	33% of Melbourne	Victoria	2012	The Wonthaggi plant has never operated.
Southern Seawater Desalination Plant	410 megalitres	20% of Perth	Binningup, Western Australia	2012	-
Port Stanvac Desalination Plant (Adelaide)	270 megalitres	50% of Adelaide	South Australia	2012	The desalination plant continues to run despite ample drinking water already being available.

* Production will recommence when dam storage levels reach 70% and will remain in production until dam storage levels reach 80%.

Table 3. Manufacturer recommendations for membrane long-term storage [27, 30, 31, 33].

Manufacturer	General storage procedures for composite polyamide RO membrane elements	
	Storage time	Chemicals (concentration)
Hydranautics	> 30 days	Sodium bisulphite (1.0%) Formaldehyde solution (0.1 to 1.0%) Glutaraldehyde solution (0.1 to 1.0%)
Dow-Filmtec	> 48 hours	Sodium bisulphite (1.0 to 1.5%)
Toray Membrane	> 4 days	Sodium bisulphite (0.05 to 0.1%)
GE Water & Process technologies	> 20 days	Sodium bisulphite (0.5 to 1.0%) GE BetzDearborn DCL30 (3.0%)

Free nitrous acid

Recent studies carried out on sewer biofilms and waste activated sludge at both laboratory and full scales, have demonstrated that free nitrous acid (FNA) is a strong biocidal agent even at parts per million levels (0.2 – 2 mgN/L), causing deactivation of microorganisms by inducing substantial cell death and biofilm detachment [34-37]. Also, it was shown that biofilm remediation was enhanced when very low doses (0.2-0.4 mgN/L) of FNA were used in combination with hydrogen peroxide (30 mg/L) [35]. The FNA technology is currently being applied to sulfide and methane control in sewer networks. In a recent trial of the technology for sewer biofilm control, it has been shown that the activities of sewer biofilms were completely suppressed, accompanied by a substantial loss of biofilm after 24 hr treatment [38]. Although sewage provided ample substrates for biofilm to regrow, the recovery of sewer biofilm activities one week after treatment was less than 20%. Preliminary results with a biofilm developed at lab-scale on a RO membrane have shown that with a nitrite dose of 50 mgN/L at pH 2, FNA achieved over 71% bacteria removal with 98.7% bacteria left being dead, demonstrating its potential to be used as a novel cleaning agent, compatible with RO membranes (Figure 2) [39]. Given the very low substrate concentration in feedwater in an RO system (in comparison to raw sewage), it is reasonable to expect that the recovery of RO membrane biofilm would be much slower in comparison to sewer biofilms.
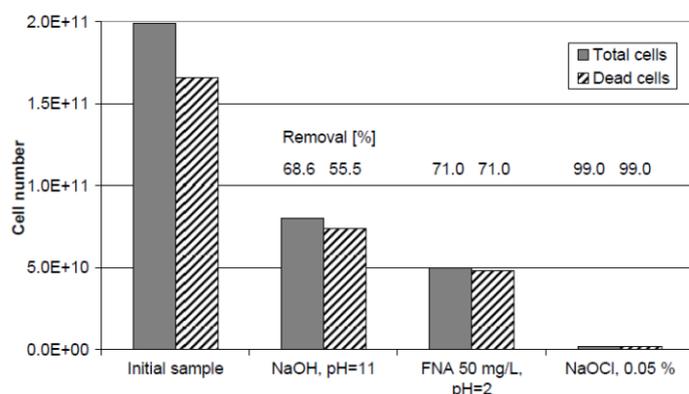
Figure 2. Average number of total and dead cells on a 9 days-old biofouled membrane sample and cleaning membrane samples from the same test (Ex-situ cleaning trials: Ratek large orbital shaker/incubator, 120 rpm, 19h of incubation). The numbers above the bars in indicate the removal of total or dead cells compared to the initial sample [39].

Objectives

The overall objective of this project was to investigate and demonstrate the effectiveness and benefits of FNA as a novel low cost membrane cleaning and preservation agent. Its utilisation, alone or in combination with hydrogen peroxide, was assessed for the removal of biofouling in RO membranes for water recycling and seawater desalination. As an acid, it is anticipated that FNA will also be effective at removing inorganics from the membrane surface. Therefore, the potential of using FNA to remove RO membrane scaling was also evaluated. The application of FNA as a cleaning agent was studied at bench-scale and pilot-scale (Task I). The application of FNA as preservation solution to prevent biofilm growth when RO plants are put in standby mode was also investigated for long-term preservation out or within the pressure vessels (Task II). In both types of applications, the compatibility with thin-film composite polyamide membrane was studied (Task III). Finally, a study of the economic and environmental impacts was assessed including life cycle analysis (Task IV).

2. Materials and Methods

2.1. Membranes

Several RO membranes were selected for this study (Table 4). The membrane selection was based on discussion with partners (Veolia and Seqwater), as well as input from other water utilities and industries (Water Corporation, CUB Yatala Brewery, Membrane Futures).

Table 4. List of RO membranes used for the project.

Reference No.	Description	Source	Justification for selection	Applications	
RO1	ESPA2 LD polyamide, thin-film composite membrane (Hydranautics/ Nitto Denko)	Municipal water recycling plant	Biofouling	Soaking cleaning tests	Membrane coupons
RO2 RO3	BW30-400-FR (DOW Filmtec)	CUB Yatala brewery recycled water plant	Biofouling	Cross-flow cleaning tests	
				Short-term preservation tests	
RO4	-	Municipal water recycling plant	Biofouling	Cross-flow cleaning tests	
RO5	-	Municipal water recycling plant	Biofouling	Cross-flow cleaning tests	
RO6	-	Seawater desalination plant	Biofouling	Cross-flow cleaning tests	
RO7	-	Coal seam gas water recycling plant	Scaling	Cross-flow cleaning tests	
RO8	TML20-400 (Toray)	Municipal water recycling plant	brackish water membrane used by Luggage point advanced water treatment plant	Long-term preservation tests	8-inch spiral wound module
ESPA-2	ESPA2 LD polyamide, thin-film composite membrane (Hydranautics/ Nitto Denko)	Unused membrane	brackish water element used by Gibson Island advanced water treatment plant	Short-term preservation tests	Membrane coupons
				Long-term preservation tests	4-inch spiral wound module
SWC-5	SWC5 polyamide, thin-film composite membrane (Hydranautics/ Nitto Denko)	Unused membrane	seawater desalination element used by Gold Coast desalination plant	Short-term preservation tests	Membrane coupons

2.2. Membrane autopsy methods

Loss on ignition (LOI)
The fouling layer was collected by scraping a known surface area of membrane (40x40 cm^2). Solids accumulated on membrane surfaces were deposited on previously cleaned (furnace, 500°C) crucibles and subsequently dried in an oven at 105°C overnight and a furnace at 500°C during 4 hours. The total and volatile solids concentrations were then calculated as follows:

Total solids concentration (g/m^2) :
$$TS = \frac{w2 - w1}{S}$$
(Equation 1)

Volatile solids concentration (g/m^2):
$$VS = \frac{w2 - w3}{S}$$
(Equation 2)

where w1 is the weight of the crucible, w2 is the weight of the crucible and deposits after oven, w3 is the weight of the crucible and deposits after furnace and S is the surface area of membrane sampled.

Elemental analysis (ICP-OES)
Metals content of the samples was determined by inductively coupled plasma optical emission spectrometry (ICP-OES) (Varian Vista Pro, Varian). A known surface area of membrane (5x5 cm²) was placed in 20 mL of 10 v/v% nitric acid solution. The solution was shaken on vortex for 1-2 minutes and stored overnight to recover all the material on the membrane surface before ICP-OES analysis. The following elements Al, As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, Pb, S, Se, and Zn were measured.

Polysaccharides and proteins
Protein and polysaccharide contents were measured using the QuantiPro^{TM} BCA Assay Kit (Sigma Aldrich) and the Phenol-Sulfuric acid method [40], respectively. The deposit recovered from a known membrane surface area (5x5 cm^2) was mechanically dispersed in Milli-Q water (20 mL) as described above. The samples were then mixed with reactants. These photometric methods are based on the fact that the colour of the mixture will vary with the concentration of the compound. The samples are typically analysed on an UV spectrometer (Cary 50 Bio, Varian) at set wavelengths. The signals are calibrated with bovine serum albumin (BSA) and D-Glucose (Sigma Aldrich) for the proteins and polysaccharides, respectively. Results are then reported as mg glucose or mg BSA per unit area. All measurements were done in triplicate.

Biomass quantification via adenosine tri-phosphate (ATP)
Total ATP was determined using the BacTiter-Glo^{TM} reagent (Promega Corporation, USA) following a protocol adapted from Hammes et al. [41]. A set volume of the mixture (300 µL) was placed in the wells of a 96 well plate, mixed with 50 µL of the reagent and then the luminescence was measured at 38°C after 20s orbital shaking. The luminescence response was read with a DTX 880 multiplate reader (Beckman coulter, USA), collected as relative light units (RLU) and converted to ATP concentrations (nM) using a calibration curve made with a known rATP standard (Promega Corporation, USA) and Ultrapure^{TM} distilled water (Invitrogen, Australia). The detection limit was set at 0.01 nM ATP, upwards of which a linear correlation of R²=0.99 was obtained. All measurements were done in triplicate.

Scanning Electron Microscopy (SEM) – Energy Dispersive X-Ray Spectroscopy (EDX)
A Phillips XL30 scanning electron microscope (secondary electrons) was used for imaging the surface of different membrane samples dried in a desiccator for at least 24h and coated with Pt to ensure the electron drainage and to avoid sample charging. Samples were cut from the coupons, placed on metal stubs, Pt coated (Centre for Microscopy and Microanalysis, UQ) and then placed in a desiccator until the measurement. The secondary electron imaging mode enables to generate high magnification images, giving information on the morphology of the surface. Typical imaging has been done at 1000x magnification rates, 15kV accelerating voltage. Energy Dispersive X-ray Spectroscopy was performed on the previously SE imaged areas with a typical working distance of 10.3 mm. Elemental percentage has been obtained after background subtraction and ZAF optimization.

2.3. Other methods conducted on membrane

Attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR)
Attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR) was used to measure chemical changes in the polyamide structure. The membrane samples were pressed on the surface of the ATR crystal, the sample was then exposed to light of different wavelength in the infrared domain. To have the focus on the polyamide top layer of the membrane the penetration depth had to be reduced. That was done by changing the standard diamond crystal with a flat plate Germanium crystal with a penetration depth of 0.5 µm. The reflected spectrum is showing chemical groups vibration and therefore chemical characteristic of the measured material (Table 5). All measurements were conducted on a Nicolet 5700 ATR-FTIR spectrometer.

Table 5. Main bonds occurring in polyamide structures [42, 43].

Wavenumber	Groups	Bonds
1538 cm^{-1}	Amide II band, amide groups	N-H in-plane bending N-C stretching vibration
1609 cm^{-1}	Aromatic amide groups	N-H deformation vibration C=C ring stretching vibration
1662 cm^{-1}	Amide I band, secondary amide groups	C=O stretching C-N stretching C-C-N deformation vibration

Confocal laser scanning microscopy (CLSM)
Confocal laser scanning microscopy was conducted to visualise biofilm and indicate the viability of bacterial cells in biofilms. A known area of membranes (1×1 cm^2) was stained using the green fluorescent SYTO®9 nucleic acid stain and the red fluorescent propidium iodide (PI) from LIVE/DEAD® BacLight™ Bacterial Viability Kits purchased from Molecular Probes® (L-7012, Invitrogen, Australia). The SYTO®9 stain labels all bacteria in a population with intact or damaged membranes. In contrast, PI stain penetrates only those bacteria with damaged membranes, causing a reduction in the SYTO®9 stain fluorescence when both dyes are present. Thus, bacteria with intact cell membranes (viable cells) are stained green, whereas bacteria with damaged membranes (dead cells) are stained red. The stained membrane coupons were incubated in a dark place for 30 min at room temperature (20^oC), allowing the staining reactions to complete and then mounted onto a glass slide for microscope observation. The stained biofilm samples were photographed using a Zeiss 510 confocal laser scanning microscopy (Australian National Fabrication Facility-QLD Node). Two excitation/emission wavelengths were used for the two florescent stains: 488 nm/500 nm for SYTO®9 and 510 nm/635 nm for PI. Twenty images were taken for randomly chosen areas of each sample. Quantification of live and dead cells was done by determining the relative abundance of green and red pixels. The pixel area counting was conducted with DAIME (Digital image analysis in microbial ecology, by Holger Daimes). The ratio of green fluorescence to the total fluorescence (red + green fluorescence) was assumed to be equal to the ratio of viable cells to the total cells (viable + dead) in the biofilm.

2.4. Membrane performance

Filtration set-up

Cross-flow lab-scale filtration unit. Clean water permeability and salt rejection tests were carried out on laboratory scale cross-flow filtration unit (Figure 3). The system included a 15 L tank, from which the source water is sent to two filtration cells in parallel with a diaphragm pump (Metering pump Z series, Tacmica). A dampener (PD36, Neptune) is fitted in the feed line after the pump to avoid flow pulsation in the system. A needle valve, to help balancing flow and pressure in the system was installed in a bypass returning the solution directly to the feed tank. A needle valve fitted in the concentrate line after the filtration cells (CF042, Sterlitech) was used as a backpressure valve to set the pressure and cross flow in the system. Pressure in the system was measured with a sensor (Cerabar M, Endress & Hauser) located in the feed line just before the filtration cells. Due to the type of pipes, valves and connectors a maximum pressure of 5 bars could be applied in the system. The system was operated in batch mode and consequently both permeate and concentrate were

recirculated back into the feed tank. The membrane coupons to be tested were placed in the cells. The system was then operated at 5 bars and a flow rate of 40 L/h.

Spiral-wound module set-up (4-inch module set-up). Some filtration experiments were performed using a single 4-inch spiral-wound module unit consisting of a stainless-steel pressure vessel (membrane shop, Australia), a Hydrovar CRN1-27 pump (Grundfos, Australia) and a 100 L feed tank (Figure 3). The feed pressure and the differential pressure between feed and concentrate lines were measured with two digital gauge transmitters. The concentrate flow rate was controlled by adjusting the speed of the pump and by adjusting a needle valve installed in the concentrate line. Permeate and concentrate flow rates were measured with a TX50 flow meter and a 1750 MPB flow meter (MPB industries Ltd, Kent, England) respectively prior to be returned to the feed tank. The temperature of the feed solution was controlled using a cooling thermostat (Lauda, Australia). Sampling points were located in feed, permeate and concentrate lines. The system was then operated at 6.5 bars and a flow rate of 2040 L/h.

a.

b.
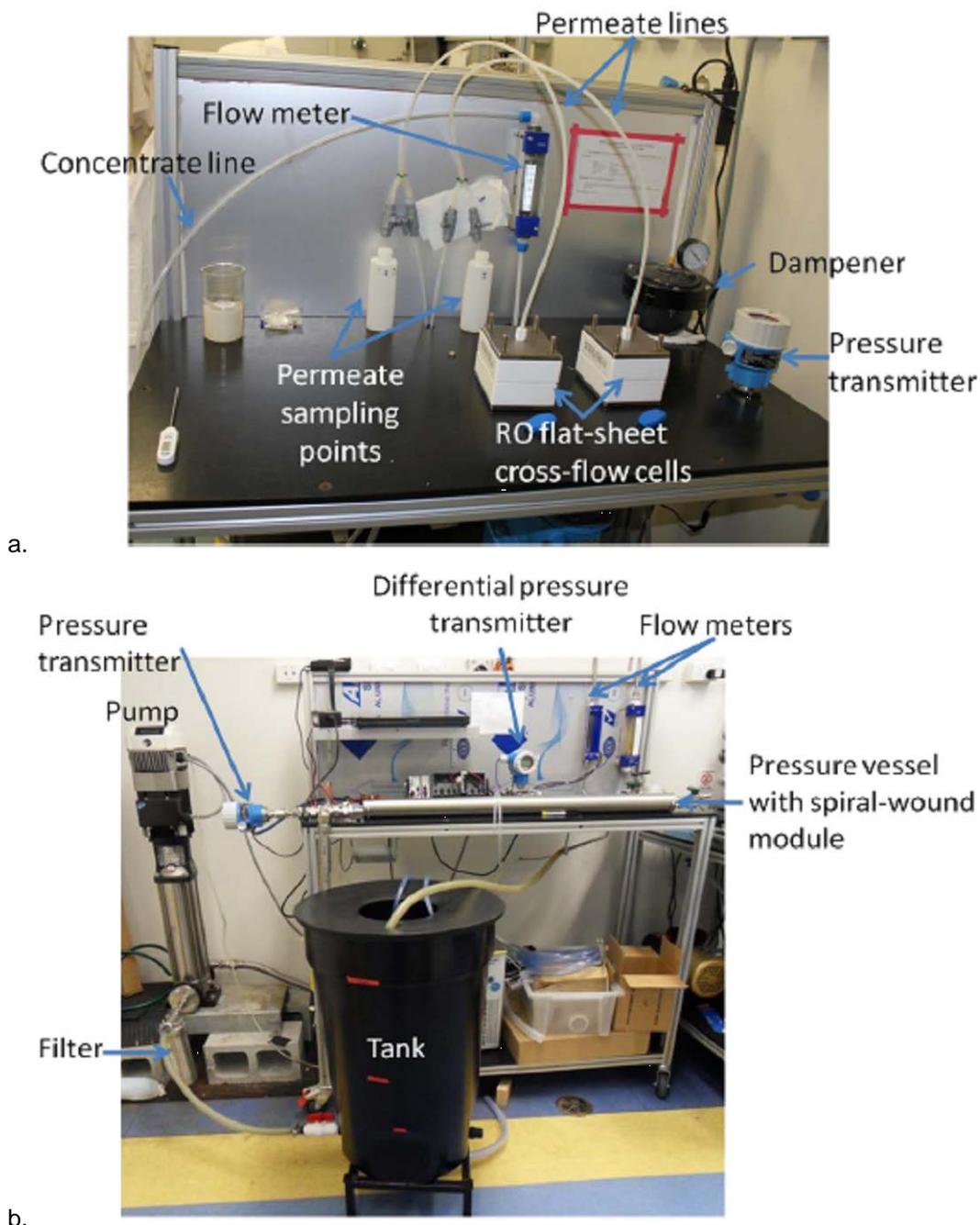
Figure 3. Photographs of (a) the cross-flow filtration unit and (b) the spiral-wound module set-up.

Permeability and salt rejection calculations

The water permeability (Kw; L/h.m^2.bar) and salt rejection (R, %) were determined using DI-water and 1500 mg/L NaCl solution, respectively.

The water permeability. The permeability (K) is corrected at 25°C to avoid the effect of the temperature:

$$J = \frac{Q_P}{S}$$ (Equation 3)

where J: Flux (L/m^2.h), Q$_p$: Permeate flow rate (L/h) and S: Membrane surface Area (m^2)

$$K = \frac{J}{NDP}$$ (Equation 4)

where K: Permeability (L/m^2.h.bar), J: Flux (L/m^2.h), NDP: Net driving pressure (bar)

$$NPD = \Delta P - \Pi$$ (Equation 5)

where ΔP: Transmembrane pressure (bar), Π: osmotic pressure (bar).

$$K_w = \frac{K}{e^{\beta \times \left(\frac{1}{273.15+T} - \frac{1}{298.15}\right)}}$$ (Equation 6)

where K$_w$: Corrected permeability (L/m^2.h.bar, 25°C), K: Permeability (L/m^2.h.bar), T: Temperature (^{o}C), β: Membrane coefficient (β = 3100)

Recovery. Recovery is an important indicator of RO performance. The recovery Rw (%) of a membrane or an overall RO system is given by:

$$Rw = \frac{Q_p}{Q_f}$$ (Equation 7)

Where, Qp: permeate volumetric flow rate (L/h) and Qf: feed volumetric flow rate (L/h).

The salt rejection. Membrane salt rejection (R) is measured using SevenEasy conductimeter (Mettler Toledo, USA) and using the equation as follow:

$$R = \left(1 - \frac{c_P}{c_f}\right) \times 100$$ (Equation 8)

Where, Cp: permeate conductivity (µS/cm) and Cf: feed conductivity (µS/cm).

2.5. Water quality analysis

Conductivity and pH
Conductivity was measured with a Mettler Toledo Seven Easy conductivity meter and pH was measured with Mettler Toledo Seven Easy pH meter.

Turbidity
A Lovibond 206020T PCCheckit Turbidity meter was used to measure turbidity and expressed as nephelometric units (NTU).

Total organic carbon (TOC)
TOC was analysed using an Analytik Jena multi N/C®-series instrument, and expressed as non-purgeable organic carbon (NPOC) which indicates the remaining acidified organic carbon after purging with an inert gas such as He, N$_2$, or CO$_2$.

Total organic nitrogen (TON)
Total organic nitrogen (TON) was calculated by subtracting the concentration of NH$_4^+$-N from the Total Kjeldahl Nitrogen (TKN).

Flow infection analysis (FIA)
A Lachat Quickchem8000 flow injection analyser (FIA) was used to determine NH_4^+-N, NO_x-N, and NO_2^--N, according to the Method 31-107-06-1-A, while TKN and TKP were measured using QuikChem method 10-107-06-2-D (analysis of ammonia) and 10-115-01-1-D (analysis of phosphate), respectively.
Samples were filtered using 1.2 µm glass fiber syringe filters. TON was calculated as the difference between TN and the sum of inorganic nitrogen species (ammonia, nitrite and nitrate).

Ionic chromatography (IC)
The ions Cl^- and SO_4^{2-} were measured by ion chromatography (IC, Dionex 2010i system).

Inductively coupled plasma optical emission spectroscopy (ICP-OES)
The metal ions were determined using inductively coupled plasma optical emission spectroscopy (ICP-OES) method (Varian Vista Pro, Varian). The following elements Ca, Fe, K, Mg, Na and P were measured.

Free chlorine
Chlorine residuals in samples were measured as free available chlorine using the N,N-diethyl-p-phenylenediamine (DPD) free chlorine colorimetric method (Hach Company, Loveland, CO, United States).

Silt Density Index (SDI)
The Standard Test Method has been described in ASTM test D 4189-82. In this test, the feedwater was passed through a filter having hydrophilic nature, usually made up of cellulose acetate, having nominal pore size of 0.45 µm and 47 mm Ø at a constant pressure of 30 psi in dead–end filtration mode. Time to filter 500 mL of water through virgin membrane was recorded as initial time (t_o) and then after water filtration for a defined length of time (T=15 min), carried out at same pressure, another reading was taken, called as final time (t_1), to filter the 500 mL of the water. SDI was calculated by the following formula:

$$SDI : [1-t_o/t_1] \times 100/15 \hspace{3cm} \text{(Equation 9)}$$

Where, t_o: the initial time necessary to filter 500 mL of water and t_1: the time necessary to filter 500 mL of water after 15 minutes.

2.6. Other methods conducted on liquid samples

Flow cytometry. Two fluorescent dyes were used alone or in combination: SYTO®9 green fluorescent nucleic acid stain and Propidium iodide (PI) from LIVE/DEAD® BacLight™ Bacterial Viability Kits purchased from Molecular Probes® (Invitrogen, Australia). Working solution of the dyes was prepared by mixing SYTO®9 (3.34 mM in DMSO) with PI (20 mM in DMSO) at a ratio of 1:1. Water samples were divided into four sub-samples: one sample was kept unstained (control 1) and three were stained with SYTO®9 only (control 2), PI only (control 3) and the mixture of SYTO®9 and PI (sample). Control 3 was incubated for 10 min at 65^oC before staining as a control measurement for inactivated bacteria. By comparing the staining pattern of heat-inactivated samples with untreated water samples, electronic gates were constructed to differentiate negatively and positively stained populations (Figure 4). 1.5 µL of dye mixture was added to 500 µL of samples. Before analysis, samples were incubated at room temperature in the dark for 15 minutes. Flow-cytometry measurements were performed with a BD FACSAria flow cytometer (BD Bioscience, USA) with 488 nm and 561 nm excitation from a blue and a yellow green laser respectively. Fluorochrome FITC (530±30 nm) and PE Texas Red® (610±20 nm) were used as optical filters in the flow cytometer to measure SYTO®9 and PI respectively. The threshold was set for the size scatter (700 and 200 for FSC and SSC respectively) channel (FITC), and data were acquired on two-parameter dot plots of green fluorescence (FITC) versus sideward scatter (SSC) or green fluorescence (FITC) versus red fluorescence (PE Texas Red®). 1 µm calibration grade Fluoresbrite® Yellow Green Microspheres (4.55 x 10^{10} particles/mL, Polysciences, USA) were used for cell counting. The ratio of live versus dead bacteria was determined for each sample on the basis of differences in cell size and green fluorescence intensities following staining with SYTO®9 [44]. The results were analysed using FlowJo.
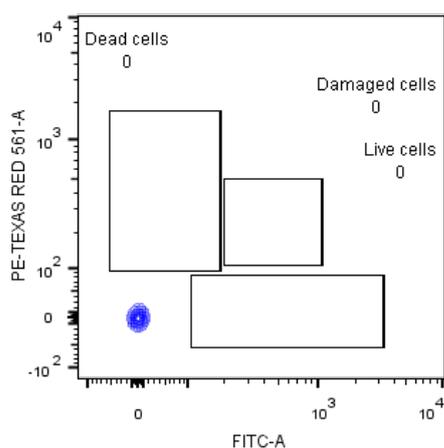
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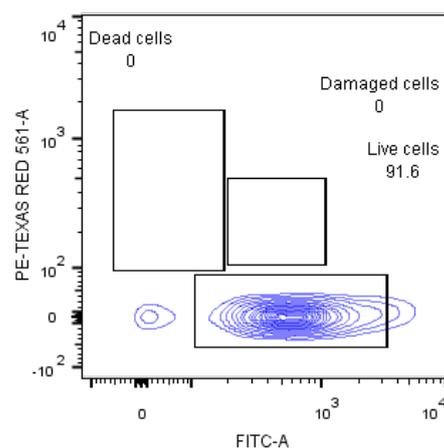
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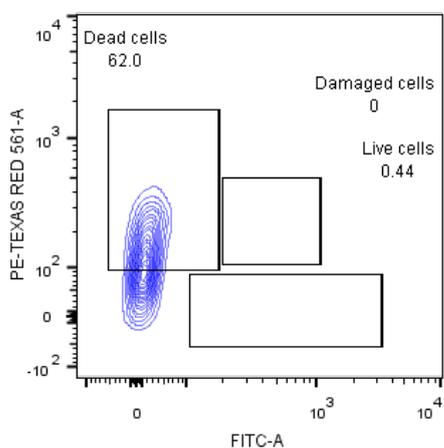
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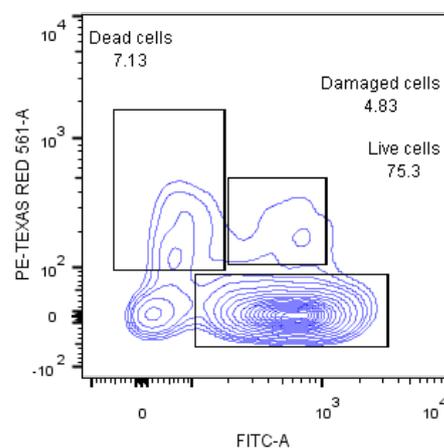
d.

Figure 4. Dot plots generated with flow cytometry: (a) the non-stained cell sample (control 1) used to determine the double negative events, (b) the SYTO®9 only stained cells (control 2) that are shifted to the right and used to adjust the FITC positive events (i.e., live cells), (c) the PI only stained sample (control 3) that are shifted to the top and used to adjust the PE Texas Red® positive events (i.e., dead cells) (d) and a SYTO®9 and PI stained sample with double positives (i.e., damaged cells).

2.7. Chemicals

Several chemicals were used for this study (Table 6).

Table 6. List of chemicals used in the project.

Chemical agent (Supplier) and conditions applied	Applications
Sodium hydroxide, NaOH (pellets, Univar)	pH adjustment Cleaning trials (benchmark)
Hydrochloric acid, HCl (32%, Univar)	pH adjustment Cleaning trials (benchmark)
Citric acid (99.5%, Chem-supply)	Cleaning trials (benchmark)
Sodium nitrite (≤99%, Sigma Aldrich)	FNA preparation Cleaning, preservation and ageing trials
Hydrogen peroxide, H_2O_2 (30 wt %, Merck)	Cleaning trials and ageing trials
Sodium metabisulphite, SMBS (powder, ACROS)	Preservation trials (benchmark)

Free nitrous acid (FNA)

FNA is related to the total nitrite concentration, the pH and the temperature and is calculated as follows [45]:

$$FNA = NO_2^- \text{-N} / (K_a \times 10^{pH}) \qquad \text{(Equation 10)}$$

where K_a is the ionization constant of the nitrous acid ($K_a = e^{-2300/(T+273)}$) and T is the temperature (°C).

The FNA concentration was achieved by varying the nitrite concentration and pH. The pH was adjusted with hydrochloric acid (HCl). Nitrite concentrations were measured by colorimetric method using TNT840 Test Kit (HACH, Lange). The kit can quantify the nitrite concentration from 2.0 mg/L to 20 mg NO_2^--N/L. The absorbance was determined at wavelength 515 nm using a Varian Cary 50 UV-Visible (USA) and directly converted to the nitrite concentration based on the calibration curve (in mg-N/L NO_2^-).

Hydrogen peroxide (H$_2$O$_2$)

The H_2O_2 concentration was determined by means of a spectrophotometric method utilising ammonium metavanadate (NH_4VO_3) [46]. UV_{450nm} was measured using a Cary 50 bio UV–vis absorption spectrophotometer (Varian, Australia). The H_2O_2 residual was eliminated by adding a specific amount of catalase (catalase from bovine liver solution C-100 mg, Sigma Aldrich) before applying routine analysis [47].

3. Application of FNA and hydrogen peroxide for fouling control

This chapter has been partially published with following reference details:

In this part of the project, the possible application of FNA and FNA/hydrogen peroxide as new RO membrane cleaning agents and their ability to remove biofouling and scaling was investigated. For that purpose, two different approaches were used:

- Lab-scale cleaning tests were used for pre-screening the optimum FNA concentrations and pH for biomass and scaling removal;
- Pilot-scale cleaning tests were conducted to validate the new RO membrane cleaning strategy using FNA and benchmark against caustic cleaning strategies.

3.1. Impact of FNA for the removal of RO membrane biofouling and scaling (lab-scale study)

3.1.1. Introduction

The effectiveness of FNA alone or in combination with hydrogen peroxide has been investigated at lab-scale without and with cross-flow. The cleaning tests were conducted using membrane coupons from fouled RO membranes from full-scale plants including industrial wastewater treatment plants, wastewater recycling plants and a desalination plant. The impact of FNA solutions on biofouling and scaling removal efficiency were investigated at different pH and with or without the addition of hydrogen peroxide. After cleaning, the same analytical tools were used to characterise the membrane coupons and evaluate the cleaning efficiency.

Along these tests the performance of the conventional cleaning agents such as sodium hydroxide (NaOH, pH 11.0) and citric acid (pH 2.0-3.0) were evaluated in order to benchmark the efficacy of FNA and FNA/hydrogen peroxide as agent(s) for biofouling and scale control, respectively. These chemical cleaning agents were tested with their optimal conditions in term of dose and pH, as commonly used in full-scale plants.

3.1.2. Material and Methods

3.1.2.1. Reverse osmosis modules and fouling characterization

The cleaning trials were conducted with fouled RO modules (RO1-RO7) collected from full-scale plants. The membranes are listed in Table 4, Chapter 2.1.

Membrane autopsies were conducted on the seven fouled membranes to characterize the fouling layer. Chemical (loss on ignition, elemental analysis, polysaccharide and protein content) and microbial (ATP) analysis were used to describe the fouling deposit. Loss of ignition (LOI) is used to determine the proportion of inorganic versus organic fraction in the fouling layer. The amount of adenosine tri-phosphate (ATP), an energy-rich biomolecule present in all active microorganisms [48], was measured to quantify the active bacterial biomass in the fouling layer. The applicability of ATP concentration as a parameter for the assessment of active biomass present in the RO membrane fouling layer has been previously reported as a robust parameter [49]. Polysaccharide and protein have been reported as extracellular polymeric substances (EPS) indicators [49]. EPS are abundant in biofilms and therefore protein and polysaccharide content were measured as proxy of microbial concentration. Elemental analysis via inductively coupled plasma optical emission spectrometry (ICP-OES) was used to determine the metals content in the fouling deposit. The analytical methods used for membrane autopsies are described in the Chapter 2.2.

3.1.2.2. Lab-scale cleaning trials

Cleaning trials were carried out at lab-scale without cross-flow recirculation (soak cleaning) and with cross-flow recirculation (cross-flow cleaning). The soak cleaning tests were used for pre-screening the optimum FNA concentrations and pH for biomass removal only, while cross-flow cleaning tests were used for assessing the impact of FNA on biomass and scaling removal.

The first set of experiments was performed by soaking membrane coupons (42 cm^2) in 300 mL of cleaning solution for 24 hours. Three replicate experiments were conducted using RO1 membrane. The beakers were placed on an orbital shaker (Ratek large orbital shaker) and agitated at 120 rpm.

The second set of experiments was conducted with cross-flow recirculation using cleaning cells made of Perspex (Figure 5) and RO2-RO7 membranes. Both membrane coupons (150 cm^2 of membrane active surface) and the respective feed spacer were placed in the cleaning cells. Cleaning cells were designed to simulate the configuration of RO filtration system and were operated with cross-flow, without permeate production. The hydraulic performance of RO membranes were conducted in a separate cross flow filtration set-up using Sterlitech CF042 cells and described in detail in the Chapter 2.4.

Figure 5. Photographs of the tailored cleaning cell (left); Cleaning system with five cleaning cells in parallel, cleaning solutions are recirculated by a peristaltic pump with five pump heads for 24 hours.

The cleaning solutions were pumped (Cole Parmer, Masterflex L/S economy drive pump) through the cleaning cell for 24 hours according to the following protocol:

- Rinse with DI water (2 hours) to remove biomass or scaling at the external layer of biofilm.
- Recirculation of cleaning solution (22 hours)
- Rinse with DI water (15 min) to remove the chemicals.

The pump was assembled with five pump heads allowing cleaning cells to run in parallel with similar flows. In order to simulate industry cleaning practice, cross flow velocity of 0.1 m/s was applied for the cleaning trials [27].

The five cleaning solutions used for the biofouling and scaling removal are described in Table 7, Table SI 1 and Table SI 2 in Appendix A. Details of the chemical used are given in Chapter 2.7. Each cleaning tests were conducted with coupons from the same membrane and in replicate (RO2, n=1-3; RO3, n=1-2; RO4, n=2; RO5, n=1-3; RO6, n=2 and RO7, n=2).

After cleaning, some of the membrane coupons (5.6 x 11.2 cm²) were rinsed with Milli-Q water and the recovery of the membrane performance in terms of permeability and salt rejection were assessed in a lab-scale cross-flow filtration unit (Figure 3, Chapter 2.4). The remaining membrane coupons were used to evaluate changes in the fouling layer.

Table 7. List of cleaning solutions and applied conditions used for the cleaning trials.

Cells	Type of cleaning	Chemical agent (Supplier) and conditions applied
#1	Water (control)	DI water
#2	Alkaline (benchmark for biomass removal)	Sodium hydroxide (NaOH), pH 11.0
	Acidic (benchmark for scaling removal)	Hydrochloric acid (HCl), pH 2.0 or 3.0
		Citric acid, pH 2.0 or 3.0
#3, #4, #5	Free nitrous acid (FNA)	50 mgNO$_2^-$-N/L* Sodium nitrite, pH 2.0, 3.0 or 4.0
	FNA/Hydrogen peroxide	50 mgNO$_2^-$-N/L* Sodium nitrite, pH 3.0 0-150 mg/L Hydrogen peroxide (H$_2$O$_2$)

* Concentrations selected based on preliminary results of soak cleaning tests (data not shown)

The biofilm was characterized before and after cleaning using ATP, Polysaccharide and protein measurements and confocal laser scanning microscopy (CLSM) to reveal the cleaning efficiency on biomass removal. The biofilm was removed mechanically from the membrane surface and suspended in MilliQ water using a Braun Oral-B Vitality electrical toothbrush (Procter & Gamble, USA). The protocol is described elsewhere [49]. Total ATP was determined using the BacTiter-Glo^{TM} reagent (Promega Corporation, USA), while Protein and polysaccharide contents were measured using the QuantiPro^{TM} BCA Assay Kit (Sigma Aldrich) and the Phenol-Sulfuric acid method [40], respectively. Confocal laser scanning microscopy was conducted to visualise biofilm and indicate the viability of bacterial cells in biofilms. The presence and removal of scaling was assessed using inductively coupled plasma optical emission spectrometry (ICP-OES) and scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM-EDS). The results were compared to the autopsy results (i.e., initial conditions obtained before cleaning) to assess the cleaning efficiency. All the above mentioned analytical methods have been described in Chapter 2.2.

3.1.3. Results and Discussions

3.1.3.1. Fouling layer characterization

Previous research on biofouling removal has mostly been conducted with laboratory-prepared biofilms using non chlorinated tap water supplied with additional nutrients (e.g., sodium acetate, nitrogen and/or phosphorus source) [25, 49], RO feedwater from full-scale water treatment plant [50] or pure culture of model bacteria such as *Pseudomonas aeruginosa* [21, 51]. The structure and composition of the fouling layer can affect the cleaning efficiency [52]. In addition, hydraulic stress during biofilm growth has been reported to have an impact on its resistance to the detachment during cleaning [53]. In this study, the chemical cleaning of RO membranes was investigated with fouled membranes collected from full-scale plants including industrial wastewater treatment plants (RO2&3), water reclamation plants (RO4&5) and a desalination plant (RO6) in order to develop a better understanding of the applicability of FNA on various fouling matrix.

The main autopsy results of the RO modules are given in Table 8 and pictures are shown in Appendix A, Figure SI 1. Although the RO membranes were collected from different plants, some similarities are observed in fouling characteristics. The fouling layers of membranes RO1 to RO6 consisted largely of organic and biological materials (proteins and polysaccharides), while the RO7 module has been exposed to mostly inorganic foulants (calcium carbonate).The natures of feedwater for the 7 membranes are presented in Table 4.

The analysis of organic versus inorganic fractions by LOI measurements revealed that the fouling layers of RO1 to RO6 membranes mainly consist of organic foulants (>85% of total solid content). The high amount of biopolymer-type compounds, such as polysaccharide (0.07-0.56 gGlucose/m²) and protein (0.04-0.35 gBSA/m²) and the detection of ATP (204-4680 pgATP/cm²), confirm the presence of biofouling on the surface of these membranes. The majority of the total solids are not identified by the analysis. The total proteins and polysaccharides represent ~20% of the total organic content. This can be due to the underestimation of the polysaccharides and proteins content but also because species such as humic acid or amino acid have not been analysed.

Table 8. Membrane autopsy results.

Membrane #	RO1	RO2	RO3	RO4	RO5	RO6	RO7
Loss of ignition (n=5)							
Total solid (g/m^2)	3.3±0. 3	1.9±0.1	3.5±0.3	4.0±0.1	0.8±0.1	0.5±0.2	n.d.
Volatile solid (g/m^2)	3.1±0. 3	1.7±0.1	3.2±0.3	3.7±0.4	0.8±0.1	0.5±0.2	n.d.
Organic fraction (%)	93.1±0.5	88.3±2.7	91.4±2.1	91.1±7.0	100±0.0	100±0.0	n.d.
Biological characterization (n=3)							
Adenosine triphosphate (pg/cm^2)	4680±854	950±288	4234±1543	919±477	204±153	339±159	n.d.
Proteins (gBSA/m^2)	0.35±0.05	0.06±0.01	n.d.	n.d.	0.04±0.01	0.07±0.03	n.d.
Polysaccharides (gGlucose/m^2)	0.56±0.09	0.07±0.01	n.d.	n.d.	0.16±0.03	0.15±0.08	n.d.
Metals (n=5)							
Calcium (g/m^2)	0.02±0.01	0.01±0.00	0.03±0.00	0.03±0.00	0.01±0.01	0.01±0.00	21.68±3.78
Iron (g/m^2)	0.02±0.01	0.04±0.01	0.04±0.01	0.004±0.001	<LOD	<LOD	<LOD
Phosphate (g/m^2)	0.01±0.01	0.005±0.001	0.02±0.01	0.01±0.00	<LOD	0.004±0.002	<LOD
ICP results (0.1-0.01 g/m²)		Na	Mg, S			Mg, Na, S	Ba, K, Mg, Na, S
ICP results (0.01-0.001 g/m²)	K, Mg	Al, K, Mg, Mn, S	K, Mn, Na	Mg, Na, S	Na	K, Mn	
Membrane performances (n=4)							
Pure water permeability (L/m^2.h.bar, 25°C)	5.3±0.3	4.1±0.2	4.4±0.1	2.3±0.1	3.6±0.2	2.5±0.1	1.2±0.4
Salt rejection (%)	96.9±0.3	95.1±1.2	95.7±1.7	98.1±0.7	98.4±0.2	99.0±0.2	98.3±0.0
Fouling	**Severe biofouling**	**Severe biofouling**	**Severe biofouling**	**Severe biofouling**	**Moderate biofouling**	**Moderate biofouling**	**Scaling**

ICP: inductively coupled plasma optical emission spectrometry; n.d.: not determined.

The total solid (TS) content (from LOI analysis) of membranes RO1 to RO4 (1.9 ± 0.1 to 4.0 ± 0.1 g/m^2) is more than double that of membranes RO5 and RO6 (0.8 ± 0.1 and 0.5 ± 0.2 g/m^2 respectively). This indicates the presence of a more severe fouling on RO1, RO2, RO3 and RO4. ATP concentrations are also higher for membranes RO1 to RO4 (4680 ± 854 to 919 ± 477 pg/cm^2) than for RO5 and RO6 (204 ± 153 and 339 ± 159 pg/cm^2 respectively). Protein and polysaccharide concentrations measured as indicators of microbial concentration support this hypothesis. According to these results, RO1 to RO4 could be classified as heavily fouled and RO5 and RO6 as moderately fouled.

Elemental analysis by ICP-OES suggests the presence of calcium ions (Ca^{2+}) in all the organically and biologically fouled membranes, with concentration up to 0.03 g/m² for RO3 and RO4. Iron (likely to be in the form of iron oxide) is also detected in membranes RO1 to RO4, with concentration up to 0.04 g/m² for RO2 and RO3. Multivalent cations promote membrane fouling by humic-like substances as they increase their adhesion force by minimizing their negative charge, creating complexes and bridges [54, 55]. Biopolymer contents of the fouling layer (i.e., polysaccharides, proteins) might also contribute significantly to the incorporation of cations [56]. Fouling layers of RO1 to RO4 contain phosphorus (P) with concentration up to 0.02 g/m² for RO3, which could be present as organic P in form of microbial cell membrane phospholipids and ATP contents [56], confirming the presence of active biofilm on the membrane. ICP data shows only trace amounts of inorganics on RO5 (Ca and Na) and RO6 (Ca, P, Mg, Na, S, K and Mn), which is in accordance with the LOI analysis indicating predominance of organic foulants (100% of TS content).

Membrane RO7 was significantly covered with inorganic scaling. ICP-OES analyses reveal that the fouling layer contains a high quantity of calcium (21.7 ± 0.4 gCa/m^2). Smaller concentrations of barium, potassium, magnesium and sodium were also present. SEM analysis conduct on the membrane surface indicated the presence of crystals (Figure 15a), that showed high Ca, C and O signals via EDS (Energy dispersive x-ray spectroscopy) elemental analysis (Figure 15a). Signals of C (15 ± 2 % as element weight percentage, wt%) and Ca (25 ± 5 %) are approximatively 2 to 4 times lower than signal of O (59 ± 3 %), based on EDS element analysis (n=18). Overall, results of SEM-EDS and ICP-OES elemental analyses performed on RO7 suggest the presence of calcium carbonate.

According to these results, RO1-RO6 are suitable for studying the effect of FNA cleaning on biofouled RO membranes, while RO7 can be used to study the effect of FNA cleaning on scaled RO membranes.

3.1.3.2. Biofouling removal

Impact on total active biomass
The cleaning efficiency was quantified mainly in terms of total biomass or ATP removal (Figure 6 and Figure 7). Control experiments were conducted in soaking conditions with RO1 to verify if the pH adjustment alone had an impact on biofilm removal (Figure 6). While lower pH alone between pH 2.0 to 6.0 is not effective in biofilm removal, addition of nitrite giving rise to the formation of FNA achieves significantly higher biomass removal (*p-values* < 0.05). The ATP residual values decreased when pH decreased (i.e., higher FNA concentration). This control experiment confirms that FNA rather than nitrite is the key agent in the cleaning process. It also confirms that ATP is not removed by low pH alone, e.g. through hydrolysis processes.

Cross-flow cleaning tests were conducted with 50 mgNO$_2^-$-N/L as nitrite concentration (based on the preliminary results of soak cleaning tests [57]). Different pH values (pH 2.0, 3.0 and 4.0) were applied resulting in various FNA concentrations (i.e. 47, 35 and 10 mgHNO$_2$-N/L respectively, T=20°C). Figure 7 shows that FNA also significantly affects the relative ATP abundance in the fouling layer after cleaning using cross-flow recirculation. Lower ATP values are found after cleaning compared to before cleaning. For all the membranes tested, the best cleaning efficiency (>85% of biomass removal) is observed at pH 3.0. Hence, 50 mgNO$_2^-$-N/L at pH 3.0 (corresponding to 35 mgHNO$_2$-N/L) are suggested as optimum conditions for biofouling removal among the conditions tested here.
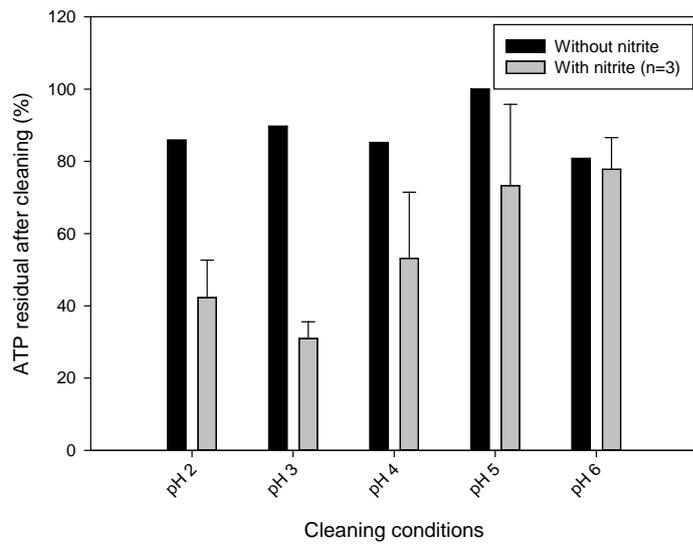
Figure 6. ATP residual after 24 hours cleaning tests performed in soaking conditions (Ratek large orbital shaker, 120 rpm) with the membranes RO1, cleaning tests were conducted with nitrite (grey) and without nitrite (black) and pH between 2.0 and 6.0. Standard test conditions: FNA (50 mgNO$_2^-$-N/L). The error bars show the standard errors of three replicate experiments. No error bars were given when less than three values were used in the calculation of the averages.

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b.

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d.
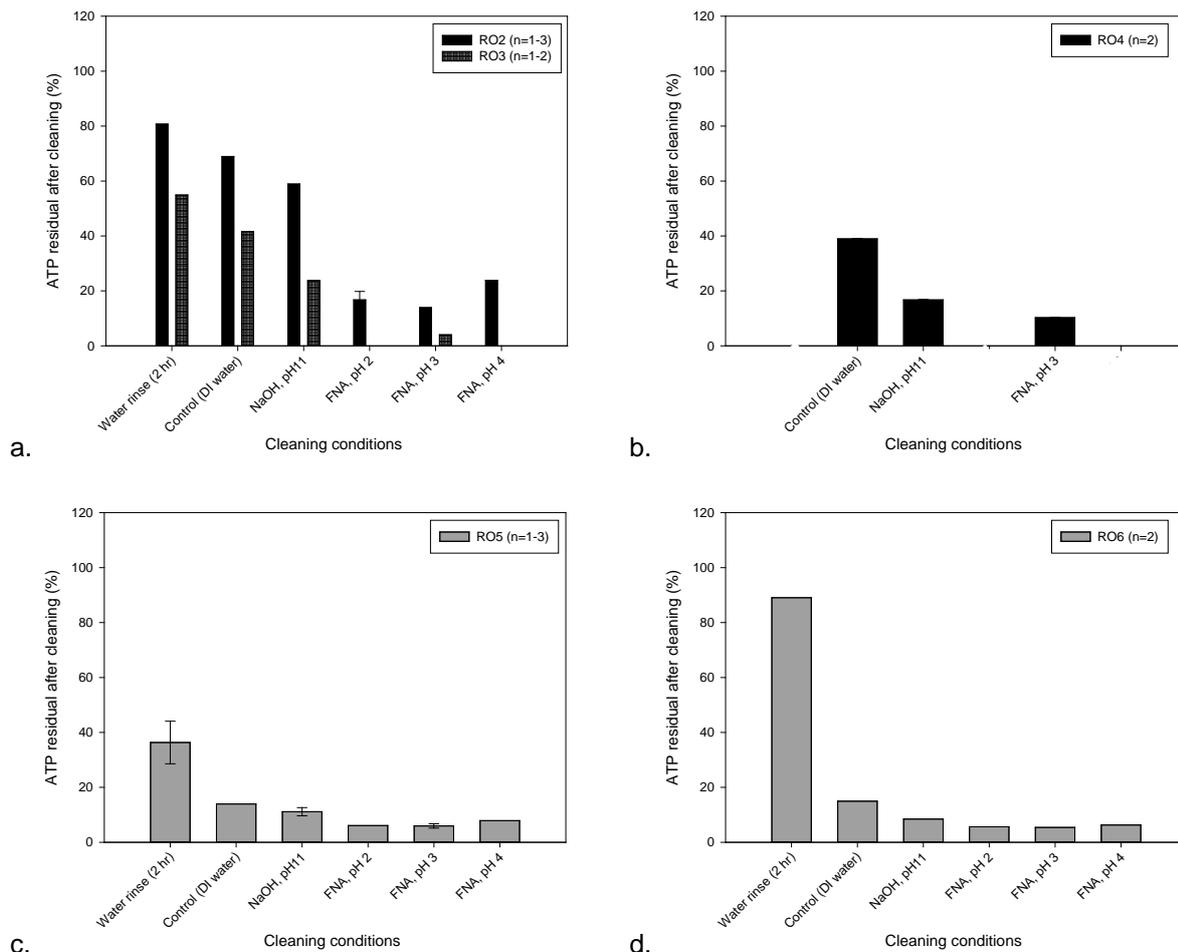
Figure 7. ATP residual after 24 hours cleaning tests performed in cross-flow conditions (cross-flow velocity 0.1 m/s) with the membranes (a) RO2 and RO3, (b) RO4, (c) RO5 and (d) RO6. Cleaning tests were conducted with heavily fouled (black) and moderately fouled (grey) RO membranes. Standard test conditions: FNA (50 mgNO$_2^-$ -N/L). The error bars show the standard errors of three replicate experiments. No error bars were given when less than three values were used in the calculation of the averages.

The effectiveness of FNA is dependent of the degree of membrane fouling. FNA acts more effectively on moderately fouled membrane (94-95% total biomass removal at pH 3.0) than on heavily fouled membrane (86-96% total biomass removal at pH 3.0). Similar observations can be made for water cleaning (control) and NaOH-based cleaning (standard treatment). Even the standard treatment (NaOH, pH 11.0) did not fully recover the membrane, indicating that membranes are fouled beyond their reversibility. According to Hijn *et al.*, biofilms develop chemical and mechanical stress resistances and their removal efficiency will vary with biofilm strength, age/maturity and history (e.g., exposure to cleaning) [49]. In this study, FNA acted more effectively on moderately fouled membranes than heavily fouled membranes, suggesting that early cleaning is preferable, or more extensive cleaning may be required for heavily fouled membranes.

Cleaning efficiency involved both chemical reaction (between the cleaning agents and the foulant) and mass transfer (from bulk phase to fouling layer) mechanisms [54]. It is possible that heavily fouled membranes have a compacted biofilm layer resulting in lower mass transfer for the permeation of the cleaning agent into the fouling layer [52]. Inversely, biofilms on the moderately fouled membranes were easily disrupted as the transfer of the cleaning solution in the fouling layer was enhanced.

Furthermore, cleaning results generated with and without cross-flow recirculation are also compared in Figure 8. A higher biomass removal efficiency (based on ATP values) is observed with cross-flow cleaning compared to soak cleaning (73-92% versus 46-77%). Therefore, the cross-flow cleaning mode is a more efficient strategy for FNA-based cleaning. The application of hydrodynamic shear due to cross-flow recirculation results in an increase of cleaning efficiency (soak versus cross-flow cleaning test results). However, biomass removal by chemical cleaning solutions is significantly higher compared to removal with a solution prepared with water only (*p-values* < 0.05). This implies that it is the combination of physical (i.e., shear) and chemical actions that aid in the biofilm removal process. In these experiments, both the soaking and cross flow conditions show no advantage or disadvantage of FNA relative to NaOH (pH 11.0) as a cleaning agent.
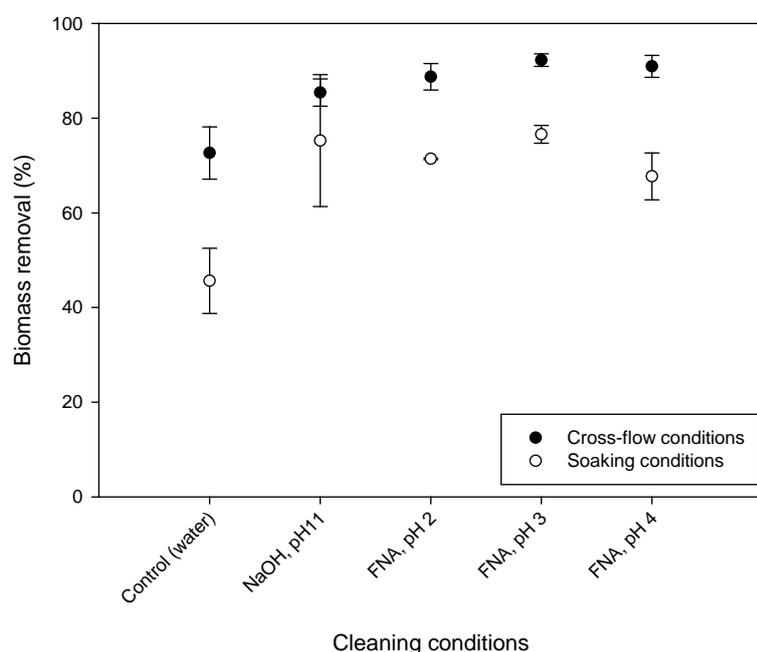
Figure 8. Biomass removal (%, based on ATP values) after 24 hours cleaning tests performed in (a) soaking (Ratek large orbital shaker, 120 rpm, RO1) and (b) cross-flow conditions (cross-flow velocity 0.1 m/s, RO2-RO6). Standard test conditions: FNA (50 mgNO$_2^-$-N/L). The error bars show the standard errors of replicate experiments (n=3-10).

Viability of bacteria remaining on membrane surfaces

Microscopic assessment of LIVE/DEAD-stained bacterial cells was used to investigate whether FNA and other cleaning solutions influenced bacteria viability in the fouling layer (Figure 9). This method was only applied to the moderately fouled membranes (RO5&6), due to lower biofilm density resulting in better quality of images. The CLSM images of RO5&6 before and after cleaning are presented in Figure 10 and Figure 11. After 24 hours cleaning, the proportion of viable cells on the membrane surface decreased for all cleaning solutions tested. These results are in accordance with biomass removal measured as ATP and presented in Figure 7.

The percentage of viable cells in the biofilm on RO5 membrane in the presence of FNA, pH 3.0 (32%) is significantly lower than that in the presence of NaOH (58%) (*p-values* < 0.05). These ratios are higher than previously reported in the literature for anaerobic sewer biofilm (FNA at 0.255 mgN/L for 6 h can induce about 80% of microbial inactivation) [35] but this could be explained by the more compact biofilm in RO application due to the applied pressure. The cleaning trials conducted with the RO6 membrane from a desalination plant show better bacteria killing efficiency. The proportions of live cells for the membrane RO6 are as follows: before cleaning (60%) > water rinse for 2 hours (52%) > water rinse for 24 hours (41%) > NaOH (38%) > FNA (6-7%). Examination of the confocal images reveals that the biofilm on membrane RO5 is denser than on membrane RO6 and might be more challenging to disrupt. However, CLSM analysis performed for the two membranes demonstrated the biocidal effect of FNA is higher than that of NaOH. A higher-level of inactivation of bacteria remaining on the membrane is expected to delay their regrowth. Quick biofilm regrowth results in a repetition of the biofouling-related system failure. Although, it is difficult to avoid bacteria regrowth, the use of a biocide can slow down/minimise this phenomenon [12].

It is important to mention that this staining protocol only show the ratio of cells with an intact versus a damaged cell membrane. This means that that the determined percentage of viable cells is a conservative estimate, but more cells may actually be dead.
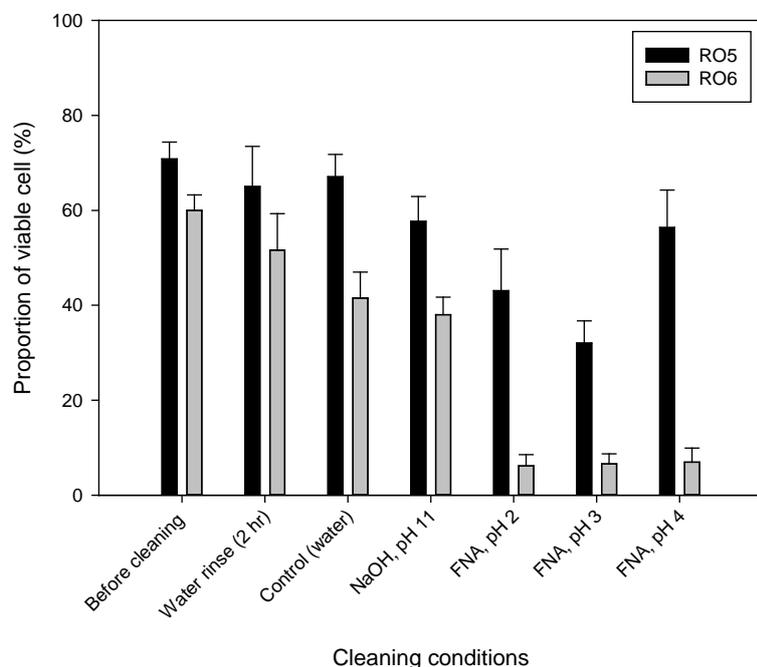
Figure 9. Proportion of viable cells in membrane biofilm before and after 24 hours cleaning tests for the membranes RO5 and RO6. Standard test conditions: FNA (50 mgNO$_2$-N/L), cross-flow velocity 0.1 m/s. The error bars show the standard errors of 15 to 60 CLSM.
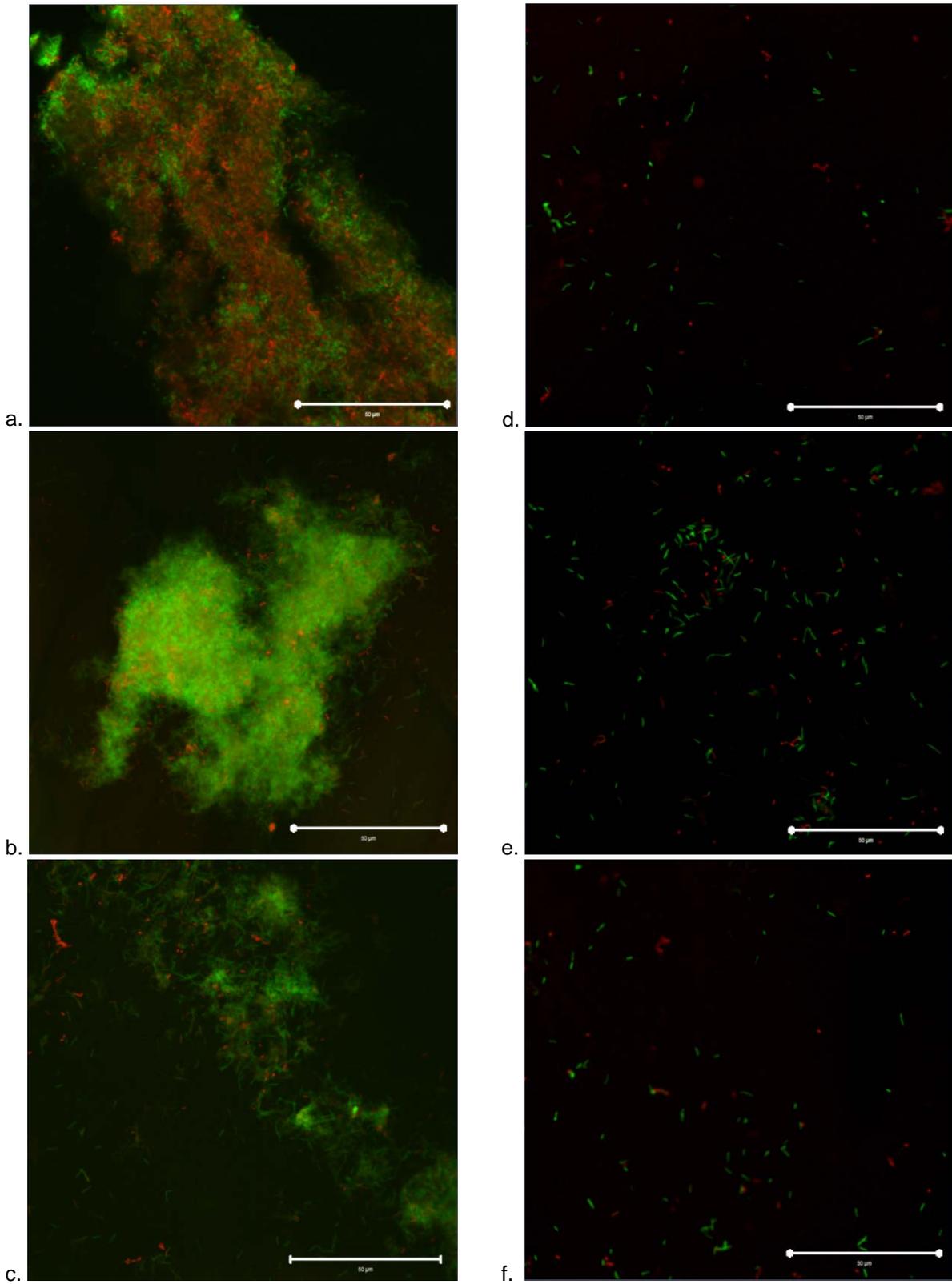
Figure 10. CLSM images of RO5 membrane, (a) before cleaning and after cleaning with (b) Control (Water), (c) NaOH, pH 11, (d) FNA, pH 2, (e) FNA, pH 3 and (f) FNA, pH 4. Standard test conditions: FNA (50 mgNO$_2^-$-N/L), cross-flow velocity 0.1 m/s. Live cells are stained in green with the DNA specific dye SYTO®9 and the dead cells are stained in red with PI.
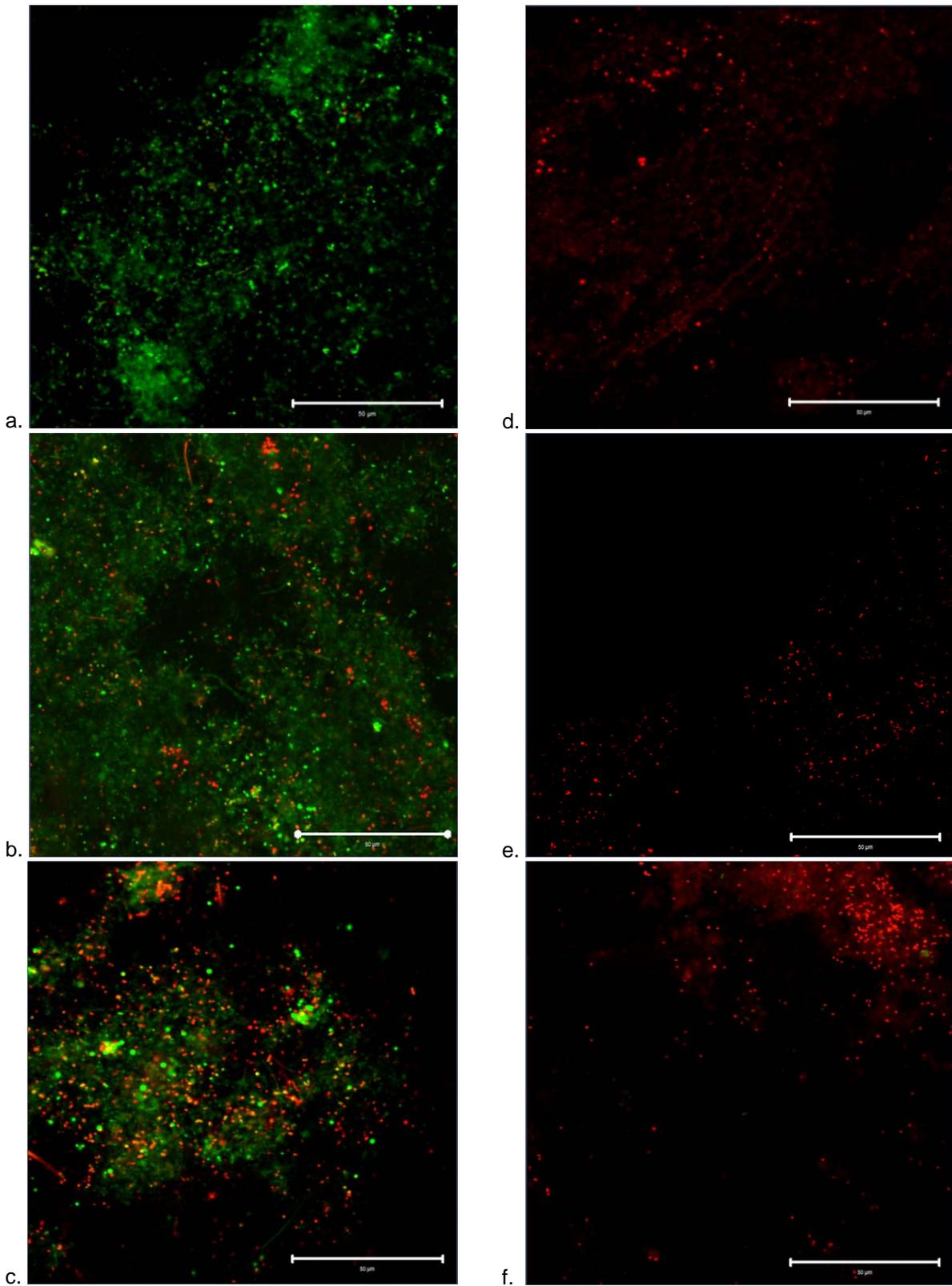
Figure 11. CLSM images of RO6 membranes, (a) before cleaning and after cleaning with (b) Control (Water), (c) NaOH, pH 11, (d) FNA, pH 2, (e) FNA, pH 3, and (f) FNA, pH 4. Standard test conditions: FNA (50 mgNO$_2^-$-N/L), cross-flow velocity 0.1 m/s. Live cells are stained in green with the DNA specific dye SYTO®9 and the dead cells are stained in red with PI.

Polysaccharide and protein removal

In addition to ATP measurement, protein and polysaccharide content was measured for the moderately fouled membranes (RO5&6) to investigate the impact of FNA and other cleaning solutions on organics (Figure 12). Although polysaccharides and proteins are components of both bacteria and EPS matrix, they are usually measured as proxy of EPS.

a.

b.
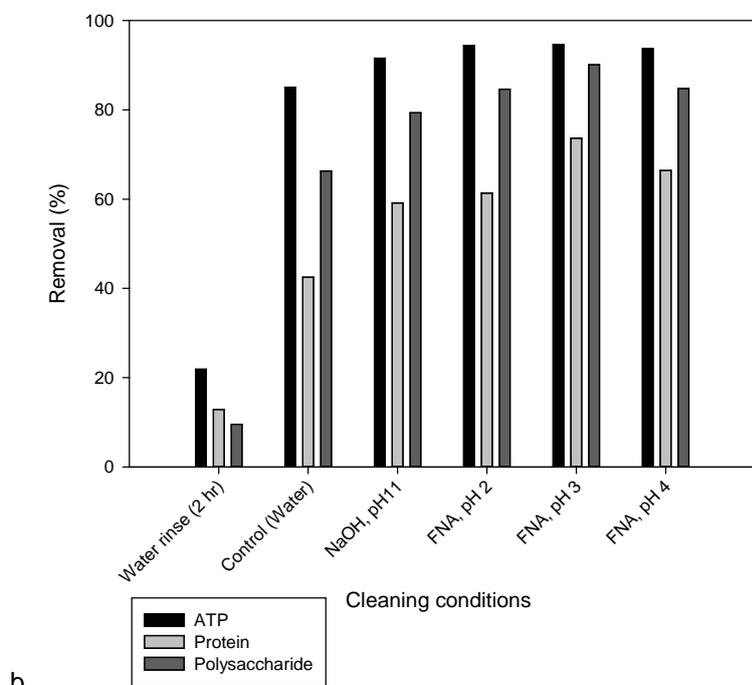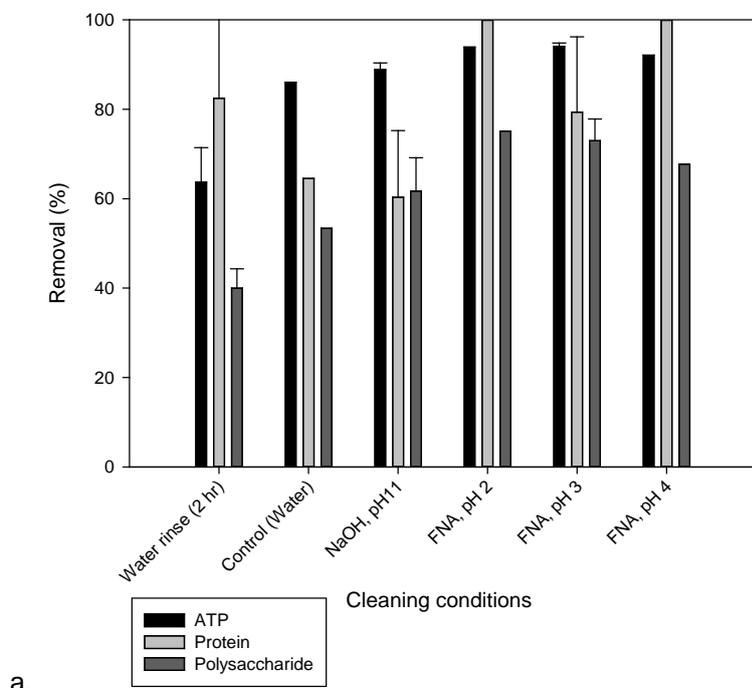
Figure 12. Biomass removal (%, based on ATP values) and protein and polysaccharide removal (%) after 24 hours cleaning tests for the membranes (a) RO5 and (b) RO6. Standard test conditions: FNA (50 mgNO$_2^-$-N/L), cross-flow velocity 0.1 m/s. The error bars show the standard errors of three replicate experiments. No error bars were given when less than three values were used in the calculation of the averages.

The removal rate of polysaccharide correlates with biomass removal rate (based on ATP values) after 24 hours cleaning tests. This suggests that FNA has an effect on both bacteria and EPS matrix. Again, the optimal cleaning conditions are observed for pH 3.0. However, each FNA cleaning solution shows a higher ATP removal (92-95%) compared to the polysaccharides removal (68-90%), implying that FNA is more efficient for bacteria than for organics removal. The resistance of the EPS matrix against chemical cleaning was demonstrated by Hijnen et al. [49].

NaOH is known to be efficient for colloidal, organic and biofouling removal and would be expected to show high polysaccharide and protein removal. However the standard cleaning solution in the trials shows average organic removals of only 59-60% and 62-79% of proteins and polysaccharides respectively, which is similar or lower than organic removal observed after FNA cleaning.

Based on the autopsy results, the RO5 and RO6 membranes present similar fouling (in terms of ATP, LOI and ICP results), although the two membranes are from different origins (Municipal water recycling plant versus seawater desalination plant) and quite different fouling removal results. This result clearly supports that the cleaning efficiency is affected by the nature and structure of the fouling layer.

Cleaning efficiency of FNA in combination with H_2O_2
RO3, RO4 and RO5 were used to study the effect of hydrogen peroxide on FNA cleaning efficiency. Figure 13 presents the biomass removal of three RO modules by using FNA in combination with H_2O_2. For all three membranes, FNA or FNA/H_2O_2 achieved better ATP removal than conventional cleaning solutions (NaOH). However, no significant improvement was observed by adding H_2O_2 in addition to FNA (*p-values > 0.05*). This is in accordance with cleaning experiments in soaking conditions [57]. In previous experiments reported on sewer biofilm [58], the biocidal effect of FNA was increased by 43–51% when FNA was combined with hydrogen peroxide compared to FNA alone. However, in this test, low FNA concentrations (e.g. 0.05 mg-N/L) were applied and the biocidal effect efficiency became smaller for higher FNA concentrations. In our project, relatively high FNA concentrations were applied and might explain why no significant improvements were observed when FNA was combined with H_2O_2.
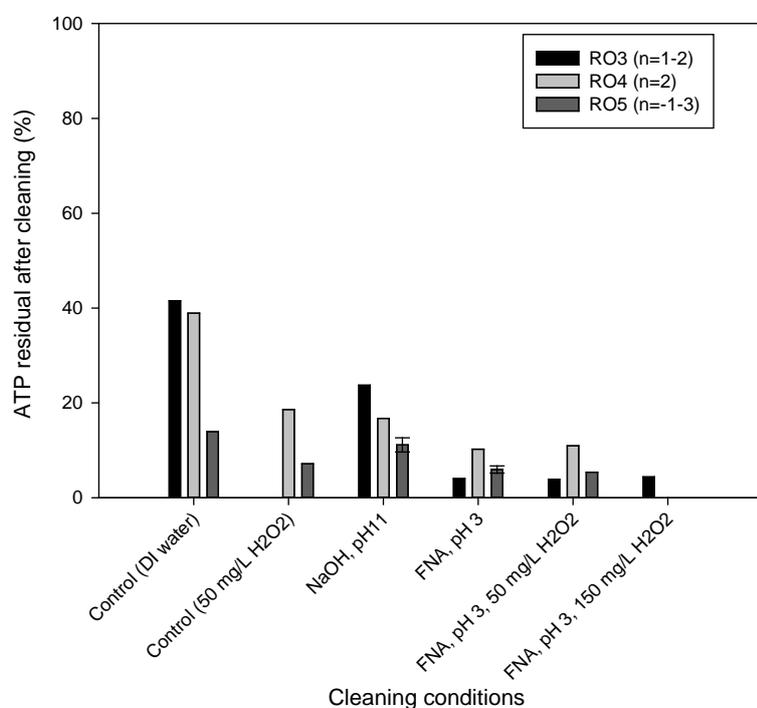
Figure 13. ATP residual after 24 hours cleaning tests for the membranes RO3, RO4 and RO5. Standard test conditions: FNA (50 mgNO$_2^-$-N/L), cross-flow velocity 0.1 m/s. The error bars show the standard errors of three replicate experiments. No error bars were given when less than three values were used in the calculation of the averages.

Hydraulic performances

Cleaning efficiency was also quantified in terms of membrane hydraulic performances (permeability recovery and salt rejection improvement). However, the results were inconclusive. The filtration trials on membrane coupons revealed no significant impact of different cleaning procedures on the performance of the membrane in terms of permeability and salt rejection for all cleaning conditions applied (Table SI 3, Appendix A). For most of the tested membranes, the permeability and salt rejection after cleaning remained constant. All permeability changes remained non-distinguishable from the natural variability of the membrane used. Membrane producers typically specify the permeability of modules with a tolerance of ±15–20% of the nominal value due to membrane manufacturing and experimental error. In addition, biofouling is mainly characterised by an increase of the differential pressure (Pressure difference between the feed and concentrate side) which is not measurable with the lab-scale filtration cells used in this study. The impact of FNA cleaning on the hydraulic performance was addressed at larger scale (see pilot-scale study).

3.1.3.3. Scaling removal

Cleaning at low pH is useful to control calcium carbonate scaling ($CaCO_3$) and possibly iron fouling (i.e., iron oxide/hydroxide) [27]. As an acid, it is anticipated that FNA will also be effective for removing inorganics from the membrane surface via hydronium ion activity. Tests were carried out to establish the efficacy of FNA to remove scaling, i.e., to verify that the addition of nitrite does not alter the efficiency of commonly used cleaning solutions in scaling mitigation (Table SI 2). A severely fouled membrane module from a full-scale coal seam gas water treatment plant (RO7) was used for this study. Based on the autopsy results, the fouling layer is mainly composed of calcium carbonate (data discussed in Chapter 3.1.3.1). According to standard manufacturers' cleaning procedures, HCl and citric acid at low pH are recommended for cleaning RO membranes with severe $CaCO_3$ fouling [27, 31]. Therefore, the efficiency of FNA for scaling removal was compared with these two alternative cleaning solutions (i.e., HCl (pH 2.0-3.0) and citric acid (pH 2.0-3.0)). Along with these cleaning agents, DI water and 10 v/v % of nitric acid (HNO_3) were applied as controls. A solution of 10 v/v% HNO_3 at pH 0.5 was used for ICP-OES analysis to dissolve/digest the fouling material, and it is reasonable to assume that $CaCO_3$ scaling would be completely removed from the membrane at this extreme pH level.
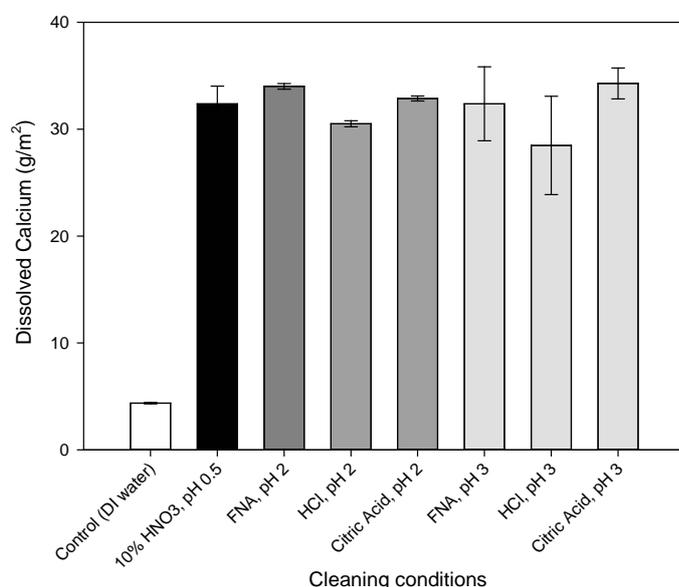
Figure 14. Dissolved calcium content removed from the membrane surface after 24 hours cleaning tests with membrane RO7. Standard test conditions: FNA (50 mgNO_2^--N/L), cross-flow velocity 0.1 m/s. The error bars show the standard errors of four measurements from two replicate experiments.
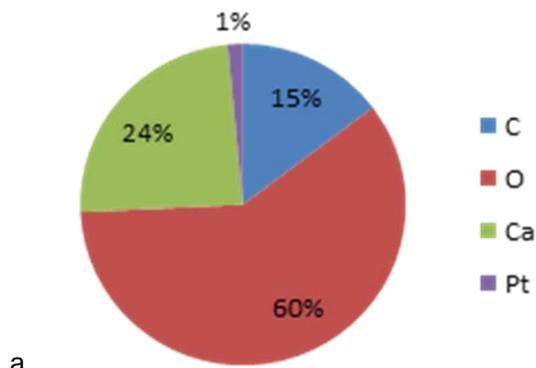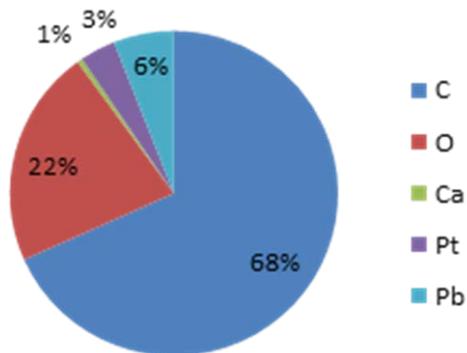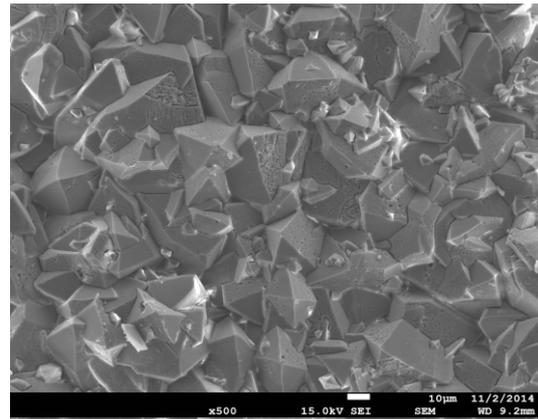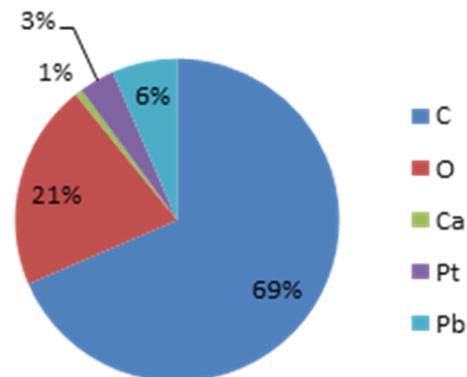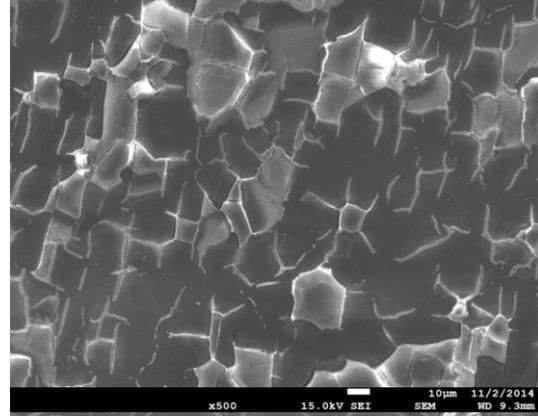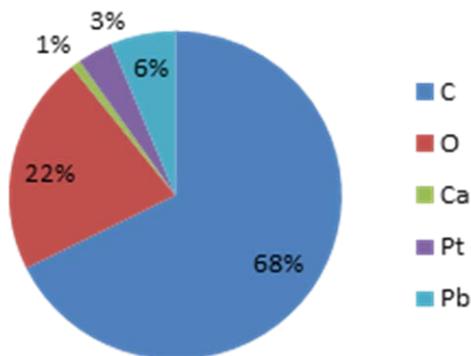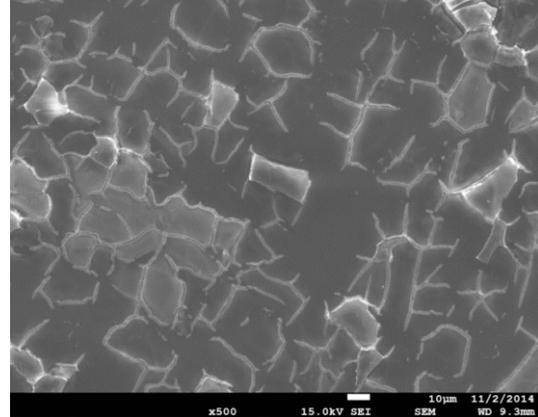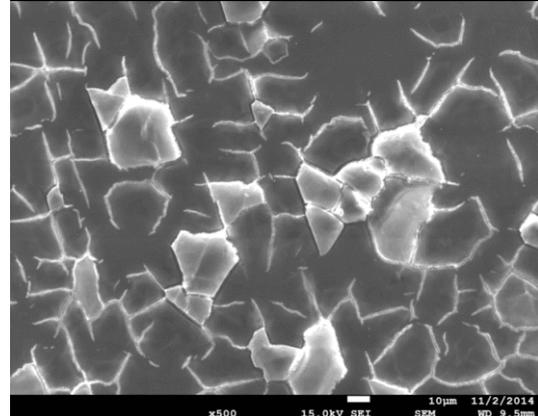
Figure 15. Element wt% (from SEM-EDS analysis) and SEM images of calcium carbonate scaled RO membrane (a) before and (b-d) after cleaning. Test conditions: (b) HCl, pH 3; (c) FNA (50 mgNO$_2^-$-N/L), pH 3; (d) Citric acid, pH 3; cross-flow velocity 0.1 m/s, No. of analysis = 5.

Figure 14 presents the dissolved calcium content (based on ICP-OES results) removed from the membrane surface after the 24 hour cleaning tests. SEM-EDS analyses were also conducted on the membrane before and after cleaning for all cleaning solutions at pH 3.0 (HCl, FNA and citric acid), which is also the pH selected for biofouling removal. SEM images and element wt% distribution are available in Figure 15.

The results shows that FNA at pH 2.0 and 3.0 are as effective as commonly used descaling agents (HCl and citric acid), implying that addition of nitrite in the acid cleaning solutions does not modify its efficiency to remove scaling. Water removed only 4.4 ± 0.1 g/m^2 of calcium from the membrane surface, while the other control (10 v/v % HNO$_3$ at pH 0.5) successfully removed 32.4 ± 1.7 g/m^2. Higher calcium removal is observed compared to the autopsy results (32.4 ± 1.7 versus 21.7 ± 3.8 g/m^2), likely due to the introduction of shear rate at the membrane surface, improving the solubility of the fouling layer. All cleaning solutions show similar calcium removal ranging between 34.3 ± 1.4 and 28.5 ± 4.6 g/m^2. These values are comparable to the calcium removed by the control (HNO$_3$), which suggests that maximum calcium removal is reached with all the cleaning solutions. This conclusion is supported by the SEM-EDS results (as SEM images and element weight percentage (wt %)) presented in Figure 15. The element wt% reveals that only 1% of calcium element is present on the membrane after cleaning with HCl, FNA and citric acid adjusted at pH 3.0 versus 24% before cleaning.

The cleaning tests conducted with the scaled membrane RO7 (no organic fouling) showed no additional benefit of using FNA rather than HCl or citric acid for scaling removal. However, in the presence of combined scaling/organic fouling the presence of FNA can lead to a better organic fouling/scale removal compared to low pH alone.

3.1.3.4. Proposed mechanisms

FNA has an effect on active bacteria cells and organics as evidenced by ATP and polysaccharide & protein removal, respectively, and also descales. NaOH at pH 11.0 removes organics by hydrolysis and solubilisation of the fouling layer [52], while the actual FNA cleaning mechanism remains unknown.

CLSM analysis showed the inactivation of cells on the membrane after FNA application. The biocidal effect of FNA has already been demonstrated on anaerobic sewer biofilm and waste activated sludge applications [34, 59]. Jiang *et al.* suggested the role of reactive derivatives, such as dinitrogen trioxide (N$_2$O$_3$), nitrogen dioxide (NO$_2$) and nitric oxide (NO), which can be generated when FNA is formed from nitrite under acidic conditions [34]. While N$_2$O$_3$ and NO$_2$ can disrupt the function of proteins or induce cell damage, respectively [60], NO is known to be a highly toxic compound for bacteria [61]. It has been reported that NO is also able to induce the dispersion of biofilm (e.g., *Pseudomonas aeruginosa* biofilms and multi-species biofilms from water distribution and treatment systems) [62, 63].

In addition to the biocidal effect, acidified nitrite (FNA) can remove organics such as proteins and polysaccharides, which can be mainly associated to EPS. EPS acts as a matrix holding microbial cells together and protecting them from external aggression/stress. Consequently it is important for a cleaning agent to remove EPS and not only inactivated bacteria cells. Biofilms are a combination of organic, inorganic and biological species. Membrane autopsies reveal the presence of multivalent ions (e.g., Ca, Fe, Mg) in biofilm, which can bind with organic molecules. As an acid, FNA was shown to dissolve this inorganic matrix (e.g., divalent cations) embedded in the biofilm thereby helping to break down the structural integrity of the fouling layer and to disperse/weaken the biofilm making it easier to remove. The metal content results (via elemental analysis using ICP-OES) before and after cleaning were low (close to the limit of detection) and no difference could be noticed to support this hypothesis. However, previous research conducted on toxic metal removal from acidified sludge and the breakdown of EPS in waste activated sludge using FNA suggested that FNA likely reacts with EPS leading to its breakdown [64, 65]. Zhang *et al.* suggested that FNA may change the chemical structure of EPS and has an impact on UV absorbing substances [65], while Du *et al.* verified that FNA efficiency resulted from the release of organically bound metals [64].

Ultimately, the effect of FNA on fouling removal is likely to be a combination of all these factors and consequently makes FNA a suitable cleaning agent for the removal of biofouling and scaling in one step. However, biofouling is dependent on feed water characteristics and processes, consequently no

unique cleaning strategy can be applied. Cleaning conditions, such as cross-flow velocity, duration or temperature need to be optimised for each individual plant, depending on feed/plant conditions. A long-term pilot-scale study would be needed to further investigate the economic potential and practical application of FNA as a new RO cleaning agent.

3.2. Impact of FNA for the removal of RO membrane biofouling (pilot-scale study)

3.2.1. Introduction

The optimal FNA-based cleaning strategy selected from the lab-scale cleaning tests (i.e., 50 $mgNO_2^-$-N/L, pH 3.0) was tested with fouled RO membrane modules generated at pilot-scale.

A biofouling protocol was defined in order to generate fouled RO membrane at pilot-scale and then to determine the efficiency of the cleaning agents and develop an application protocol. For this, a pilot unit with 4-inch commercially available thin-film composite RO membranes were fed with a RO feedwater from a full-scale reclamation plant (municipal secondary effluent filtered through PALL microfiltration system using 0.1 micron PVDF membranes). The performance (i.e., permeability, salt retention and pressure drop) were monitored during the duration of the experiments. Then, the efficiency of FNA on fouling removal was investigated at two different temperatures and benchmarked against caustic cleaning (NaOH, pH 11.0).

3.2.2. Material and Methods

3.2.2.1. Description of the Pilot Plant

A pilot plant (donated by Seqwater as its in-kind contribution to the project) was used to generate fouled RO membranes. The pilot was located at the Luggage Point Water Reclamation Plant (Myrtletown, QLD, Australia) operated by Queensland Urban Utilities. The process flow diagram for the membrane pilot plant and pictures are shown in the Figure SI 2, Appendix B and Figure 16, respectively.

The pilot plant unit was fed with feedwater for the full-scale RO units, transported via gravity to the pilot-plant feed tank (500L). The feed tank supplies three pressure vessels (CodeLine DIVISION, USA) operated in parallel, called RO-A, RO-B and RO-C below, each equipped with one spiral wound element (4-inch diameter ESPA-2, Hydranautics, USA). The three units have been designed identically in terms of instrumentations, materials, pipe diameter. The feedwater was pumped via a high-pressure pump (CRNE10-22, Grundfos, Denmark) positioned after a 5 μm catridge filter (CC100 Cartridge filter vessel and C50 Cartridge filter, Waterco–Trimline, Australia). The RO lines were operated at 7.0 bar feed water pressure, 20 L/m^2h average permeate flux and 11% recovery rate. The feedwater flow rate for the RO system was 1.4 m^3/h with a cross-flow velocity of 0.08 m/s. The wastes (i.e., concentrates and permeates) and overflow from the feed tank were discharged to the underground storage tank through pipes and the effluents returned to the wastewater treatment plant inlet.

Table 9. Initial characteristics of the high-pressure membranes applied in the pilot-plant study.

Parameter	Characteristics given by the manufacturer	New membrane		
		RO-A	RO-B	RO-C
Initial RO Permeability (Kw_0)* (L/h.m².bar at 25°C) (n=3)	-	6.1±0.4	5.5±0.5	6.7±0.3
Initial RO salt rejection (R_0)** (%) (n=3)	99.6 (99.4 min.)***	98.6±0.0	98.9±0.0	99.3±0.0

*Based on the following conditions: DI-water, 6.5±0.1 bar applied pressure, 31.2±2.5 °C operating temperature, 10.5±0.3% permeate recovery; ** Based on the following conditions: 1500ppm NaCl solution, 6.5±0.2 bar applied pressure, 31.4±1.3 °C operating temperature, 8.7±0.3% permeate recovery; ** Based on the following conditions: 1500ppm NaCl solution, 10.3 bar (150 psi) applied pressure, 25 °C operating temperature, 15% permeate recovery.

The RO membrane characteristics provided by the manufacturer and the initial characteristics are given in Table 9. The salt rejections of the new membranes were lower than the characteristics given by the manufacturer (98.6-99.3% versus 99.4% salt rejection). The operating pressure (7 bar) is lower than applied by the manufacturer (10 bar), which may explain the low salt rejections. A higher pressure would lead to increased water flux. The water dilutes the salt and decreases the permeate concentration, leading to a higher rejection.

Figure 16. Photographs of the RO pilot-plant.

The pilot plant had not been in use by Seqwater for a long time and many damaged materials and sensors had to be replaced in addition of electrical plugging, plumbing/hydraulic connection, testing and tagging of the switch board and testing sensors (flow meters, pressure transmitters). The four existing fibreglass pressure vessels designed to suit four 4.0" x 40" membranes have also been replaced by three fibreglass pressure vessels designed to suit one 4.0" x 40" membrane in order to reduce the number of membrane and feed flow necessary for the study.

3.2.2.2. Pilot monitoring

The feedwater samples collected weekly after the 5 μm cartridge filter were subjected to physicochemical analyses. Parameters analysed included pH, Conductivity, Turbidity, Silt Density Index (SDI_{15}) and TOC, with analytical methods described in Chapter 2.5. The feed water quality data are presented in Table 10.

Technique	Element	Average concentration
Conductivity (uS/cm)		2588±591
Temperature (^{o}C)		28.8±2.5
pH		7.3±0.3
Turbidity (NTU)		0.3±0.2
TOC (mg/L)		9.7±1.0
TN (mg/L)		4 ± 2 (1.5±0.9 TON)
TKN (mgN/L)		2.7±1.6
TP (mgP/L)		2.9±2.0
FIA (mg/L)	$N\_NH_4^+$	1.1±1.2
	$N\_NO_3^-$	2.1±1.7
	$N\_NO_2^-$	0.3±0.2
	$P\_PO_4^{3-}$	3.5±2.0
IC (mg/L)	Cl^-	582 ± 190
	SO_4^{2-}	40±10
ICP-OES (mg/L)	Ca	40±5
	Fe	0.12±0.04
	K	30±7
	Mg	43±14
	Na	357±108
	P	3.3±2.2
Free Cl (mgCl$_2$/L)		0.06±0.02
SDI$_{15}$		3.9±1.3

TN = Total Nitrogen, TON = Total Organic Nitrogen, TKN = Total Kjeldahl Nitrogen, TP = Total Phosphorus, FIA = Flow Injection Analysis, IC = Ion Chromatography, ICP-OES = Inductively Coupled Plasma Optical Emission Spectrometry, SDI = Silt Density Index.

The hydraulic membrane performances (i.e., differential pressure drop, filtration flow rate, permeability and salt rejection) with regards to occurrence of membrane fouling were also monitored weekly. The formula to calculate the permeability (K_w, L/m^2.h.bar, 25°C) and salt rejection (R, %) are described in Chapter 2.4.

3.2.2.3. Cleaning trials

The fouling trial was stopped after 4 months and 2 months and the fouled RO modules were cleaned (cleaning #1 and #2, respectively) following the protocol given below, using the spiral-wound module set-up described in Chapter 2.4.

- Measuring the permeability and salt rejection before cleaning (Kw_1 and R_1),
- Rinsing with DI-water,
- Recirculating the cleaning solution for 1h (approx. 37 L/min, <4bar),
- Soaking for 14 hours,
- Recirculating the cleaning solution for 1h (approx. 37 L/min, <4bar),
- Rinsing with DI water,
- Measuring the permeability and salt rejection after cleaning (Kw_2 and R_2).

For the first cleaning trial, RO-A and RO-B were cleaned using 60L FNA-based cleaning solution (i.e., 50 mgNO$_2^-$-N/L, pH 3.0) at 25 and 35°C, respectively, while RO-C was cleaned using NaOH, pH 11.0 at 35°C. For the second cleaning trial, RO-A was cleaned using NaOH, pH 11.0 at 35°C, while RO-B and RO-C were cleaned using FNA-based cleaning solution at 35 and 25°C, respectively. After cleaning, the RO modules were re-installed in the RO pilot-plant and run under the same conditions to evaluate the impact of cleaning.

3.2.3. Results and Discussions

3.2.3.1. Fouling development

Results from membrane pilot plant operation between January 2014 and August 2015 are presented and discussed in this section. Figure 17 presents the normalised permeability, pressure drop and salt rejection for the three RO membranes.

a.

b.

c.
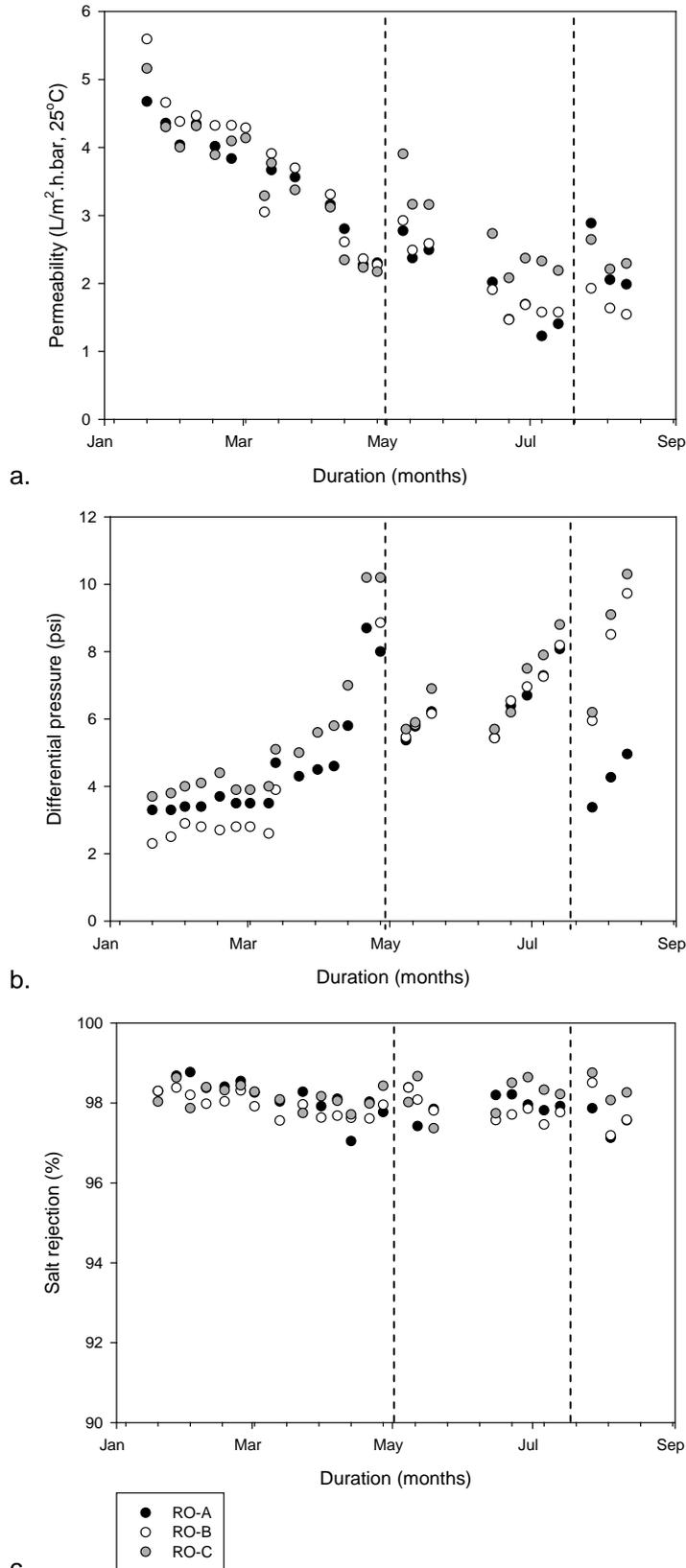
Figure 17. (a) Normalised permeability, (b) differential pressure and (c) salt rejection for the RO membranes from the pilot-plant. Standard test conditions: feed water pressure 7.0 bar, average permeate flux 20 L/m²h and cross-flow velocity 0.08 m/s. The dashed line represents the cleanings.

No significant changes could be observed in the monitored water quality parameters during the pilot-plant operation. The average quality of RO feedwater, after the 5 μm cartridge pre–filtration, was as follows: SDI_{15} ≤5; TOC: 9.7 mg/L; TN: 4.2 mg/L; TP: 2.9 mg/L. The RO feedwater in this study has high carbon and total phosphate concentrations, suggesting that the RO feedwater could significantly promote membrane biofouling in the pilot plant.

During the four-month operation, the permeability decreased of 50%, 40% and 40% for RO-A, RO-B and RO-C, respectively, while no variation in salt rejection was observed. The differential pressure increased by 2.4, 3.9 and 2.8 times for RO-A, RO-B and RO-C, respectively. The decreased permeate flow, unchanged salt rejection and increased differential pressure are typical symptoms for biofouling..

The RO-pilot was moved at the Luggage Point Water Reclamation Plant in December 2013. However it only started running continuously in January 2015 due to several technical difficulties. As mentioned before, the pilot plant had not been in use by Seqwater for many years and several materials and sensors broke down and had to be replaced. In addition of repair work, some maintenance performed on the full-scale plant also prevents the pilot to be run continuously and it was difficult to predict how long it will take for the membrane to be fouled. This resulted in delay on the project and only one cleaning tests could be carried out.

3.2.3.2. Cleaning trial

The effect of the cleaning process on the cleaning efficiency is shown in Table 11 and Figure SI 3, Appendix B. After the first cleaning, the differential pressure dropped for the three cleaning applied (28-38% for FNA-based cleaning and 44% for the NaOH-based cleaning) and reached similar differential pressures 5.4-5.7 psi. FNA cleaning did also increase permeability by 20-29%. Again, after the second cleaning, the differential pressure dropped for the three types of cleaning. However, the pressure after NaOH-based cleaning was higher than for FNA-based cleaning (58% versus 27-30%). Similarly, the permeability recovery was more significant after caustic cleaning (105%), than FNA-based cleaning (74% and 21% for cleaning at $35^{o}C$ and $25^{o}C$, respectively. The three cleaning strategies did not impact the salt rejection.

Table 11. Permeability and salt rejection gain and differential pressure drop after cleaning.

Cleaning trial	Cleaning strategy	RO feed, Pilot-plant				
		Permeability gain*	Salt rejection gain**	Differential pressure drop***	Differential pressure (psi)	
					Before cleaning	After cleaning
#1	RO-A (FNA-based, $25^{o}C$)	20%	1%	33%	8	5.4
	RO-B (FNA-based, $35^{o}C$)	29%	0%	38%	8.9	5.5
	RO-C (NaOH-based, $35^{o}C$)	80%	0%	44%	10.2	5.7
#2	RO-A (NaOH-based, $35^{o}C$)	105%	0%	58%	8.1	3.4
	RO-B (FNA-based, $35^{o}C$)	22%	1%	27%	8.2	6.0
	RO-C (FNA-based, $25^{o}C$)	21%	1%	30%	8.8	6.2

* Permeability gain after cleaning = $\dfrac{Kw_{T2} - Kw_{T1}}{Kw_{T1}}$ (Kw_{T1}: Permeability of the fouled membrane (L/m^2.h.bar, 25°C); Kw_{T2}:

Permeability immediately after cleaning (L/m^2.h.bar, 25°C), ** Salt rejection gain after cleaning (%) = $\dfrac{R_{T2} - R_{T1}}{R_{T1}}$ (R_{T1}: Salt

rejection of the fouled membrane (%); R_{T2}: Salt rejection immediately after cleaning (%), *** Differential pressure reduction after

cleaning (%) = $\dfrac{dP_{T1} - dP_{T2}}{dP_{T1}}$ (dP_{T1}: Differential pressure of the fouled membrane (psi); dP_{T2}: Differential pressure immediately

after cleaning (psi).

The FNA-based cleaning solutions in this trial were less effective than NaOH-based one at pH 11.0 (20-74% versus 80-105% permeability recovery). The higher efficiency achieved with NaOH could be explained by the membrane fouling nature (high proportion of EPS in the biofouling layer). NaOH is known to be highly efficient for colloidal and organics removal. Hydroxide ions can break the fouling layer due to increased ionic strength and pH, increasing the organic matter solubility and enhancing deprotonation of carboxylic and phenolic groups (i.e., increase negative charge). At high pH, EPS are negatively charged and less dense due to repulsion (weaker interactions).

The cleaning trial also revealed that the temperature does not have a major effect on the efficiency of FNA-based cleaning (20-21% versus 22-29% for 25^oC and 35^oC, respectively). However, these results are based on two cleaning trials only. It is essential to repeat the cleaning trials to validate these results.

3.3. Conclusions

Lab-scale tests

The impact of FNA and FNA/H_2O_2 on biofouling and scaling removal was investigated at different pH levels using fouled RO membranes from full-scale plants including industrial and municipal water recycling plants and also a seawater desalination plant. The following conclusions can be drawn:

- FNA cleaning is effective in removing bacteria and organics from membrane surfaces; it also causes substantial inactivation of bacterial cells remaining on the membrane surface after cleaning. FNA cleaning has a superior performance in bacteria removal than the current method of NaOH cleaning at pH 11 (based on 11 cleaning trials).
- A nitrite concentration of 50 $mgNO_2^-$-N/L and a pH level of 3.0 are suitable conditions for biofouling removal.
- For scale removal, FNA at pH 2.0 and 3.0 is as efficient as the commonly used descaling agent (HCl and citric acid). This effect, along with the effect of FNA on biofouling removal, implies that the use of FNA as a cleaning solution can simultaneously achieve both biofouling removal and descaling.
- The results from the lab-scale cleaning tests in dynamic conditions suggested that FNA could be used as a single cleaning agent for both biofouling and scaling removal.
- The cleaning trials revealed that hydrogen peroxide does not further enhance the membrane biofouling removal, when applied with FNA.
- The filtration trials on membrane coupons revealed no significant impact of different cleaning procedures on the performance of the membrane in terms of permeability and salt rejection for all cleaning conditions applied. All permeability changes remained non-distinguishable from the natural variability of the membrane used.

The optimal FNA-based cleaning strategy selected from the lab-scale cleaning tests (i.e., 50 $mgNO_2^-$-N/L, pH 3.0) was tested with fouled RO membrane modules generated at pilot-scale. A biofouling protocol was applied in order to generate fouled RO membrane at pilot-scale and then to validate the efficiency of the cleaning agents.

Pilot-scale tests

- FNA-based cleaning improves permeability by 20-29% and reduces differential pressure drop by 27-38%. FNA-based cleaning showed similar differential pressure drop after cleaning compared to the current method of NaOH cleaning at pH 11 for the first cleaning, but did not show better permeability recovery. FNA cleaning may not be as effective as NaOH cleaning in removing biofouling fouling due to the large proportion of organics (e.g., EPS).
- The cleaning trials revealed that a higher temperature does not further enhance the efficiency of FNA-based cleaning.
- Only two cleaning trials have been carried out. Considering the good results collected form the lab-scale studies, it is highly recommended to repeat the cleaning trials at pilot-scale to confirm the results.

4. Application of FNA for the preservation of RO membranes during long-term storage

In this part of the project, the possible application of FNA as a biocide for the preservation of RO membranes during long-term storage was investigated. For that purpose, two different approached were used:

- Short term membrane preservation trials were conducted at lab-scale using RO membrane coupons to determine the optimum dose of FNA for storage;
- Long term membrane preservation trials (6 months) were conducted using 4- and 8-inch commercially available RO modules to validate the results from the short-term preservation tests and benchmark against sodium metabisulphite (SMBS) preservation strategy. Two storage approaches were evaluated; preservation out of the plant (in bags) and within membrane pressure vessels.

4.1. Impact of FNA for the preservation of RO membrane (lab-scale study)

4.1.1. Introduction

The objective of this sub-project was to evaluate the effectiveness and benefits of FNA for membrane storage in order to prevent microbiological growth and/or membrane degradation during long-term RO plant shut-down.

Lab-scale stability tests were first carrying out to study the potential of FNA as storage solution in terms of solution stability. Then short-term trials of one month were conducted to determine the low-end dosages to preserve membrane without biofilm development. The work was performed using unused membranes (ESPA-2 and SWC-5) and used (fouled and cleaned) membranes from the field (industrial wastewater recycling plant, RO2). RO membrane coupons were stored at varying FNA concentrations. At the end of the one-month test, the preservation solutions were analysed to evaluate their biomass content (adenosine tri-phosphate (ATP) measurements). Membrane performances (water permeability and salt rejection) were measured at bench-scale and compared to the initial conditions.

4.1.2. Material and Methods

4.1.2.1. Preservation solutions

Free nitrous acid (FNA). The preservation trials were conducted with FNA concentrations of 0.1, 1, 3 and 10 $mgHNO_2$-N/L at pH 5.0 (adjusted with hydrochloric acid). The FNA concentration was achieved by varying the nitrite concentration as described previously in chapter 2.7.

Deionised water (DI). DI water and DI water adjusted at pH 5.0 (adjusted with hydrochloric acid) were chosen as negative controls.

4.1.2.2. Reverse osmosis membranes

Membrane preservation trials were performed using two unused and one used RO membranes (see Table 4 in chapter 2.1). The unused membranes were commercially available thin-film composite RO membranes from Hydranautics Corporate (San Diego, USA) designed for seawater desalination (SWC-5) and water recycling applications (ESPA-2), respectively. The used membrane (RO2, BW30-400-FR, Filmtec membranes) was collected from a full-scale industrial water recycling plant. Before preservation the used membrane was cleaned with caustic and acid consecutively as follows:

- DI water rinsing (1h soaking)
- Caustic cleaning (NaOH, pH 11.0 for 1h + 2h soaking)
- DI water rinsing (15 min)
- Acid cleaning (Citric acid, pH 3.0 for 1h + 2h soaking)
- DI water rinsing (15 min)

Membrane coupons (54 in total) were cut out from the three RO modules (Table 12). Baseline performance measurements were taken to characterize membrane performances (water permeability and salt rejection).

Table 12. RO membrane coupons used for the short-term membrane preservation trials.

Preservation solutions	Replicate (n)		
	ESPA-2	SWC-5	RO2
Baseline (DI water)	2	2	2
DI water	3	3	3
DI water, pH 5.0	3	3	3
0.1 mgHNO$_2$-N/L FNA, pH 5.0	3	3	3
1 mgHNO$_2$-N/L FNA, pH 5.0	3	3	-
3 mgHNO$_2$-N/L FNA, pH 5.0	3	3	-
10 mgHNO$_2$-N/L FNA, pH 5.0	3	3	3

4.1.2.3. Protocol

Stability study. FNA stability was studied during 30 days in MilliQ-water at different pH (pH 3.0, 4.0, 5.0 and 6.0) and temperature levels (23±1, 30±1 and 37±1°C). The experiments were performed in brown-glass bottles to prevent from light exposure. Each condition was tested in duplicated. Bottles were filled with 100mL MilliQ-water and conditioned by adding hydrochloric acid (1M), sodium hydroxide solution (1M) and sodium nitrite to achieve the desired pH and FNA concentration (10 mgHNO$_2$-N/L). Bottles with temperature set point at 23±1°C were kept in laboratory with temperature controlled at 23±1°C. Bottles with higher temperature set point were kept in two incubators with temperature set at 30°C and 37°C, respectively. On day 1, 2, 3, 4, 7, 15 and 30, pH and nitrite concentrations were measured.

Preservation tests. For each FNA preservation solution, membrane coupons were soaked in the membrane preservation solution for 30 min. Then the coupons were enclosed in resealable plastic bags with 20 mL of the preservation solution added to the bag. Triplicates were prepared for each preservation solution/membrane combination to assess the repeatability on the measures. The resealable bags were then enclosed in vacuum sealed bags; a vacuum pump (FoodSaver®, Australia) was used to remove the air from the plastic bags (Figure 18). The bags were stored in the dark and at room temperature (i.e. 20-25°C) to reproduce conditions generally used on site. As controls, parallel trials were carried out with DI water and DI water at pH 5.0.

After one-month storage time, the membranes were removed from the plastic bag and rinsed for 30 min with DI water to remove the remainder of the preservation solution. The performance of the membrane coupons was measured (permeability and salt rejection). The preservation solutions were analysed to evaluate their potential for biological growth (e.g., biomass quantification using ATP measurement). Modification of membrane structure by the solution was also being investigated using infrared spectroscopy (ATR-FTIR), while the presence of biofilm was assessed employing scanning electron microscopy (SEM).

Step 1. The coupons were enclosed in oxygen barrier plastic bags (resealable bags) with 20 mL

Step 2. The resealable bags were then enclosed in vacuum sealed bags

Figure 18. Protocol description of the short-term preservation trials.

4.1.2.4. Analytical methods

The preservation solutions were characterized before and after storage. The biomass quantification was assessed via adenosine triphosphate (ATP). Nitrite concentration and pH were measured to discuss the preservation solution stability. Modification of membrane structure by the solution was also being investigated using infrared spectroscopy (ATR-FTIR), while the presence of biofilm was assessed employing scanning electron microscopy (SEM). All the above mentioned analytical methods used in this study have been described in Chapter 2.

4.1.3. Results and Discussions

4.1.3.1. FNA solution stability

The effect of pH, temperature on FNA concentration was studied to determine the stability of FNA solution over 30 days. Figure 19 represents the FNA concentration as a function of time for pH 3.0, 4.0, 5.0 and 6.0.

a.

b.

c.

d.
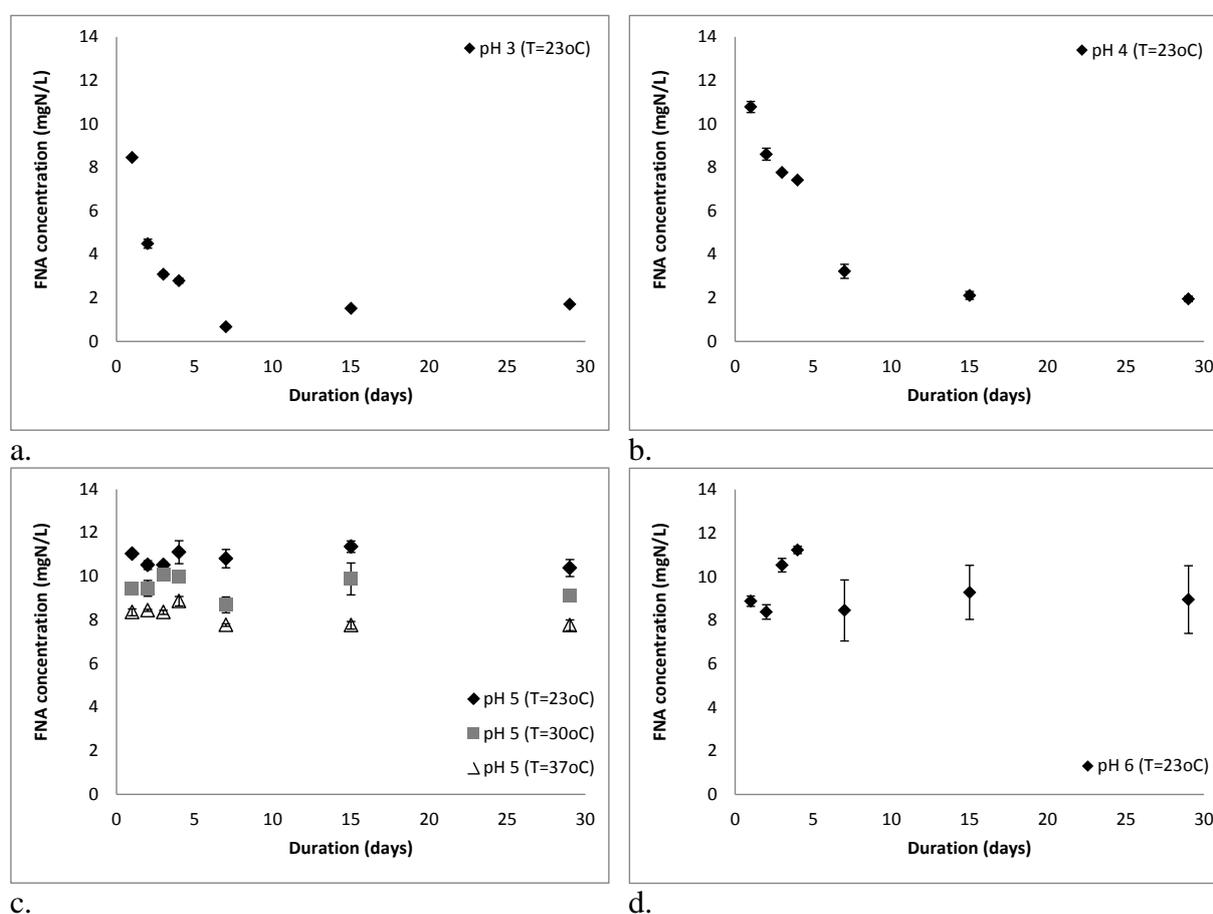
Figure 19. Change of FNA concentration as mgHNO$_2$-N/L for different pH during 30 days incubation tests: a) pH 3 (T=23°C), b) pH 4 (T=23°C), c) pH 5 (T=23, 30 or 37°C) and d) pH 6 (T=23°C). Standard conditions: 10 mgHNO$_2$-N/L initial FNA concentration. The error bars show the standard errors of six measurements from duplicate experiments.

While the pH values were stable over the time (data not shown), the total nitrite concentration decreased resulting in a decrease of FNA concentration at pH 4.0 and below. The lower the pH, the greater FNA concentration decreased. Both pH 5.0 and 6.0 did not demonstrate FNA degradation after 30 days. Experiments conducted at pH 4.0 and 3.0 showed a total nitrite concentration decreasing of 80%. These results are in accordance with the disproportionation of nitrous acid in aqueous solution giving nitric acid and nitric oxide (Eq. 11):

$$2x[2\ HNO_2 \leftrightarrow H_2O + N_2O_3]$$
$$2x[N_2O_3 \leftrightarrow NO + NO_2] \qquad \text{(reversible decomposition)}$$
$$2\ NO_2 \leftrightarrow N_2O_4 \qquad\qquad\qquad\qquad\qquad \text{(Equation 11)}$$
$$N_2O_4 + H_2O \leftrightarrow HNO_3 + HNO_2 \qquad \text{(slower step)}$$
$$\mathbf{3\ HNO_2 \leftrightarrow H^+ + NO_3^- + 2\ NO + H_2O}$$
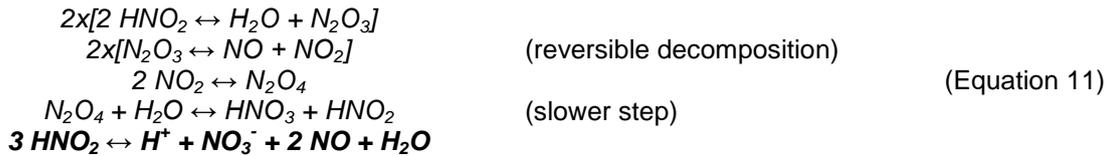
The stability of FNA solution is strongly pH dependant. FNA solutions are stable at pH 5.0 and above over 30 days. The temperature only has a slight effect on FNA concentration; lower the temperature more stable the FNA solution.

Sodium metabisulphite (SMBS) is the current standard preservation chemical used. However SMBS can oxidize easily inside pressure vessels [28]. Regular pH checks of the preservation solution are required (e.g., once a week), which is a time-consuming operation. Furthermore, total isolation of the RO system from air is difficult to control. FNA solution at pH 5.0 presented potential biocide properties and showed to be stable over 30 days. Furthermore, absence of head-space in the bottles employed for the test and buffer did not show any impact on FNA stability (data not shown). Based on the results, this pH 5.0 can be applied for membrane preservation without FNA degradation.

4.1.3.2. Preservation trials with unused and used RO membranes

Hydraulic performances
Figure 20 illustrates the results of the trials conducted with unused and used membranes in terms of pure water permeability and the salt rejection, respectively. The raw data are presented in Appendix C (Table SI 4).

Before membrane storage, membrane performance were measured to establish the membrane coupons performance baselines and to further discuss the impact of the preservation solutions on the membranes. The pure water permeability baselines were 4.8 ± 0.4, 1.6 ± 0.0 and 4.1 ± 0.6 $L/m^2.h.bar$ for ESPA-2 (n=2), SWC-5 (n=2) and RO2 (n=2) respectively. The salt rejection baselines were 97.9 ± 1.6, 98.6 ± 0.7 and $95.5\pm1.8\%$ for ESPA-2 (n=2), SWC-5 (n=2), RO2 (n=2) respectively. After one-month storage, filtration trials were performed with the stored membrane coupons (n=2) and compared with the baseline values.

Figure 20 shows that permeabilities were stable after one-month storage for ESPA-2 membranes, with permeabilities values between 4.3 and 4.9 $L/m^2.h.bar$. The permeability variation is below the 15% permeability variability acceptable due to membrane manufacturing and experimental error. Membrane producers typically specify the permeability of modules with a tolerance of ±15–20% of the nominal value [66]. Only the two membranes stored in DI-water (control) showed lower permeation compared to the baseline (3.5 ± 0.1 versus 4.8 ± 0.4 $L/m^2.h.bar$). During the storage, the resealable bags were not closed properly resulting in leaking of preservation solutions in the vacuum sealed bag. This permeability drop after could be explained by a drying of the membrane coupons. Similarly, no loss of performance after short-term storage was observed for SWC-5 membranes. Pure water permeabilities vary between 1.3 and 1.7 $L/m^2.h.bar$.

In terms of salt passage, no performance difference was observed for all the membrane/preservation solution combinations tested. Salt rejections were between 98.1 and 99.2% for ESPA-2 membrane (higher than under baseline conditions) and 97.6 to 99.0% for SWC-5.

For RO2, the permeability increased after one-month storage, indicating an improvement of the hydraulic performances of the membranes, while no clear trend could be observed for salt rejection. However, no significant difference could be observed between the different preservation solutions tested. Pure water permeabilities vary between 5.4 and 5.6 $L/m^2.h.bar$ and the salt rejections vary between 96.1 and 99.8%. The increase of pure water permeability observed is likely due to a positive cleaning effect of the one-month soaking. The performance could not be measured for the control with DI water at pH 5.0.
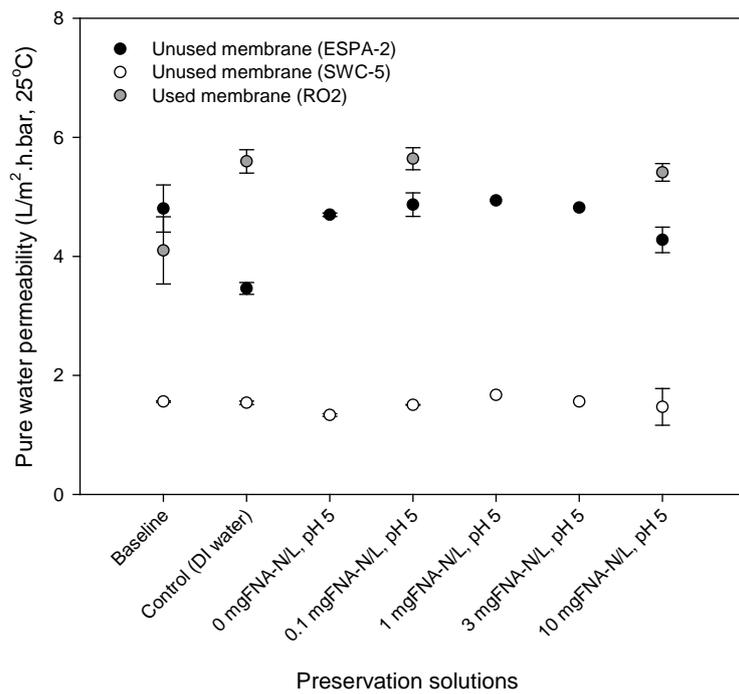
a.
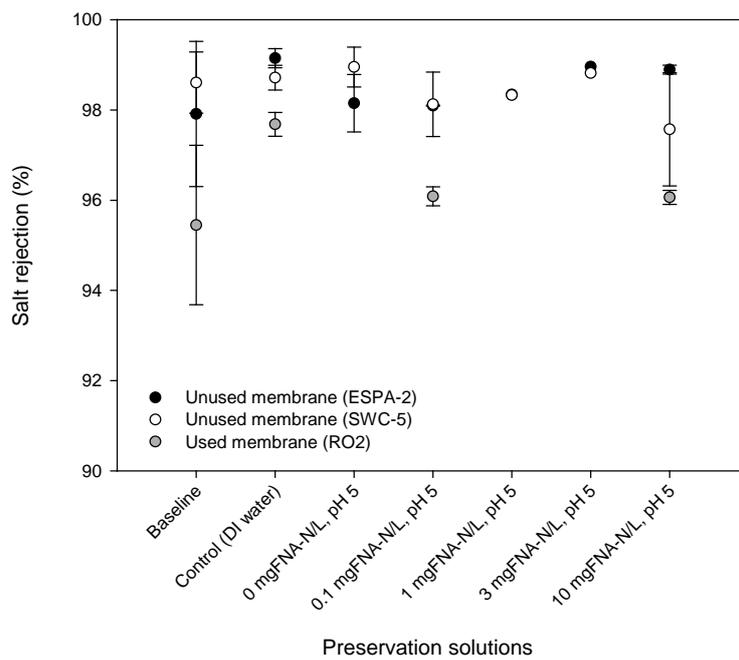
b.

Figure 20. (a) Pure water permeability and (b) salt rejection of ESPA-2, SWC-5 and RO2 membranes before (baseline) and after one-month storage in different preservation solutions (n=2). Standard filtration test conditions: 5bar, 25±2°C.

Biomass quantification

ATP was measured to quantify and compared the bio contamination in the different preservation solutions.
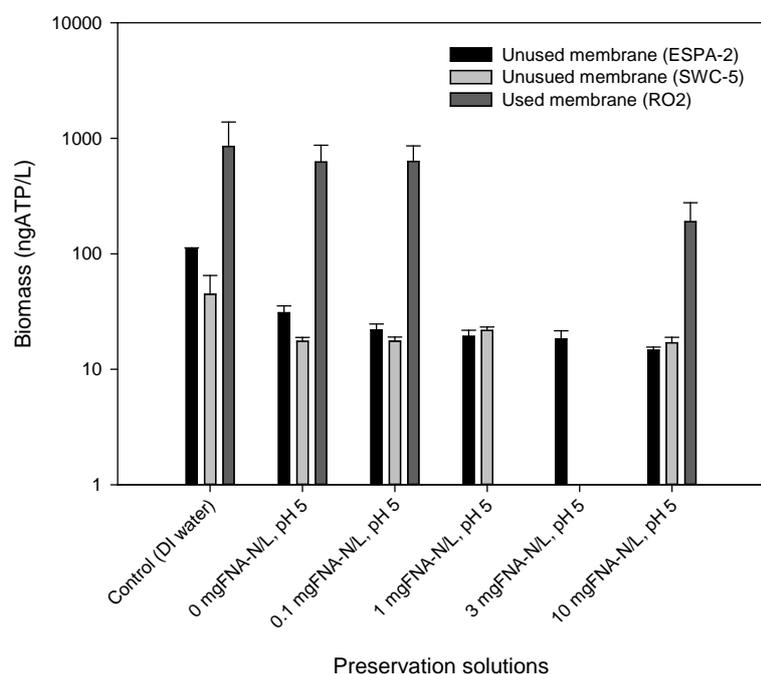
Figure 21 presents the biomass concentration (as ngATP/L) in the different preservation solutions after one-month storage. The highest ATP value was observed in the control solution with DI-water only, indicating a higher total bacteria count compared to the other preservation solutions. This result was observed for the two unused membranes tested (110±3 and 45±20 ngATP/L for ESPA-2 and SWC-5, respectively). Very low biomass concentrations were measured in the other preservation solutions (< 40 ngATP/L). However, the ATP concentration seems to decrease with FNA concentration increase. The lower biomass concentrations were observed for 10 $mgHNO_2$-N/L solution at pH 5.0 (15±1 and 17±2 ngATP/L for ESPA-2 and SWC-5, respectively).

For RO2, ATP values showed higher biomass concentrations compared to the ones conducted for the unused membranes. This is likely due to a detachment of foulant from the used membranes during storage. However similar trends were observed compared to the short-term membrane preservation trials performed with unused membranes. The highest ATP value was also observed in the control solution with DI-water only (852±532 ngATP/L), indicating a larger bio-contamination/bacterial growth and the lowest biomass concentrations were observed for 10 $mgHNO_2$-N/L solution at pH 5.0 (190±88 ngATP/L). This FNA concentration was selected as optimum concentration for preservation tests.

Complementary analysis

SEM analysis. SEM images were conducted on the SWC-5 membrane coupons to identify the adherence of bacteria on the membrane surface (Figure 22). The presence of bacteria could be observed only on the membrane stored with 0.1 $mgHNO_2$-N/L of FNA, pH 5.0, and only on low proportion. These results support the ATP results: no/low bacterial growth/contamination could be observed after one-month storage, whatever the preservation solution applied.
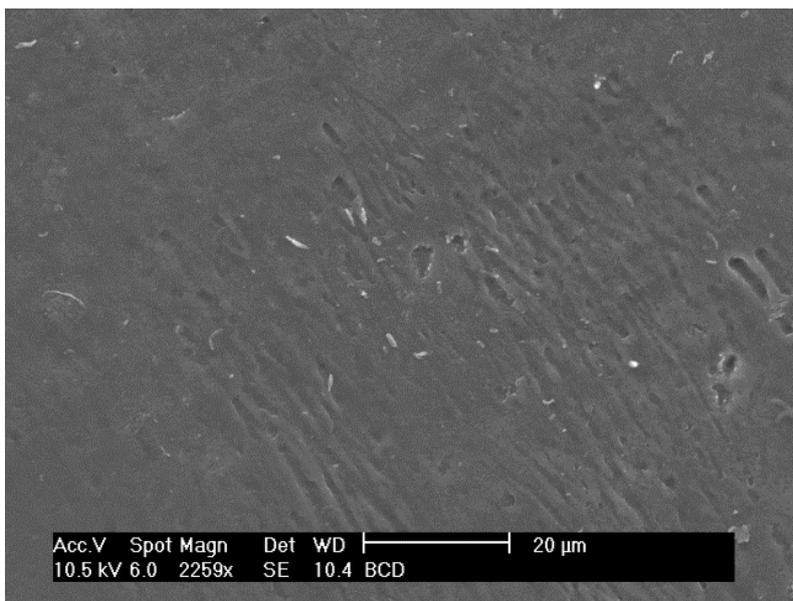
Figure 22. SEM (Pt coating) analysis of the membranes (feed side) after one-month storage in FNA solution. Standard test conditions: 0.1 mgHNO$_2$-N/L FNA, pH 5.0.

ATR-FTIR analysis. Although no hydraulic performance decline were observed, ATR-FTIR spectroscopy was applied to investigate if membrane modifications occurred during the storage, i.e., to study the compatibility of the preservation solutions and the membranes. The spectra measured for the membrane coupons after one-month storage are presented in Figure SI 4, in Appendix C. No variations in absorbance intensity were observed between the different samples. No clear chemical conversion of the membrane active layer could be observed on the samples tested. The ATR-FTIR peak intensities collected were similar to the ones obtained for the baseline membrane. These results suggested that no structural damages to the active layer of the membranes occurred during short term preservation using FNA up to 10 mgHNO$_2$-N/L. The compatibility of FNA with polyamide membrane was investigating more in details and results were reported in Chapter 5.

4.2. Long term trials

4.2.1. Introduction

The suitability of FNA for long-term membrane preservation (due to its potential biocide properties) was evaluated using both new and used (fouled and cleaned) spiral wound modules and the optimum dose of FNA selected from the lab-scale trials (i.e., 10 mgHNO$_2$-N/L, pH 5.0). The efficiency of FNA on was benchmarked against 1% sodium metabisulphite solution.

4.2.2. Material and Methods

4.2.2.1. Preservation solutions

Free nitrous acid (FNA). The preservation trials were conducted with FNA concentrations of 10 mgHNO$_2$-N/L at pH 5.0.

Sodium metabisulphite (SMBS). A standard 1% SMBS solution was chosen as a reference preservation solution (benchmark).

Deionised water (DI). DI water was used as negative controls.

4.2.2.2. Reverse osmosis membranes

Long-term membrane preservation trials were performed using unused and used RO membranes (Table 4 in chapter 2.1). The unused membranes (18 ESPA-2 modules, Toray) were commercially available 4-inch RO modules from Hydranautics Corporate (San Diego, USA) designed for water

recycling applications (ESPA-2). The used membranes (18 TML20-400 modules) were collected from the Luggage point advanced water treatment plant, as an in-kind contribution from Veolia Water.

4.2.2.3. Protocol

Long-term trials of six months were conducted on unused and used (fouled and cleaned) spiral-wound RO membranes. The modules were collected and their initial performance in terms of permeability and salt rejection were determined on a single module test rigs. Two methods were applied for the membrane preservation trials: (1) the first batch of membranes (unused membranes) was soaked overnight in different preservation solutions, then drained to remove the excess of preservation solutions and bagged in vacuum sealed bags (FoodSaver® bag, Sunbeam) with 1L of the solution to simulate *membrane preservation for storage out of the plan*t; (2) the second batch of membranes (used membranes) was stored in PVC tubes designed to simulate *membrane preservation for storage within membrane pressure vessels* (Figure 23). The PVC tubes were filled completely (head-space free) with preservation solution. For each method, the impact of FNA (10 mgHNO$_2$-N/L, pH 5.0) on membrane preservation was compared with control (Deionised water, pH 5.0) and standard preservation solution (1% SMBS solution). The pH of the preservation solutions was checked weekly via a tap and the SMBS solution would be changed when pH drop below 3.0. The membranes were stored away from direct sun light. The room temperature was monitored using a data logger (Tinytag, UK). The temperature was 24.8±3.0^{o}C (n=4437) over the six-month period (Figure SI 5, Appendix D), which is in the range of recommended temperature from membrane supplier (i.e., 0-40°C) [31].

Method 1. Module stored in vacuum sealed bags to simulate membrane preservation for storage out of the plant.

Method 2. PVC tubes designed to simulate membrane preservation for storage within membrane pressure vessels.

Figure 23. Protocol description of the long-term preservation trials.

After 2, 4 and 6 months of preservation the solutions were analysed to monitor the pH, nitrite concentration and microbial development. Two modules of each solution tested were rinsed for 30 min with DI water to remove the preservation solution and re-tested to discuss the impact of storage on membrane performances (permeability and salt rejection). Three test times, and duplicate elements, result in 6 elements per selected preservation solution to test (i.e., 18 modules in total for each method).

4.2.2.4. Analytical methods

Nitrite concentrations and pH were measured to investigate the FNA solution stability. Biomass content and cell counts were assessed using adenosine tri-phosphate (ATP) measurements and flow cytometry, respectively. All the above mentioned analytical methods used in this study have been described in Chapter 2.

4.2.3. Results and Discussions

4.2.3.1. Preservation trials for preservation out of the plant

Preservation solution stability
The pH values and nitrite concentrations of the preservation solutions in the vacuum sealed bags were measured after 2, 4 and 6 months (Table SI 6, Appendix D). The pH values of the DI-water and SMBS-based preservation solutions decreased of 31% and 29% respectively. After 6-month storage, SMBS solution reached pH of 2.7, which is below the pH recommended by supplier (i.e., pH 3.0) to prevent membrane damage.

The pH of the FNA-based preservation solution increased by 31% after storage, while nitrite concentration decreased by 20%. This indicates that denitrification occurred. After 6-month storage, FNA residual of 0.06 mgHNO$_2$-N/L remains in the solution.

Hydraulic performances
Before membrane preservation, the membrane performances were measured to establish the performance baselines and further discuss the impact of the long-term preservation on the membrane after 2, 4 and 6 months. The raw data are presented in Table SI 5, Appendix D.

a.   b.
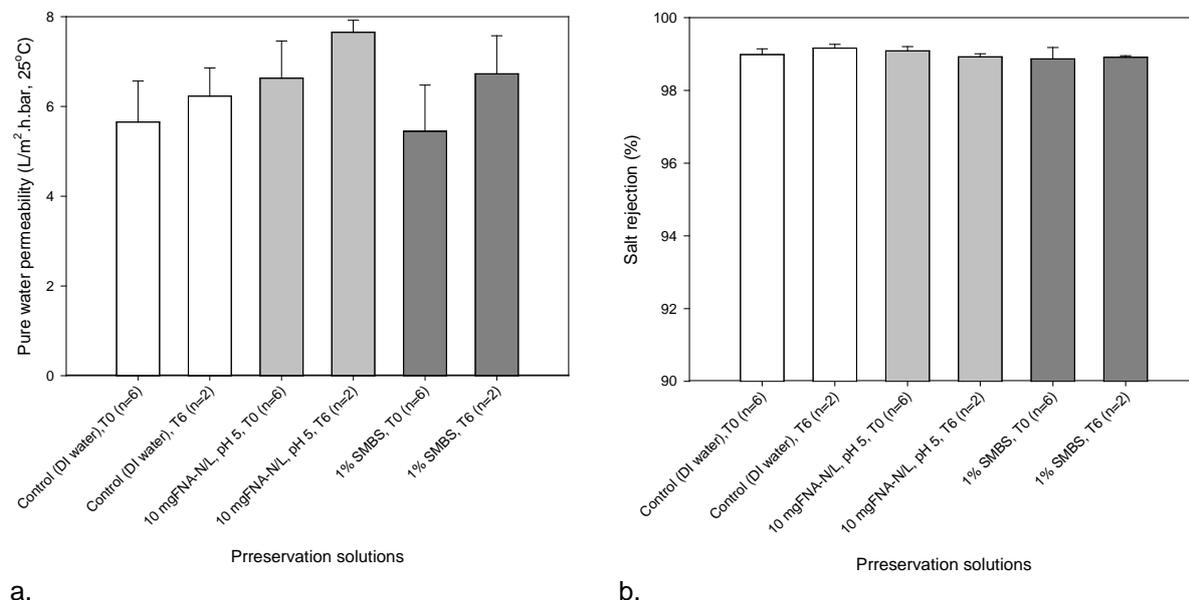
Figure 24. (a) Pure water permeability and (b) salt rejection of RO membranes before and after long-term preservation trials (6-month storage) in three different preservation solutions. Standard conditions: 4-inch RO modules (ESPA-2, Hydranautics) stored in vacuum sealed bag to simulated "Preservation out of the plant".

Figure 24 compares the pure water permeability and salt rejection before and after 6 months preservation for the three preservation solutions. While the permeability increased for all the membrane/preservation solution combinations tested (10%, 15% and 24% for DI-water, FNA and SMBS solutions, respectively), no impact on the slat rejection could be observed. The permeability increases together with no loss in salt rejection could be due to a reduction in the thickness of the polyamide layer, through deterioration of the coating or just membrane swelling.

Biomass quantification
Figure 25 shows live and dead cell concentration for each membrane/preservation solution combination after 2, 4 and 6-month storage in vacuum sealed bags. The biomass concentration (as ngATP/L) was also measured and the data are presented in Figure SI 6, Appendix D. The highest cell counts were observed in the FNA-based preservation solution, indicating a higher bacteria activity compared to the other preservation solutions. This result is in accordance with ATP concentrations and the presence of algea collected in vacuum sealed bags after 4 and 6-month storage.
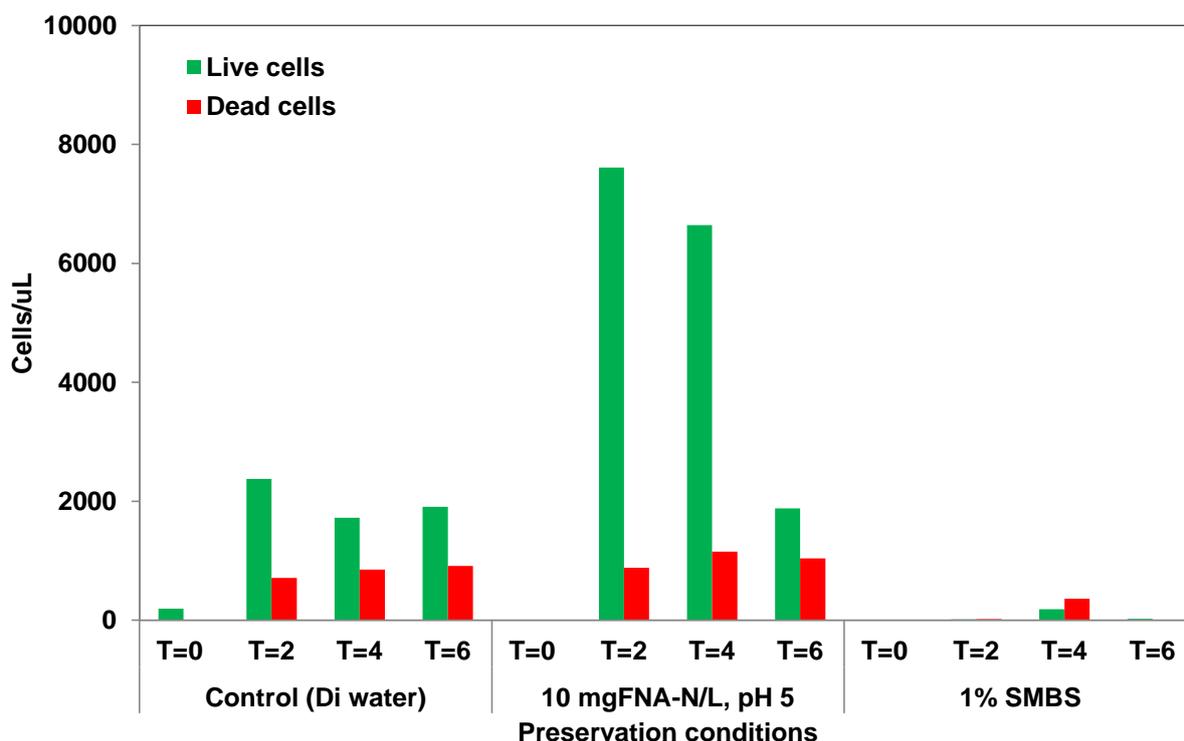
Figure 25. Live and dead cell concentrations during long-term preservation tests. Standard conditions: 4-inch RO modules (ESPA-2, Hydranautics) stored in vacuum sealed bag to simulated "Preservation out of the plant".

4.2.3.2. Preservation trials for preservation within membrane pressure vessels

Preservation solution stability
The stability of the preservation solution was studied over six months. The pH values and nitrite concentrations of the preservation solutions in the PVC tubes were measured every month (Table SI 8, Appendix D). The three solutions showed to be more stable in PVC tubes than in the vacuum sealed bags. The pH values of the DI-water and SMBS-based preservation solutions decreased of 4% and 14%, respectively. After 6-month storage, SMBS solution reached pH of 3.6, which is above the pH recommended by supplier before solution replacement (i.e., pH 3.0).
For the FNA-based preservation solution, denitrification still occurred in duration. The pH of the FNA-based preservation solution increased by 32% after storage, while nitrite concentration decreased only by 4%. After 6-month storage, FNA residual of 0.25 mgHNO$_2$-N/L remains in the solution.

Hydraulic performances
The membrane performances were also measured before and after preservation to discuss the impact of the long-term preservation on the membrane after 2, 4 and 6 months. The raw data are presented in Table SI 7, Appendix D. Figure 26 compares the pure water permeability and salt

rejection before and after 6-month preservation for the three preservation solutions. The membranes presented small variation in pure water permeability after 6-month preservation. However, these variations remain in the error range of the baselines. All the salt rejections are above 99% after preservation, which is the minimum salt rejection reported by Toray for these membranes.

a.   b.
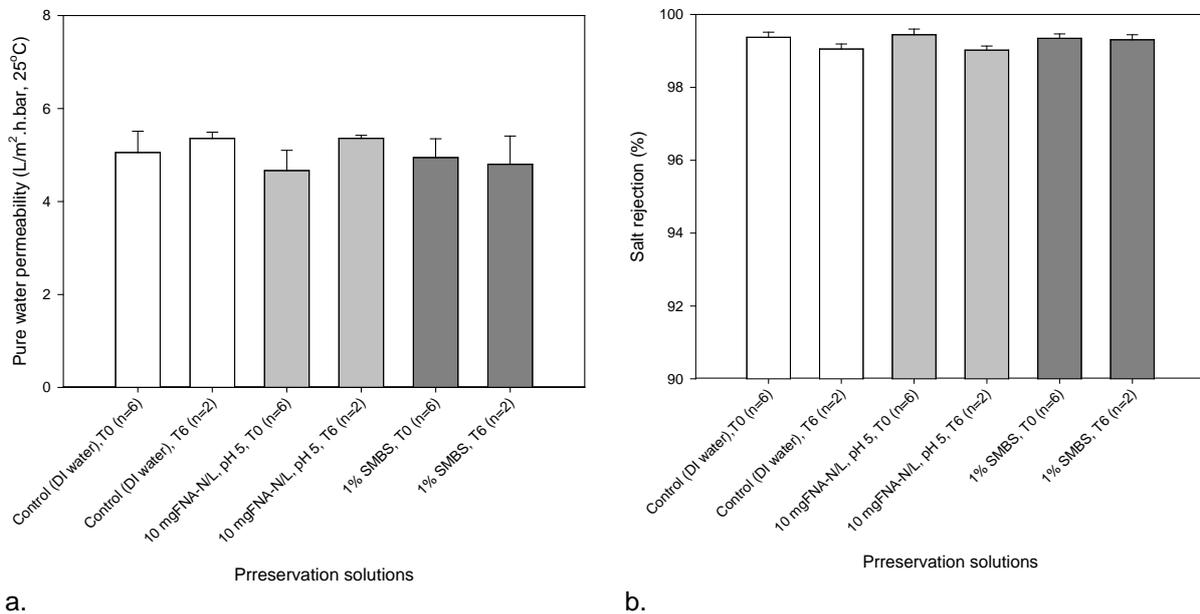
Figure 26. (a) Pure water permeability and (b) salt rejection of RO membranes before and after long-term preservation trials (6-month storage) in three different preservation solutions. Standard conditions: 8-inch RO modules (TML20, Toray) stored in PVC tubes to simulated "Preservation within membrane pressure vessels".

Biomass quantification

Figure 27 shows live and dead cell concentration for each membrane/preservation solution combination after 1, 2, 3, 4, 5 and 6-month storage in PVC tubes. The biomass concentration (as ngATP/L) was also measured and the data are presented in Figure SI 7, Appendix D.
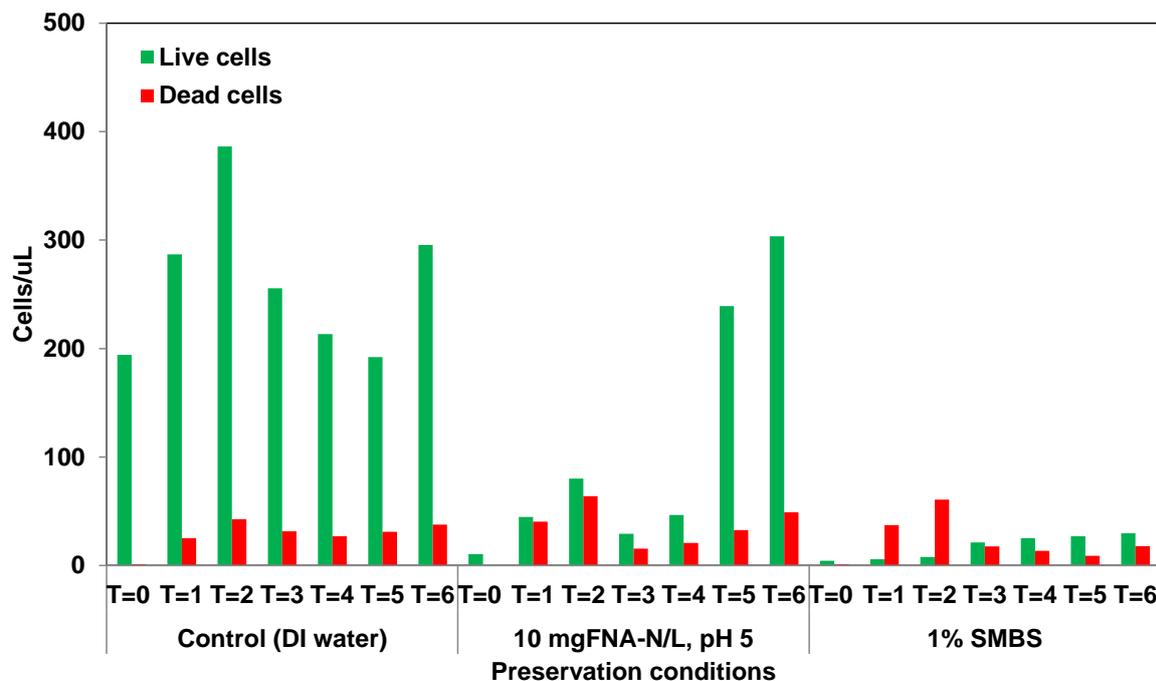
Figure 27. Live and dead cell concentrations during long-term preservation tests. Standard conditions: 8-inch RO modules (TML20, Toray) stored in PVC tubes to simulated "Preservation within membrane pressure vessels".

All the cell counts and ATP values are much lower than for the membrane stored in bags, indicating than storage within the pressure vessels is a better approach for long-term preservation. The highest cell counts were observed in the control solution with DI-water only (200-400 cells/μL), while very low cell concentrations (<100 cells/μL) were measured in the FNA-based and SMBS-based preservation solutions after 4 months. After 5 and 6 months, the cell concentrations in FNA-based preservation solution increased, although no negative impact was observed in term of membrane performances.

4.3. Conclusions

FNA for membrane storage was evaluated in order to prevent microbiological growth and/or membrane degradation.

Short-term preservation tests

The potential of using FNA as new agent for membrane preservation was first investigated over a month at lab-scale using used and unused RO membrane coupons. The following conclusions can be drawn:

- FNA can be used as a preservation solution for short-term storage of unused and used RO membranes. FNA-based preservation solution showed better performance for bacteria growth prevention than DI-water only (control).
- A FNA concentration of 10 mgHNO$_2$-N/L and a pH level of 5.0 showed to be stable over a month and a high biocidal effect.
- No structural damages to the active layer of the membranes occurred during short term preservation using FNA up to 10 mgN/L.

Long-term preservation tests

The optimal FNA concentration and pH selected from the short-term preservation tests (i.e., 10 mgHNO$_2$-N/L, pH 5.0) was tested with full-scale RO modules for 6 months. The following conclusions can be drawn:

- Long-term preservation tests conducted in PVC tubes to simulate "storage within membrane pressure vessels" showed that FNA can be used as a preservation solution equivalent to SMBS for long-term storage up to 4 months. Very low biomass and cell concentrations were measured in the preservation solutions, demonstrating that no significant microbial growth occurs, whatever the preservation solution applied. However, higher ATP value was observed in the control solution with DI water only, indicating biological contamination of the preservation solution during membrane storage.
- After long-term preservation, the filtration trials revealed no significant impact of different preservation strategies on the performance of the membrane in terms of permeability and salt rejection. The permeability and salt rejection after preservation remained constant. All permeability variabilities remained non-distinguishable from the natural variability of the RO membranes, while all the salt rejections are conformed to supplier specification (above 99%).
- After 5 months, FNA-based preservation solution showed increased of bacteria activity; although no negative impact was observed in terms of membrane performances. In this case, SMBS seems more suitable; however there is always a risk of oxidation and pH drop resulting in membrane damage. For shorter preservation period, FNA could be more stable than SMBS and then would be more applicable due to the reduction of the frequency of solution replacement leading to a significant cost benefit. Further studies should be carried out to confirm this hypothesis.

5. Impact of FNA on membrane integrity

In this part of the project, the impact of FNA and FNA/H_2O_2 on membrane life expectancy was investigated in order to evaluate the compatibility of the cleaning/preservation agents with thin-film composite polyamide membranes and determine the high-end dosages to clean or preserve membrane with FNA without damaging the membrane. For this purpose, two different approaches were used:

- Short-term tests at lab-scale in both static and dynamic conditions, to determine the ppm·hour (dose x contact time) threshold above which RO membrane performance are significantly affected during membrane cleaning application,
- Long-term tests, to validate the compatibility of FNA with the membrane during preservation. In this approach, long contact time and RO modules were used instead of high dose and membrane coupons, respectively.

5.1. Short-term ageing trials - membrane cleaning application

5.1.1. Introduction

The continuous or repetitive exposure of membranes to some biofouling control and/or removal chemicals has been shown to have a negative impact at long term [42, 43, 67]. Indeed, as a consequence to this extended exposure, the membrane performance can deteriorate with increased salt passage and/or decreased water permeability due to membrane ageing [15]. It is necessary to evaluate the compatibility of FNA and FNA/H_2O_2 with the membranes and the possible impact on their performance and structure (as suggested for example by Filmtec [27]). Accelerated ageing tests were then conducted to determine chemical compatibility, i.e. determine the ppm·hour (dose x contact time) threshold above which performance are significantly affected.

5.1.2. Material and Methods

5.1.2.1. Chemicals

Free nitrous acid (FNA). The ageing trials were conducted with 100 mgHNO$_2$-N/L FNA at pH 2.0 or 4.0 (adjusted with hydrochloric acid). The FNA concentration was achieved by varying the nitrite concentration as described previously (see chapter 2.7).

Hydrogen peroxide. The impact of hydrogen peroxide was investigated with 150 mg/L of H_2O_2. Details regarding the solution preparation are given elsewhere (see chapter 2.7).

Deionised water (DI). DI water and DI water adjusted at pH 2.0 or 4.0 (adjusted with hydrochloric acid) were chosen as negative controls.

5.1.2.2. Reverse osmosis membranes

Lab-scale membrane ageing trials were performed using an unused RO membrane from Hydranautics Corporate (San Diego, USA) and designed for water recycling applications, ESPA-2. This membrane, a commercially available thin-film composite polyamide membrane, is majorly developed using aromatic polyamides and a supporting porous layer made of polysulfone [68]. Membrane coupons were cut out of the spiral-wound membrane module.

5.1.2.3. Protocols

Membrane ageing. Accelerated ageing tests in static and dynamic conditions were conducted with 100 mgHNO$_2$-N/L FNA. The membrane samples were exposed to higher cleaning agent doses than required for the actual cleanings for short durations (hours or days). From the results obtained, the impact of the cleaning chemicals on the membranes was assessed and extrapolated to eventual long term effect on membranes life using the ppm·hour concept (dose x contact time). Membrane coupons were exposed to a range of doses of FNA alone or in combination with hydrogen peroxide and contact times to quantify the impact of the solutions on the membranes structure.

The accelerated ageing tests were conducted to simulate 21600 mgN.h/L exposures. The number of mgN.h/L exposure was calculated based on the following hypothesis:

- Membrane life time : 6 years
- Cleaning frequency: three times per month (i.e., every 10 days)
- Cleaning condition: FNA 50 $mgNO_2^-$/L, pH 2.0 (equivalent to 50 $mgHNO_2$-N/L)
- Cleaning duration: 2 hours

Control experiments were conducted to verify if the pH adjustment only has an impact on membrane performances.

Static membrane ageing tests were first carried out in chemical resistant jar (e.g. glass jars) at room temperature (i.e. 20-25°C). The active side of the membrane coupons was brought into contact with the cleaning solutions for a range of concentrations and contact times.

Dynamic membrane ageing tests were then conducted by continual exposure of a membrane to the chemical cleaning solution with cross-flow recirculation. Two Sterlitech CF042 cross-flow test cell-units were used. The filtration set-up is described elsewhere (see chapter 2.4). During these tests, the water permeability, saline solution permeability or salt rejection (using 1.5 g/L NaCl solution) were monitored to determine the maximum (continuous) hours of exposure at the determined dosage level of the chemicals without loss of membrane performance. The system was operated in a recycling configuration, i.e., concentrate and permeate was directed back in the feed tank, at 5 bars, a feed flow of 80 L/h and temperatures of 25-28 °C. The impact of pH (2.0-4.0) and hydrogen peroxide (150 mg/L) has been investigated.

All the ageing tests can be divided into four steps: (a) membrane compaction, (b) initial membrane characterisation, (c) membrane ageing and (d) membrane characterisation after ageing. The pH of the solutions and nitrite concentration were monitored to measure the FNA concentration during the tests and the FNA solution was replaced with a freshly prepared one every day. The performance of the membrane coupons (salt rejection and permeability) before and after ageing were carried out on a bench-scale filtration unit, described in chapter 2.4, and, the changes in membrane structure was monitored with infrared spectroscopy. Duplicate or quadruplicate tests were conducted in parallel to determine the representativeness of the results, as described in Table 13.

Table 13. RO membrane coupons used for the ageing tests in static and dynamic conditions.

Ageing solutions	Replicate (n)	Membrane ageing tests
DI water, pH 2.0 (control)	2	Static ageing tests
DI water, pH 7.0 (control)	2	
100 $mgHNO_2$-N/L FNA, pH 2.0	2	
DI water, pH 2.0 (+1.5 g/L NaCl) (control)	2	Dynamic ageing tests
DI water, pH 4.0 (+1.5 g/L NaCl) (control)	2	
100 $mgHNO_2$-N/L FNA, pH 2.0 (+1.5 g/L NaCl)	4	
100 $mgHNO_2$-N/L FNA, pH 4.0 (+1.5 g/L NaCl)	2	
100 $mgHNO_2$-N/L FNA, pH 4.0, 150 mg/L H_2O_2 (+1.5 g/L NaCl)	2	

5.1.2.4. Analytical methods

Nitrite and hydrogen peroxide concentrations, pH and conductivity were measured to investigate the FNA solution stability. Modification of membrane structure by the solution was also being investigated using infrared spectroscopy (ATR-FTIR). All the above mentioned analytical methods used in this study have been described in Chapter 2.3.

5.1.3. Results and Discussions

5.1.3.1. Membrane baseline

Before accelerating ageing tests, membrane performances of 18 membrane coupons were measured to define the baseline in terms of water permeability, saline solution permeability and salt rejection

(Figure 28). The membrane shows an average of 4.3±0.7 $L/m^2 \cdot h \cdot bar$ water permeability, 3.1±0.4 $L/m^2 \cdot h \cdot bar$ saline solution permeability and 98.6±0.3% salt rejection.
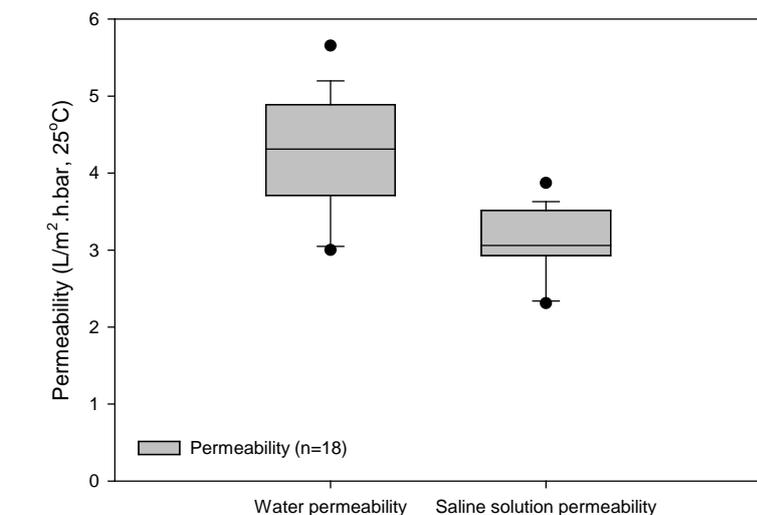
a.
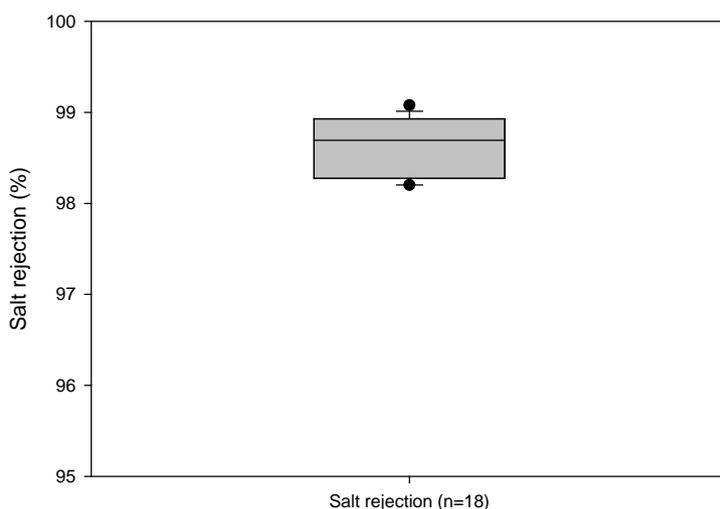
b.

Figure 28. Performance baseline of the RO membrane (ESPA-2), in terms of (a) water and saline solution permeabilities and (b) salt rejection. Data were analysed using box plots. In each graph the vertical lines represents the limits of maximum and minimum values; the box the 25th to 75th percentile values and the median. The number of samples is n = 18. Saline solution permeability and salt rejection were measured using 1.5 g/L NaCl solution. Standard test conditions: 4.9±0.8 bar, 25±1°C.

Large variability between the different membrane coupons were observed with coefficient of variation of 17%, 14% and 0.3% for the water permeability, saline solution permeability and salt rejection, respectively (n=18). These data are comparable with supplier variability, which claim permeate flow for individual elements may vary + or - 15 percent [ESPA-2, Hydranautics]. After accelerated ageing tests, membrane performances were compared to the membrane coupons performance baselines to further discuss the impact of the FNA solutions on membrane integrity.

5.1.3.2. Ageing tests in static conditions

In the static ageing tests, membrane coupons were exposed to 100 $mgHNO_2$-N/L FNA at pH 2.0 for 216 hours in order to simulate 21600 mgN.h/L total exposures. Due to FNA decay (Figure SI 8, Appendix E), the FNA solution was replaced daily to maintain a constant concentration. Control experiments were conducted in parallele with DI-water adjusted at pH 7.0 and pH 2.0.

The pure water permeability and salt rejection baselines for these trials were 3.0±0.0 L/m^2.h.bar and 98.5±0.7 %, respectively (n=2). After 5, 24, 120, 168 and 216 hours of exposure, filtration trials were carried out with the aged membrane coupons (n=2) and compared with the initiale membrane performances.

The results of the hydraulic performances are shown in Figure 29 and Figure 30, which illustrate the normalized pure water permeability and the salt rejectionafter FNA exposure up to 21600 mgN.h/L. The raw data are presented in Table SI 9, Appendix F.
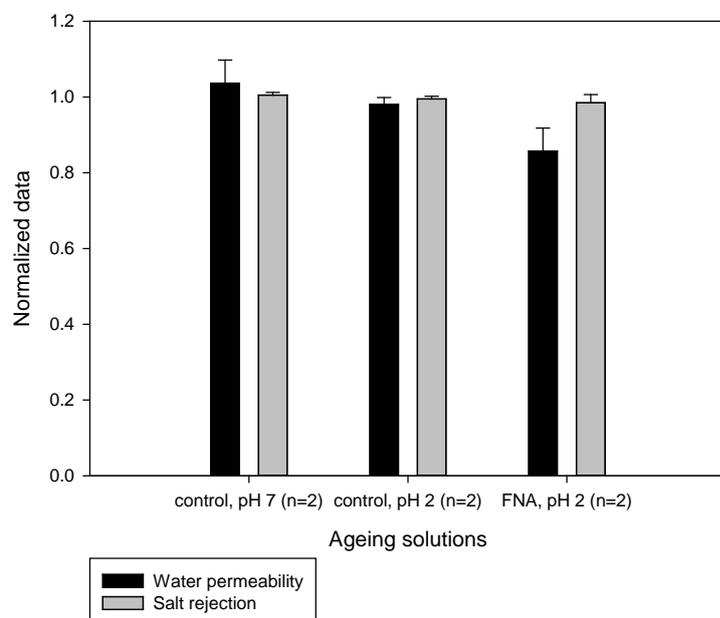
Figure 29. Normalized water permeability and salt rejection of RO membranes exposed to DI-water at pH 7, DI-water at pH 2.0 and 100 mgHNO2-N/L FNA at pH 2.0 for 216 hours, i.e, 21600 mgN.h/L FNA total exposures. Salt rejection was measured using 1.5 g/L NaCl solution. Standard filtration test conditions: 5.2±0.4 bar, 26±1°C.
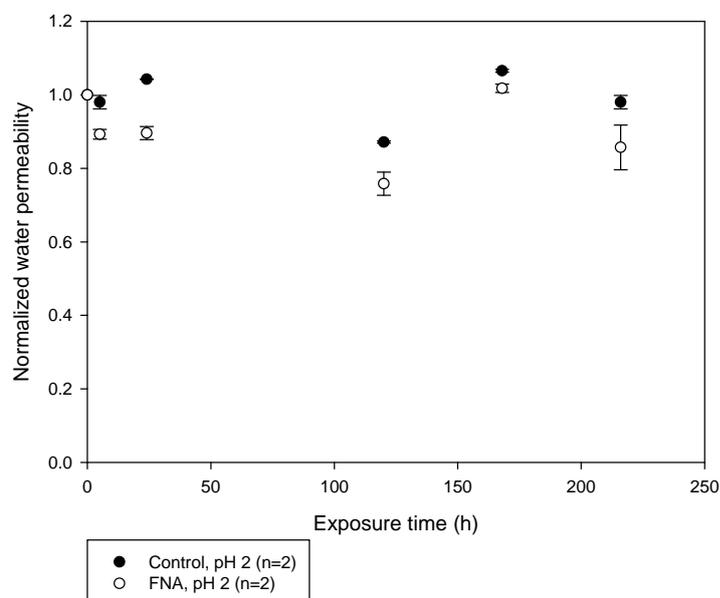
Figure 30. Normalized water permeability of RO membranes exposed to 0, 500, 2400, 12000, 16800 and 21600 mgN.h/L FNA at pH 2.0. The performances were measured at 0, 5, 24, 120, 168 and 216 h exposure time. Standard filtration test conditions: 5.2±0.4 bar, 26±1°C.

No significant effects in permeability and salt rejection were observed after static ageing tests. The salt rejection values before and after accelerated ageing are not statistically different (*p-value > 0.05*) regardless the ageing solutions applied. Similar results were observed with permeability values before

and after exposure to the two control ageing solutions (i.e., DI-water at pH 7.0 and 2.0), while the permeability mean value of the membrane aged with FNA at pH 2.0 showed a decrease of permeability (14%). The permeability variation is below the 15% permeability variability acceptable due to membrane manufacturing and experimental error.

The normalised water permeability for the control (pH adjustment alone, pH 2.0) and the FNA solution (100 mgHNO$_2$-N/L FNA, pH 2.0) as a function of exposure time was plotted to verify if the nitrite addition can explain the permeability loss (Figure 30). Normalized water permeability was defined as the permeability of the aged membrane divided by the permeability of the new membrane under comparable conditions. No clear trend of permeability loss due to extended exposure to FNA, as compared to the control at pH 2.0, could be observed. It is difficult to confirm if the permeability decrease is due to addition of nitrite. The two experiments showed a similar trend suggesting that the variation is related to the filtration set up and operation rather than the membrane coupon itself.

Although no hydraulic performance decline was observed, ATR-FTIR spectroscopy was applied to investigate if membrane modifications occurred during the ageing tests. Measurements with ATR-FTIR showed to be a sensitive and useful tool for analysing membrane ageing induced by chloramination and chlorine [13, 69]. The wavenumber of interest are 1538 cm^{-1}, 1609 cm^{-1} and 1662 cm^{-1} which are the wavelengths of amide II (N-H bonding), aromatic amide (C=C bonds) and amide I (C=O bonds), respectively (Table 5). These bonds are occurring in polyamide structures (Table 5). The light probe can penetrate the polyamide layer and reach the polysulphone support layer [15]. The FT-IR spectra include bands of the polysulfone support layer as well. All the spectra were normalised using the stronger band near 1250 cm^{-1}, associated with the C-O-C asymmetric stretching vibration from Aryl-O-aryl group. The spectra measured for the membrane coupons after 216 hours exposure are presented in Figure SI 9, Appendix F.

Variations in absorbance intensity were observed between the different analysed membrane coupons (Figure 31). The absorbance intensities decreased for the three aged samples at 1538 and 1609 cm^{-1}. However, the membrane samples aged with FNA (pH 2) showed similar peak intensities than the control, pH 2. No clear chemical conversion of the membrane active layer could be observed on the samples tested. These results suggested that no additional structural damages to the active layer of the membranes occurred during static ageing tests using FNA up to 21600 mgN.h/L exposure compared to the exposure to pH 2 only.
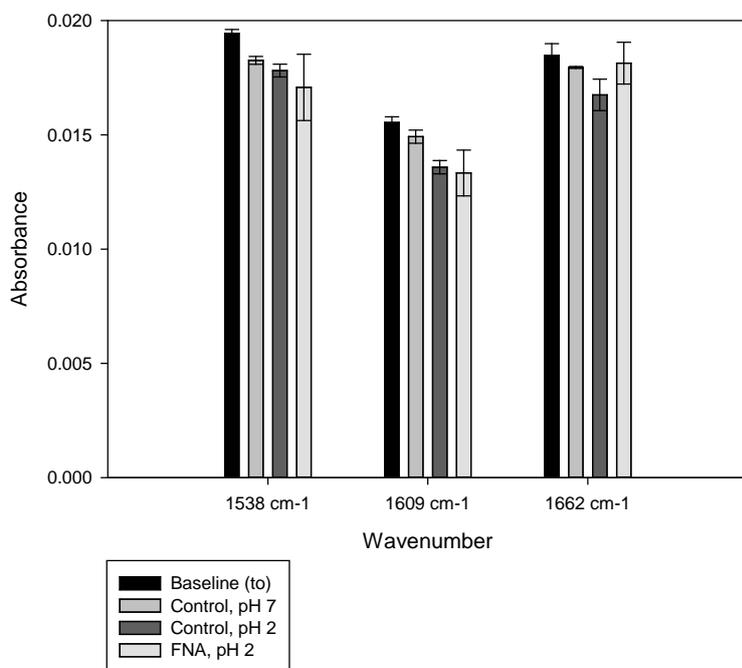
Figure 31. Peak shift of amide II (1538 cm^{-1}), aromatic amide (1609 cm^{-1}) and amide I (1662 cm^{-1}), respectively, after static ageing test using RO polyamide membrane. Normalisation of the spectra has been made on maximum peak near 1250 cm^{-1}, associated with the C, associated with the C-O-C asymmetric stretching vibration from Aryl-O-aryl group.

5.1.3.3. Ageing tests in dynamic conditions

A previous membrane ageing study looking at the impact of chlorine on polyamide membrane showed different results from static versus dynamic ageing tests. While actively damaged membrane showed increase in permeability and salt passage, passively damaged membranes showed reasonable increase in permeability, but not the salt passage [13]. Therefore, the impact of the cleaning agents on membrane life expectancy was investigated in dynamic mode by continual exposure of a membrane to the chemical cleaning solution with cross-flow recirculation. During these dynamic ageing tests, the decrease in water permeability or salt rejection was monitored. The membrane coupons were also exposed to $mgHNO_2$-N/L FNA for 216 hours in order to simulate 21600 mgN.h/L total exposures. Due to FNA decay (Figure SI 8, Appendix E), the FNA solution was replaced daily to maintain the FNA concentration constant. FNA with pH values between 2 and 4 showed the best cleaning efficiency in terms of ATP removal during the lab-scale cleaning trials. Then ageing trials were conducted with FNA solutions at pH 2.0 and 4.0. Control experiments were conducted in parallele with DI-water adjusted at pH 4.0 and pH 2.0.

The performance of the membrane was measured with clean water (DI-water) and saline solution (1.5 g/L NaCl solution) before the ageing was conducted. After ageing the system was flushed with water and performance tests with clean water and saline solution were completed. The comparison between the performance before and after ageing is shown in Figure 32. The raw data are presented in Table SI 10, Appendix G. Figure SI 10 compares the normalized permeaility as a function of the FNA exposure in static versus dynamic conditions at pH 2.0.
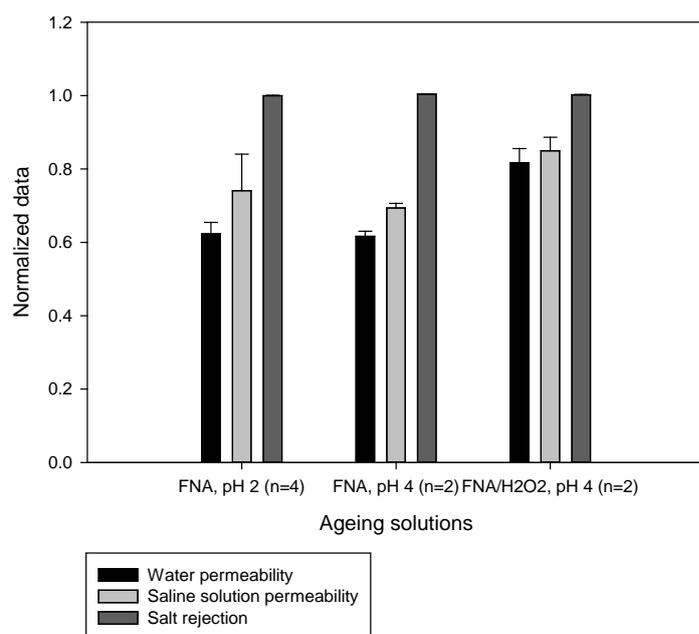
Figure 32. Normalized water permeabilities, saline solution permeability and salt rejection of RO membranes exposed to 100 mgHNO2-N/L FNA solutions for 216 hours, i.e., 21600 mgN.h/L FNA total exposure in dynamic conditions (cross-flow velocity of 0.1 m/s). Saline solution permeability and salt rejection were measured using 1.5 g/L NaCl solution. Standard filtration test conditions: 5.2±0.4 bar, 26±1°C. The error bars show the standard errors of 2-4 replicate experiments.

No significant effects on salt rejection were observed after dynamic ageing tests. The salt rejection values before and after accelerated ageing are not statistically different (*p-value > 0.05*) regardless the ageing solutions applied. However, water and saline solution permeabilities decreased significantly after dynamic ageing tests for the three ageing solutions. Exposure of the membrane to FNA-based solutions resulted in water permeability loss of 38%, 38% and 18% for the FNA solution at pH 2.0, pH 4.0 and pH 4.0 with hydrogen peroxide solutions, respectively.
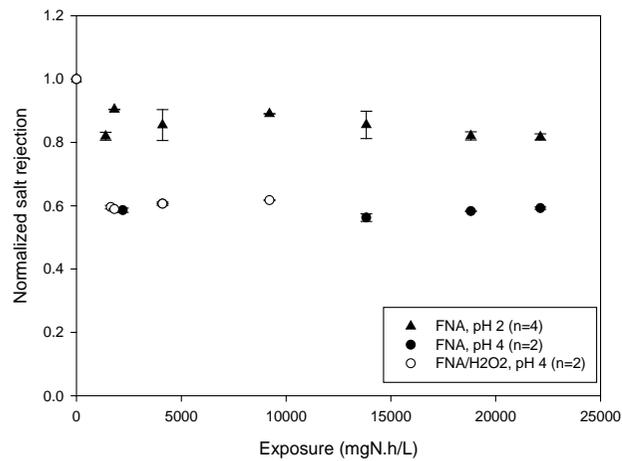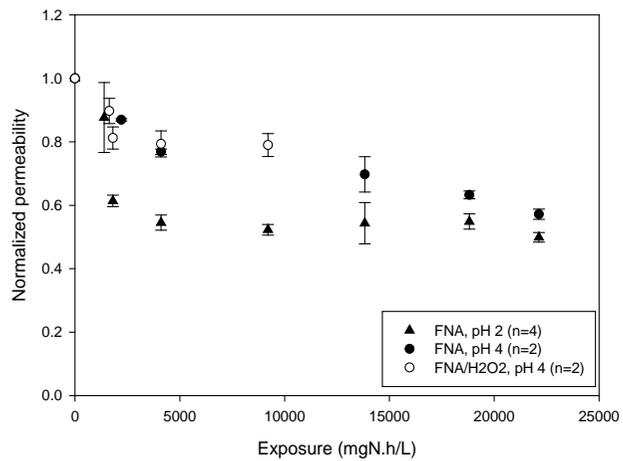
Figure 33. Normalized (a) saline solution permeability and (b) salt rejection of RO membrane exposed to 100 mgHNO2-N/L FNA solutions at pH 2.0 and 4.0 in dynamic conditions (cross-flow velocity of 0.1 m/s). Saline solution permeability and salt rejection were measured using 1.5 g/L NaCl solution. Standard filtration test conditions: 5.2±0.4 bar, 26±1°C. The error bars show the standard errors of 2-4 replicate experiments.
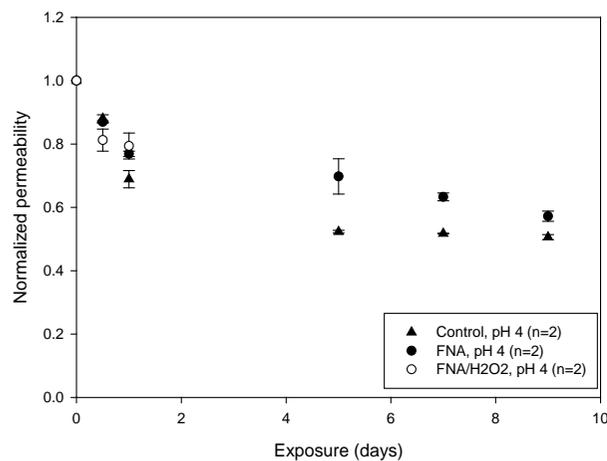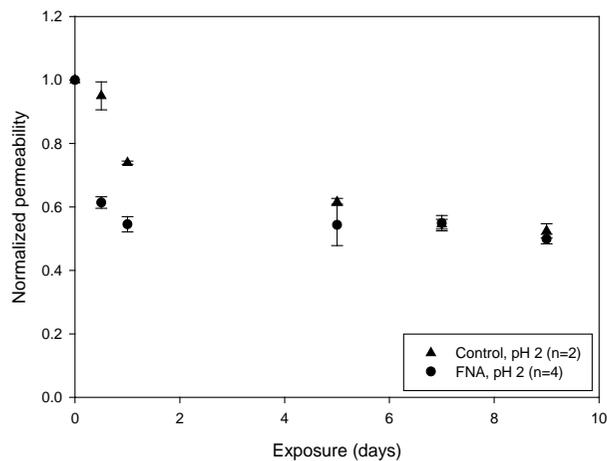
Figure 34. Normalized saline solution permeability of RO membrane exposed to DI-water and 100 mgHNO2-N/L FNA solutions at (a) pH 2.0 and (b) pH 4.0 in dynamic conditions (cross-flow velocity of 0.1 m/s). The performances were measured at 0, 0.5, 1, 5, 7 and 9 days. Standard filtration test conditions: 5.2±0.4 bar, 26±1°C. The error bars show the standard errors of 2-4 replicate experiments.

Figure 33 and Figure 34 show the normalized saline solution permeability and salt rejection during the ageing tests in cross-flow conditions. Normalized saline solution permeability was defined as the permeability of the aged membrane divided by the saline solution permeability of the new membrane before FNA addition under comparable conditions. Exposure of the membrane to FNA-based ageing solution resulted in reduction in the membrane permeability (up to 50 and 43% for FNA solution at pH 2.0 and 4.0, respectively) and salt rejection (up to 18 and 41% for FNA solution at pH 2.0 and 4.0, respectively) after 21600 mgN.h/L of exposure. While the salt rejection decrease showed to be reversible, the permeability did not recover to the initial values at the end of the ageing tests. At pH 2.0, where ageing solution is consisted mainly of free nitrous acid, permeability is decreasing up to 50%, which could be the result of membrane compaction (the blockage of the pores). At pH 4.0, the conversion from nitrite to free nitrous acid is lower and a larger amount of nitrite is needed to reach 100 $mgHNO_2$-N/L of FNA in solution (2520 mg/L $NaNO_2$ or 511 $mgNO_2^-$.N/L at pH 4.0 versus 525 mg/L $NaNO_2$ or 106 $mgNO_2^-$.N/L at pH 2.0), resulting in a higher TDS content. In addition of membrane compaction, the permeability decrease could also be due to concentration polarization due to high salt concentration. This also explains the salt rejection drop.

The membrane aged with FNA solutions at pH 4.0 with addition of hydrogen peroxide showed similar membrane performance compare to the ones aged with FNA solution at pH 4.0 only. The addition of hydrogen peroxide does not seem to enhance membrane performance loss.

Control experiments were conducted to verify if the pH alone was responsible for permeability loss rather than nitrite addition. The RO membrane coupons were exposed to 1.5g/L NaCl solution for 0.5, 1, 5, 7 and 9 days at pH 2.0 and 4.0. The Figure 34 compares the normalised permeability of the membrane exposed to ageing solution with and without nitrite addition. A drop in permeability for both applied pH was observed, indicating that the major change in the performances is only observed as a pH effect.

The ATR-FTIR spectra of new membranes were compared with the ATR-FTIR spectra of the aged membranes (Figure SI 11, Appendix G). No change in the chemical structure of the membrane could be observed after FNA exposure.

5.2. Conclusion

The impact of FNA and FNA/H_2O_2 solutions on membrane life expectancy was investigated to evaluate the compatibility of the cleaning/preservation agents with thin-film composite polyamide membranes. The following conclusions were reached:

Short-term ageing trials - membrane cleaning application

- FNA-based solutions are compatible with RO polyamide membranes up to 21600 mgN.h/L (based on high dose and short contact time ageing test).
- While accelerated ageing tests in both static and dynamic conditions at low pH (pH 2.0 and 4.0) did not show dramatic changes in membrane performances in terms of salt rejection, decrease of permeability was observed; pH appeared to be a major factor affecting the flux reduction.
- Lab-scale tests showed large variation of permeability and salt rejection between different initial membrane coupons, resulting in challenging interpretation of the data (distinguishing the real impact from experimental error).

Long-term ageing trials - membrane preservation application

- FNA-based solutions are compatible with RO polyamide membranes up to 43200 mgN.h/L (based on low dose and long contact time ageing test).
- Accelerated ageing tests at pH 5.0 (pH as recommended for membrane preservation applications) did not show dramatic changes in membrane performances in terms of salt rejection and permeability.
- This study showed the feasibility of using FNA at pH 3.0 and pH 5.0 for biomass removal and long-term preservation, respectively. No negative effect on polyamide RO membranes was observed when FNA was applied.

6. Economic and environmental impacts

In this part of the project, the environmental and economic benefits of the new cleaning chemicals were determined.

Based on the optimum conditions for RO membrane cleaning and preservation determined in the previous chapters and in comparison to current practice, quantification of the cost savings and environmental benefits achievable was carried out. Based on a life cycle analysis approach, the economic and environmental impact of the new cleaning agents was determined for the production, transport, use and disposal of the chemicals and compared to the costs of the commonly used cleaning chemicals.

6.1. Cost benefit analysis

Chemical cleaning. The FNA cleaning solution can be prepared by addition of sodium nitrite and HCl. According to the experimental results, 50 $mgNO_2^-$-N/L at pH 3.0 is the optimum condition for RO biofouling removal among the conditions applied in this study. A biofouling removal efficiency of up to 96% was obtained, and the scaling removal efficiency was comparable to the conventional acid cleaning solution (up to 100%). Therefore these conditions were used for cost calculation. As similar cleaning conditions were used for the different cleaning solutions, such as volume, cross-flow velocity, duration and temperature, the economic study was based on the chemical cost only. The results are presented in Table 14. The chemical costs of the FNA cleaning solution (Strategy A) was compared with the benchmarks used in this study (i.e., Strategy B: NaOH treatment at pH 11.0 followed by HCl treatment at pH 2.0). The acid consumption to maintain low pH for scaling removal is dependent on the amount of calcium carbonate present on the membrane surface. Hence, the total acid required to maintain a pH of 2.0 (Strategy B) and 3.0 (Strategy A) was calculated from the cleaning solution titration using the scaled membrane RO7 and presented in Figure SI 12, Figure SI 13 and Figure SI 14, Appendix H. The chemical cost associated with the two-step cleaning strategy to control biofouling and scaling appears to be significantly higher than using FNA alone ($2.3/m^3$ versus $1.7/m^3$). Membrane manufactures recommend 0.04-0.08 m^3 of cleaning solution per 8-inch RO element depending on the severity of the fouling [31]. In addition to cost effectiveness, the one-step cleaning strategies simplify the cleaning operation compared with the two-step strategies and lower the risk of irreversible fouling and membrane damage. FNA is readily available at low costs as it can be formed from the commonly available sodium nitrite and HCl, or even produced from ammonium containing wastewater as recently demonstrated for wastewater recycling applications [70].

The most common form of fouling in a wastewater application is biofouling on the lead membranes. For this reason, in some cases, NaOH cleaning only can be applied. When the acidic cleaning step does not take place (e.g., Strategy C), FNA might not be more economic in comparison with alkaline cleaning. However, FNA has the advantage to remove scaling as well. In addition, alkaline cleaning are often used in combination with others chemicals (e.g., chelating agents or surfactants), that are expensive chemicals. It should be highlighted that a full economic assessment must be done on a case by case basis considering the specific conditions of the systems and also the chemical supplier, delivery and waste disposal options.

Table 14. Comparison of chemical costs for RO biofouling and scaling control. Chemical costs are calculated based on average from 10 suppliers in US$/ton ($450/ton for sodium hydroxide, $240/ton for 32% hydrochloric acid, $440/ton for $NaNO_2$, $840/ton for citric acid and $1310/ton for SDS) (http://www.alibaba.com). Chemical cost for strategies A** and B** are calculated to simulated scaling removal based on membrane RO7.

Cleaning strategies	Prices (US$/m³)		
	Cleaning step #1	Cleaning step #2	Total
Strategy A (FNA, pH 3.0)*	0.22	-	**0.22**
Strategy A (FNA, pH 3.0)**	1.73	-	**1.73**
Strategy B (NaOH, pH 11.0/HCl, pH 2.0)*	0.05	0.48	**0.53**
Strategy B (NaOH, pH 11.0/HCl, pH 2.0)**	0.05	2.29	**2.34**
Strategy C (NaOH, pH 11.0)*	0.05		**0.05**

* Biofouling, ** Biofouling + Scaling

In addition, the expenses for cleaning chemicals are low compared to the cost of RO modules, pressure vessels and the lost production due to offline time. One of the crucial aspects is the

compatibility of FNA with polyamide membrane, which can allow a more frequent usage and at an earlier stage of fouling compared to other chemicals (such as cationic and nonionic cleaning agents), without membrane damage [71]. Decreasing cost related to membrane replacement would is another key aspect of the cost benefit of FNA application.

Membrane preservation. According to the experimental results, 10 mgHNO$_2$-N/L at pH 5.0 (419 mgNO$_2^-$-N/L) can be applied for long-term preservation of RO membrane within the pressure vessels (up to 6 months). These conditions were used for cost calculation (based on the chemical cost only). The chemical costs of the FNA preservation solution (Strategy C) was compared with the benchmarks used in this study (i.e., Strategy D: 1% SMBS solution). The total acid required to reach pH of 5.0 was calculated from the titration presented in Figure SI 13, Appendix H. The chemical cost associated with the application of FNA as a preservation agent appears to be lower than using SMBS ($0.9/m^3 versus $3.3/m^3). Chemical costs are calculated based on average chemical supplies from 10 suppliers in US$/ton ($332/ton for sodium metabisulphite, $240/ton for 32% hydrochloric acid, $440/ton for NaNO$_2$, http://www.alibaba.com). Furthermore, the benefit of the better chemical stability of FNA can contribute to a decrease of the operating cost. Any contact of SMBS solution with atmospheric oxygen will oxidize SMBS to sulphate. Then the preservative solution pH will drop and the potential for biological activity will increase due to nutrition supply for anaerobic bacteria [31]. FNA showed to be stable at pH 5.0 over a month (Figure 19). This would decrease the number of pH check needed and same time and operating time, particularly for large plants.

6.2. OH&S considerations

6.2.1. Potential OH&S implications

This section discusses the chemicals handling, storage and discharge when using FNA.

Transport and storage. As FNA will be generated from sodium nitrite and hydrochloric acid, it should be noted that, according to the material safety data sheet (source: Chemwatch), concentrated sodium nitrite (40%) is classified Dangerous Goods by the criteria of the Australian Dangerous Goods Code (ADG Code) for transport by Road and Rail because it is toxic if swallowed (see MSDS, Appendix I). Specific transport would then be required for sodium nitrite; however, this is also the case for sodium hydroxide (CAUSTIC SODA - LIQUID (46%-50%)) and Hydrochloric acid (33%), commonly used for membrane cleanings, due to their corrosive nature and, toxicity and body contact risk level, respectively. Interestingly, FNA itself is reported as Non-Hazardous Substance and Non-Dangerous Goods according to the National Occupational Health and Safety Commission Criteria and ADG Code. Sodium nitrite is classed as Packing Group III (= minor danger) which is the lowest level on a 3 point scale, while hydrochloric acid 33% is classed as Packing Group II (= medium danger). However, hydrochloric acid with concentrations up to 10% is classed as Packing Group III, consistently across a number of countries, including Australia. Although lower concentration requires a larger volume to be dosed to achieve the same pH reduction, the benefits in terms of convenience of transport and handling outweigh this as an issue. Similarly, Sodium nitrite with concentration of 0.5% is classified as non-dangerous goods according to the Model WHS Regulations and the ADG Code. Then, it would be strongly recommended to purchase/use lower concentrations of chemicals in order to increase safe handling and storage conditions.

When using FNA as a preservation agent, NaNO$_2$ is needed less frequently, and then powder can be used because it is easier to be transported. SMBS is not classified as Dangerous Goods by the criteria of the Australian Dangerous Goods Code (ADG Code) for transport by Road and Rail. However, this material is hazardous according to Safe Work Australia. In some case, FNA could even be produced from ammonium containing wastewater as recently demonstrated for wastewater recycling applications [70].

The conditions for storage of the different chemicals considered are similar as they all have to be stored in cool, dry and well ventilated places and away from heat sources.

Utilization. Nitrite is considered a major contaminant in wastewater, as are other nitrogenous compounds (e.g. ammonia and nitrate). However, no permeate filtration is expected during the cleaning process; the risk of releasing nitrite through the RO permeate is minimal. The nitrite will be concentrated and discharged either in the concentrate or the waste cleaning solution. However,

sodium nitrite, which will be used to form the FNA, is known to react with secondary amines under acidic conditions to produce carcinogenic nitrosamines [72]. Dimethylamine (DMA) is known to be present in wastewater from faeces (at fairly high levels ~ 1 ug/L) and would be one of the most common precursor [73]. The potential for nitrosamines formation during biofilm removal using FNA is reported in the following section.

Discharge. After cleaning, cleaning solutions can be discharged to a storage tank before to be either mixed and neutralised or directly discharged. For wastewater recycling application, spent cleaning solutions will be likely blended with WWTP effluent or discharged to the wastewater collection system or local sewer system. However, the FNA cleaning solution could also be subject to denitrification treatment before surface water discharge complying with discharge permits. For example at the Bundamba Advanced Water Treatment Plant (Ipswich, Australia), which was built as part of the Western Corridor Recycled Water Project to solve the water scarcity problem in south-east Queensland, a Moving Bed Biofilm Reactor (MBBR™) technology (nitrification) coupled with anoxic sand filtration (denitrification) were applied to treat RO concentrate and remove nitrogen. Then, the cleaning solution could be mixed with the RO concentrate and be biologically treated before being discharged to the river or other surface water sources. Effluent polishing allows meeting stringent EPA environmental requirements for environmental discharges. For seawater application, it is unlikely that the spent cleaning solutions can be discharged to ocean with brine due to the pH levels and organic/scale content. Special treatment would be needed.

However, for both applications, it is important to mention that typical cleaning frequency of RO membranes is two to four times per year and one membrane train is cleaned at a time [74]. Furthermore, spent cleaning solution tank including the waste cleaning solutions and the flush water. Then spent cleaning solution has already been diluted when discharged. Finally, the FNA cleaning solution is readily biodegradable (to N_2) through denitrification after dilution with other wastewater streams. Therefore its disposal after use will not likely cause environmental problems such as increased salt load and can be simply discharged after dilution.

6.2.2. Nitrosamine formation potential

The potential for nitrosamines formation during biofilm removal using FNA was investigated. A full detailed report has been submitted (Technical report, T2). The formation potential of five nitrosamines, including N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitrosomorpholine (NMOR), N-nitrosopiperidine (NPIP) and N-nitrosodibutylamine (NDnBA) was investigated during the application of free nitrous acid (FNA) for reverse osmosis (RO) membrane cleaning and biofilm removal. The five nitrosamines are described in Table SI 11, Appendix J. First, a nitrosamine formation potential test (30 mg/L NO_2-N, pH 5.0, 7 days incubation) was developed at the laboratory scale for FNA application in order to quantify and compare the concentration of precursors in different matrices under controlled conditions (i.e. ideally different RO membrane cleaning solutions). Based on the results, pH 5.0 was selected as the recommended pH values that can be used for the formation potential test without significant FNA degradation after 7 days of contact time.

Preliminary experiments were performed with two model precursors. For this aim, dimethylamine (DMA) and doxylamine (DOX) were employed as surrogates for secondary and tertiary amines, respectively. Their nitrosamine formation potential when using FNA was compared with the one observed when using monochloramine, already well reported in the literature. In general, the formation of NDMA via nitrosation was found to be less than the NDMA formation during chloramination. Results indicate that NDMA formation from FNA depends on pH. The highest NDMA concentration after 7 days of contact time was generated at pH 4.0-4.5 regardless of the DMA/NO_2 ratio. Although the initial reaction rate for nitrosation of secondary amines reaches its maximum at pH 3.0-3.4 (pKa of HNO_2), the yield for prolonged periods is largest at pH 4.0-5.0 due to a faster decomposition of nitrous acid at lower pH values.

Formation potential tests with both chloramine and FNA were conducted in simulated RO membrane cleaning solutions. For all conditions applied, the generated NDMA concentrations were measured between 5 and 20 ng/L, indicating that NDMA precursors were present in very limited amounts in membrane foulant and consequently in the cleaning solution. Although the NDMA formation potential with FNA was much lower than during chloramination, the formation of other nitrosamines was observed. In particular, NDEA was measured up to 110 ng/L during formation potential test at $37^{o}C$.

High temperature (37°C), high nitrite concentrations (500 mg/L as NO_2) and pH values of 4.0-5.0 enhanced nitrosamine formation, in particular NDEA ((max 110 ng/L) and NDnBA (max. 45 ng/L). Lower concentrations of NMOR (max. 25 ng/L) were detected under these specific conditions, while NPIP was not measured above the limit of detection. No significant effect of H_2O_2 addition was noticed for NDMA and NDnBA formation potential, while the addition of H_2O_2 inhibited the formation of NDEA.

The data shows that small amounts of nitrosamines may be formed during the membrane cleaning process with FNA (up to 20 ng/L). However, the quantity will not accumulate to a level that would affect the environment because the cleaning solution will be washed out and discharged in the RO concentrate. During the cleaning process, the FNA cleaning solution would not be in direct contact with the RO feed water. However, nitrosamines precursors (e.g. proteins, secondary amines) can be accumulated on the membrane surface or trapped in the biofilm. In this case, nitrosamines formation potential has to be considered in case they may pass through the membrane. Commonly, RO membrane cleaning is conducted at low pressure to compensate for the pressure drop from feed to concentrate. Consequently, little permeate can be produced. NDMA rejection by RO membranes was reported between 10 and 70% in the literature [75, 76] and may pass through the membrane. Nevertheless, the concentration from the cleaning solution formation potential test with FNA was low (max 20 ng/L). The other nitrosamines (i.e. NDEA, NMOR, NDnBA) were detected at much higher concentrations (especially NDEA). However, they have shown 90% or greater rejection by four RO membranes [75].

At the end of the cleaning procedure, RO permeate or deionized water is used for flushing out the cleaning solution and membrane foulants using high flow rate. Therefore, the nitrosamines potentially formed during RO membrane cleaning process using FNA will be flushed out similarly and are likely to be discharged with the cleaning solution to a sewer with a high dilution effect.

To summarize, the higher risk of nitrosamine formation is related to the low rejection of NDMA by RO membranes. However, NDMA formation is low and other nitrosamines will be rejected even if they are formed at higher concentrations. Based on these results, the application of FNA alone or in combination with hydrogen peroxide shows a low risk level for nitrosamine formation, including NDMA, NDEA, NMOR, NPip and NDnBA.

6.3. Life cycle analysis

6.3.1. Introduction

Along with the cost comparison, the life-cycle GHG implications of the proposed FNA cleaning system have been assessed.

6.3.2. Methodology

System Boundary & Functional Unit
The two systems being compared are consistent with those included in the cost analysis section of this report. Table 15 provides a summary of the steps modelled for each of the two cleaning regimes. Important considerations are that:

- Given the likely variation in actual cleaning regimes employed at wastewater recycling facilities around the world, the 'conventional' scenario represents a hypothetical cleaning regime in order to provide indicative results.
- The FNA cleaning regime is based on a number of important assumptions that could vary depending on actual plant circumstances.

Table 15. The 'Conventional' and 'FNA' cleaning regimes considered in the life-cycle analysis.

Cleaning Step	'Conventional' scenario	'FNA' scenario
Initial membrane flush	Low pressure flush with RO permeate for 10min. All flush water discharged to a sump, then pumped to the head of the STP.	Low pressure flush with RO permeate for 10min. All flush water discharged to a sump, then pumped to the head of the STP.
Prepare the primary cleaning agent	Pump 50%ww caustic solution from bulk storage tank into the chemicals mixing tank, and dilute to 0.1%ww with RO permeate. Heat the solution to 35°C.	Mix solid $NaNO_2$ & 33%ww liquid HCl with RO permeate in the mixing tank, to achieve a nitrite concentration of 50 mg/L at pH 3.
Circulate the primary cleaning agent	Circulate the solution through the membranes for 60min, and then soak.	Circulate the solution through the membranes for 120min, and then soak.
Flush the cleaning agent out of the membranes	Low pressure flush with RO permeate for 10min. All flush water discharged to a sump, then pumped to the head of the STP.	--
Prepare the secondary cleaning agent	Dilute 33% liquid HCl with RO permeate in the chemicals mixing tank, to achieve a 0.2% HCl solution at pH 1-2. Heat the solution to 35°C.	--
Circulate the secondary cleaning agent	Circulate the solution through the membranes for 60min, and then soak.	--
Final membrane flush	High pressure flush with RO permeate for 10min. Low pressure flush with RO permeate for 60min. All flush water discharged to a sump, then pumped to the head of the STP.	High pressure flush with RO permeate for 10min. Low pressure flush with RO permeate for 60min. All flush water discharged to a sump, then pumped to the head of the STP.
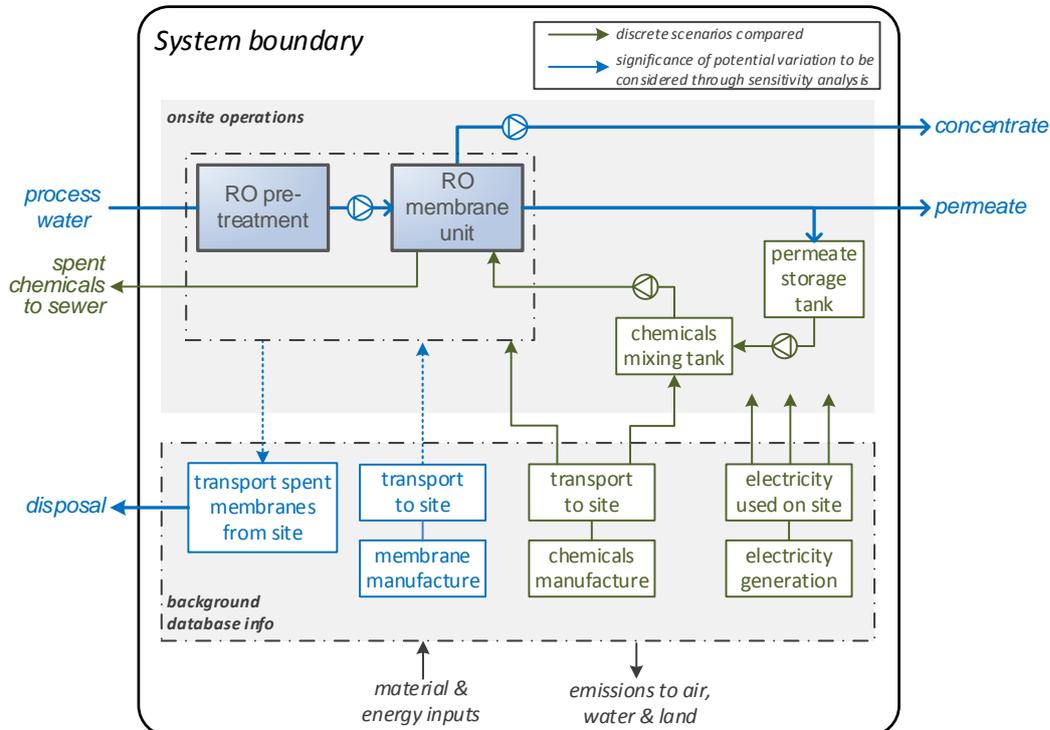
Figure 35. The scope of the system flows that were included in the analysis for each of the two scenarios.

Key elements that define the system boundary are:

- Only operational flows were included in this analysis. Construction of the treatment plant infrastructure, and end-of-life disposal of that infrastructure, were excluded on the grounds that (a) they are typically only a small contributor to the overall life-cycle impacts associated with urban water systems [see 77, 78, 79]; and (b) the requirement for infrastructure (e.g. tanks & pumps) is expected to be largely the same for the two different cleaning regimes.
- The life-cycle inventory includes the generation & supply of the chemicals used for the cleaning regime. While the discharge of spent cleaning fluids is included in the modelling, any downstream implications of treating that waste are not considered in this analysis.
- The generation of RO permeate is also included as an input to the system, incorporating a full life-cycle model of the RO permeate production system. This includes all material and energy inputs to the production plant, along with the disposal of MF backwash and RO concentrate flows. The model used here excludes any operations downstream of the RO membrane (e.g. advanced oxidation, disinfection & product water discharge pumping).
- This recognises that if less permeate is used for the cleaning process (flushing & mixing with chemicals), then that permeate would become available as product water that can be delivered to users.
- Reducing the permeate requirements for membrane cleaning would therefore reduce the overall production required by the treatment plant, and the environmental implications of that reduced production are attributed to the scenarios shown here.

The basis for analysing and comparing the two scenarios (the functional unit) is a single cleaning cycle. Implicit in this choice of functional unit is the assumption that the FNA-based cleaning regime would be implemented with the same frequency as the more conventional alternative. The implications of that assumption are considered further below.

Results are presented on a per-membrane or per-plant basis depending on the question being addressed in each section of this chapter - in all cases; it was assumed that the input flows are proportional to the number of membranes included.

Inventory data
For each of the system flows described in Table 15, mass and energy flows were estimated using equipment specifications taken from an existing facility for cleaning RO membranes in an advanced wastewater treatment plant.

Database and literature sources were used to estimate the material inputs, energy inputs, and environmental emissions associated with providing the inputs (chemicals; RO permeate; membranes; electricity) to the cleaning process (Table 17).

Impact Assessment
The analysis in this chapter focusses primarily on greenhouse gas (GHG) footprints, using 100yr equivalency factors taken from the most recent IPCC report [80].

Twelve other life-cycle impact assessment (LCIA) categories were also considered briefly in the final part of the results section below. The indicators are described in Table SI 12 (Appendix K). The suite of categories, and the models chosen for each, followed the default approach chosen in [77].

Software
The inventory models, and impact assessment results, were implemented in the Simapro (v8.05) software.

Table 16. Estimates of the foreground inventory flows for each of the scenarios.

Inventory item	Conventional scenario	FNA scenario
Initial membrane flush	336 L of permeate used per membrane being cleaned (0.56 L/s per membrane, for 10min)	
Prepare the primary cleaning agent	0.2 kg of 50%ww caustic per membrane 112 L of RO permeate per membrane 1.3kWh of electricity per membrane	0.03 kg of solid $NaNO_2$ per membrane 0.05 kg of 33%ww HCl per membrane 111 L of RO permeate per membrane
Flush the cleaning agent out of the membranes	336 L of permeate used per membrane being cleaned (0.56 L/s per membrane, for 10min)	--
Prepare the secondary cleaning agent	0.7 kg of 33%ww HCl per membrane 111 L of RO permeate per membrane 1.3kWh of electricity per membrane	--
Final membrane flush	2750 L of permeate used per membrane being cleaned (1.22 L/s per membrane for 10min; then 0.56 L/s per membrane for 60min)	
Bulk chemical transfer pump	60 W motor operating at 1.5 L/h	
Permeate supply pump	18.5 kW motor operating at 22 kL/s	
Chemical circulation pump	1.2 kW power required per membrane being cleaned	
Sump pump	7.5 kW motor operating at 35 L/s	

Table 17. Sources & assumptions used for the background inventory estimates.

Inventory item	Source	Key assumptions
Manufacturing & transport of chemicals used in the cleaning process	Australasian LCI database [81]	All chemicals are manufactured in Australia. The manufacturing inventories are based on the Ecovinvent v3 database [82], modified to use Australian inputs (e.g. power & transport models)
Generation & supply of electricity used in the cleaning process	Australasian LCI database [81]	Electricity is assumed to be from a system average mix of Queensland generators
Production of the RO permeate	Data from Seawater desalination & Advanced Wastewater Treatment Plant to recycle wastewater for Direct Non-potable Reuse [77]	

6.3.3. Results & Discussion

Greenhouse Gas (GHG) analysis

The primary chemical input to the FNA cleaning regime is sodium nitrite ($NaNO_2$). The majority of global $NaNO_2$ production uses sodium hydroxide as the primary feedstock, hence the manufacture of $NaNO_2$ would be expected to have a higher environmental footprint than the manufacture of caustic. Using the manufacturing inventories from the Ecoinvent database [82] as a guide, the GHG footprint of producing 1kg of solid $NaNO_2$ is ~3 times bigger than that for producing 1kg of 50% caustic solution.

Note also that, in terms of the GHG footprint, the transport of the chemicals manufacturing plant to the water utility is only of minor relevance. Assuming that the $NaNO_2$ is sourced with a transport distance of ~1000km, the road freight of the chemical products adds only 4% to the GHG footprint of the supplied product. If the chemical is imported from overseas, the GHG emission would be substantially higher, depending on the transport distance. For this analysis, it is assumed that the caustic soda is manufactured in Brisbane, hence incurs only a negligible transport distance.

While the FNA product has a higher GHG intensity per-kg, much less chemical product is required for the FNA scenario, hence the GHG footprint associated with chemicals procurement in the FNA scenario is actually much lower (Table 18).

Furthermore, when the full system boundary (as in Figure 35) is considered, chemicals supply contributes only a very small fraction (1-6%) of the overall GHG footprint for either system (Table 18). It is therefore likely that a comparison across the different cleaning approaches would be more sensitive to key operational parameters (e.g. circulation times; heating requirements; flushing requirements) than it would be to the choice of chemicals in use.

For some wastewater applications, scaling may not be an issue. In this case, the acid cleaning step is not needed in the conventional method. Then, the overall GHG footprint would be reduced from 10.9% to 8.2%, which is still higher than the FNA strategy (7.3%).

Table 18. Breakdown of the greenhouse gas footprint for the (a) conventional scenario, and (b) FNA scenario. Totals are shown both in absolute units, and as a % of the overall GHG footprint for each scenario. Impact Assessment categories and respective units are described in Appendix K.

(a)

		chemicals supply	permeate supply	direct power use heating	direct power use pumping	total
initial flush		--	0.45	--	0.07	0.5 (5%)
caustic	preparation	0.41	0.15	1.2	0.03	1.8 (16%)
caustic	circulation	--	--	--	1.1	1.1 (10%)
caustic	flush	--	0.45	--	0.07	0.5 (5%)
acid	preparation	0.23	0.15	1.2	0.02	1.6 (15%)
acid	circulation	--	--	--	1.1	1.1 (10%)
final flush	HP	--	0.97	--	0.2	1.1 (10%)
final flush	LP	--	2.69	--	0.4	3.1 (29%)
Total		0.6 (6%)	4.9 (44%)	2.4 (22%)	3.0 (28%)	10.9

(b)

		chemicals supply	permeate supply	direct power use heating	direct power use pumping	total
initial flush		--	0.45	--	0.07	0.5 (5%)
FNA	preparation	0.10	0.15	--	0.02	0.3 (2%)
FNA	circulation	--	--	--	2.2	2.2 (21%)
FNA	flush	--	--	--	--	--
acid	preparation	--	--	--	--	--
acid	circulation	--	--	--	--	--
final flush	HP	--	0.97	--	0.2	1.1 (10%)
final flush	LP	--	2.69	--	0.4	3.1 (29%)
Total		0.1 (1%)	4.3 (59%)	--	2.9 (40%)	7.3

It is worth noting the significant contribution (44-59%) of the permeate supply to the overall GHG footprint of the both cleaning approaches. While the permeate volumes required to prepare the chemicals and flush the system during the cleaning process are very small compared to the overall plant production, so too the chemical requirements for a cleaning cycle are small compared to the chemical inputs required for other aspects of the treatment plant operations. The estimated GHG footprint for permeate supply in this study was 1.3 kg-CO2e/kL. This value incorporates the power use, chemicals use and waste disposal associated with the RO permeate production, and is based on operating data from a large scale RO based plant [see 77].

The contribution of chemicals supply to the life-cycle burden of a membrane cleaning process for a sea-water RO desalination (SWRO) plant would likely be even lower. This is because the life cycle 'burden' associated with the diversion of permeate to the cleaning process, would be even greater for a SWRO plant, which requires a much higher energy demand for pumping through the RO membranes.

Analysis across a broad set of Life Cycle Impact Assessment (LCIA) indicators
A direct comparison between the 'conventional' and 'FNA' cleaning regimes, shows that our FNA scenario has a lower environmental footprint for all impact categories considered (Table 19).

Table 19. Impact Assessment results for the two scenarios. (a) The absolute results are provided, along with the overall difference between the two scenarios (a negative difference implies that the FNA scenario had a lower impact). (b) The overall difference for each impact category is allocated across changes in the choice of chemicals, use of power onsite, and use of permeate. For each impact category, the largest contributor to the differences is highlighted in grey. Impact Assessment categories and respective units are described in Appendix K.

(a)

		Scenario		Overall Difference
		Convent-ional	FNA	
Freshwater Extraction	(L H2O)	0.02152	0.0065	-70%
Eutrophication	(kg O2e)	0.00821	0.0069	-16%
Eco-toxicity — Marine	(kg 1-4-DCBe)	0.00589	0.0046	-22%
Eco-toxicity — Freshwater		0.0081	0.0076	-7%
Eco-toxicity — Terrestrial		6.6E-05	5.7E-05	-14%
Terrestrial Acidification	(kg SO2e)	0.062	0.042	-33%
Global Warming	(kg CO2e)	10.9	7.3	-33%
Ozone Depletion	(kg (CFC11e)	1.4E-05	9.6E-06	-33%
Resource Depletion — Fossil Fuels	(kg oil-e)	2.8	1.8	-34%
Resource Depletion — Minerals	(kg Sb-e)	2.2E+04	1.2E+04	-46%
Human Health — Toxicity	(kg 1-4-DCBe)	0.64	0.43	-32%
Human Health — Photochemical Oxidants	(kg NMVOC)	0.049	0.033	-33%
Human Health — Particulate Matter	(kg PM10e)	0.020	0.013	-33%

(b)

	Overall Difference	Difference attributed to…		
		choice of chemicals	direct power use	permeate use
Freshwater Extraction	-70%	-64%	-2%	-4%
Eutrophication	-16%	0%	-5%	-11%
Eco-toxicity — Marine	-22%	-10%	-3%	-8%
Eco-toxicity — Freshwater	-7%	5%	0%	-12%
Eco-toxicity — Terrestrial	-14%	6%	-13%	-7%
Terrestrial Acidification	-33%	-5%	-22%	-6%
Global Warming	-33%	-5%	-23%	-5%
Ozone Depletion	-33%	-7%	-20%	-6%
Resource Depletion — Fossil Fuels	-34%	-6%	-22%	-5%
Resource Depletion — Minerals	-46%	-28%	-13%	-5%
Human Health — Toxicity	-32%	-19%	-6%	-8%
Human Health — Photochemical Oxidants	-33%	-4%	-24%	-5%
Human Health — Particulate Matter	-33%	-5%	-23%	-5%

Differences in direct (onsite) power use between the two scenarios explain the majority of the differences for 7 of the 13 impact categories, whereas the difference choice of chemicals explains the majority of the overall difference for 4 of the 13 impact categories (Table 19). This does provide some further support for the conclusion that it is onsite characteristics of the cleaning regime (e.g. heating requirements/circulation times), rather than the choice of chemical agents, which most strongly influences the life-cycle analysis.

However, it is clear that the choice of chemicals could have a stronger influence on a comparative study of cleaning regimes, if the focus is on a broader set of impact categories than just greenhouse gas emissions. Future studies should therefore not overlook the importance of refining the quality of those key inputs relating to the chemicals supply – inventories for the manufacturing of the chemicals, and the distance and mode of transport involved in getting those chemicals to site. Since the 'conventional' choice of cleaning chemicals, and the proximity to various chemical manufacturing plants, could vary greatly depending on the water treatment plant under consideration, all those factors could vary greatly from one case study to another.

The implications of any change to membrane performance
Finally, the RO membrane cleaning process is such a tiny part of the overall life-cycle GHG footprint for the treatment plant operations. It is therefore likely that the most influential GHG effect of the FNA cleaning regime would be any change it causes to fouling rates over the long term, the effect this has on the long term average filtration efficiency of the RO membranes and the potential increased membrane life.

6.4. Conclusion

The environmental and economic benefits of the new cleaning chemicals were determined. Based on the optimum conditions for RO membrane cleaning and preservation determined in the previous chapters and in comparison to the current practice, quantification of the cost savings and environmental benefits achievable was carried out. Based on a life cycle analysis approach, the economic and environmental impact of the new cleaning agents was determined for the production, transport, use and disposal of chemicals and compared to the current costs of commonly used cleaning chemicals.

Cost benefit analysis

- Due to the potential for both biofouling and scaling removal, FNA is a cost-effective solution for biofouling removal and long-term preservation in RO filtration applications compared to two-step cleaning process (alkaline/acid cleaning).
- One of the key benefits would be the compatibility of FNA with polyamide membrane, which can allow a more frequent usage and at an earlier stage of fouling compared to other chemicals (such as cationic and non-ionic cleaning agents) or biocide (such as chlorine and monochloramine), without membrane damage. Decreased cost related to membrane replacement s another crucial aspect of the cost benefit of FNA application.

OH&S considerations

- No significant changes would be required for the chemicals handling, storage and discharge when using FNA compared to commonly used chemicals.
- Sodium nitrite used to generate FNA is classified as Dangerous Goods by the criteria of the Australian Dangerous Goods Code (ADG Code) for transport by Road and Rail because it is toxic if swallowed. However similar specific transport is also required for sodium hydroxide (50%) and Hydrochloric acid (33%), commonly used for membrane cleanings.
- Formation potential tests of nitrosamines disapproved hypothesis that nitrosamines may be formed in excessive amounts during membrane cleaning.
- After cleaning, cleaning solutions can be discharged to a storage tank before to be either mixed and neutralised or directly discharged. Different options are suggested depending of the membrane application and location of the plants. However, due to the low frequency of membrane cleaning and/or long-term preservation and mixing with flush water, spent FNA-based solution has already been diluted when discharged. FNA solution is readily biodegradable (to N_2) through denitrification after dilution with other wastewater streams. Therefore its disposal after use will likely not cause environmental problems such as increased salt load and can be simply discharged after dilution.

Life cycle assessment

- While the proposed FNA-based cleaning regime had the lowest life-cycle environmental footprint of the different scenarios considered in this study, it is not possible to provide a definitive statement on whether RO membrane cleaning using the FNA process will be a more 'environmentally friendly' option. FNA has not been applied in real application and lot of assumptions have been done for the calculation.
- The analysis indicates that the LCA results are more sensitive to onsite operational conditions than to the choice of cleaning chemicals in use. The proposed FNA cleaning regime will therefore reduce the environmental footprint of the treatment plant; if it can be implemented with reduced heating requirements, reduced circulation times and/or reduced demand for flushing of the membranes with RO permeate (e.g., when one-step cleaning can be applied instead of two step alkaline/acid cleaning). All these characteristics may vary substantially from site to site.
- More importantly, the life-cycle environmental burdens associated with the RO membrane cleaning process are very small compared to those associated with the energy required to pump water through RO membranes. Consequently, the greatest environmental benefits would occur if the proposed FNA cleaning regime could enable lower membrane fouling rates, hence lower (on average) pumping power requirements. However, this still needs to be proven at large-scale.
- Further research should focus on whether the proposed FNA approach can reduce overall chemical and biofouling rates. Given the proposed one step FNA-based cleaning regime would greatly reduce the time (and potentially labour) required for each cleaning step, consideration should be given into whether this makes possible a more frequent cleaning implementation, assuming that could help reduce average fouling over the long term.

Appendix

APPENDIX A- LAB-SCALE CLEANING TRIALS

RO1

RO2

RO3

RO4

RO5

RO6

RO7

Figure SI 1. Photographs of the RO membranes from seven full-scale RO plants.

Table SI 1. List of the cleaning trials conducted for biofouling removal.

Cleaning test #	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11
Membrane module	RO2				RO3	RO4		RO5		RO6	
Cleaning Solutions	FNA, pH 2	FNA, pH 2	FNA, pH 2	FNA, pH 3	FNA, pH 3	FNA, pH 3	FNA, pH 2	FNA, pH 3	FNA, pH 3	FNA, pH 2	FNA, pH 2
	FNA, pH 3	FNA, pH 3	NaOH, pH 11	NaOH, pH 11	FNA, pH 3, 50 mg g/L H_2O_2	FNA, pH 3, 50 mg g/L H_2O_2	FNA, pH 3	FNA, pH 3, 50 mg g/L H_2O_2		FNA, pH 3	FNA, pH 3
	FNA, pH 4	FNA, pH 4	Water (2hr Rinse)	Water	FNA, pH 3, 150 mg g/L H_2O_2	50 mg g/L H_2O_2	FNA, pH 4	50 mg g/L H_2O_2		FNA, pH 4	FNA, pH 4
	NaOH, pH 11	Water (2hr Rinse) /Water	-	Water (2hr Rinse)	NaOH, pH 11	NaOH, pH 11	NaOH, pH 11	NaOH, pH 11	NaOH, pH 11	NaOH, pH 11	NaOH, pH 11
	Water	-	-	-	Water (2hr Rinse) /Water	Water (2hr Rinse) /Water	Water (2hr Rinse) /Water	Water (2hr Rinse) /Water	Water (2hr Rinse)	Water (2hr Rinse) /Water	Water (2hr Rinse) /Water

Table SI 2. List of the cleaning trials conducted for scaling removal.

Cleaning test #	#1	#2	#3	#4
Membrane module	RO7			
Cleaning Solutions	10 v/v% HNO_3	Water	10 v/v% HNO_3	Water
	HCl pH 2	HCl pH 3	HCl pH 2	HCl pH 3
	FNA, HCl pH 2	FNA, HCl pH 3	FNA, HCl pH 2	FNA, HCl pH 3
	Citric Acid pH 2	Citric Acid pH 3	Citric Acid pH 2	Citric Acid pH 3

Table SI 3. Hydraulic performances of membranes after cleaning tests (Pristine permeabilities have been measured using membrane coupons from unused membranes).

Membrane #	Pristine Permeability ($L/m^2.h.bar$, 25^oC)	Membrane autopsy Permeability ($L/m^2.h.bar$, 25^oC) (n=4)	Water Rinse (2hr)	Water	NaOH pH 11	FNA pH 2	FNA pH 3	FNA pH 4
RO1	4.5±0.9 (n=19)	5.3±0.3	-	-	-	-	-	-
RO2	3.1±0.3 (n=4)	4.1±0.2	-	-	1.3	-	3.7	-
			-	4.7	2.9	-	4.8	4.6
RO3	3.1±0.3 (n=4)	4.3±0.1	4.0	3.8	4.7	-	4.3	-
			4.0	4.2	4.2	-	3.9	-
RO4	-	2.3±0.1	2.2	1.9	2.2	-	2.2	-
			-	3.6	3.6	3.4	3.7	3.2
RO5	-	3. 6±0.2	-	-	4.0	-	2.7	-
			-	3.7	2.9	-	3.7	-
RO6	-	2.5±0.1	-	2.8	2.7	2.7	2.8	2.8
			-	-	-	-	2.6	2.6
RO7	-	1.2±0.4	-	-	-	-	-	-

Membrane #	Rejection (%)	Rejection (%) (n=4)	Water Rinse (2hr)	Water	NaOH pH 11	FNA pH 2	FNA pH 3	FNA pH 4
RO1	98.6±0.3 (n=19)	96.9±0.3	-	-	-	-	-	-
RO2	91.5±2.3 (n=4)	95.1±1.2	-	-	95.9	-	95.1	-
			-	95.4	96.5	-	96.1	95.6
RO3	91.5±2.3 (n=4)	95.7±1.7	95.4	95.5	96.4	-	97.5	-
			92.0	96.3	95.9	-	97.6	-
RO4	-	98.1±0.7	98.6	98.5	98.7	-	98.2	-
			-	97.3	98.8	99.4	96.3	99.1
RO5	-	98.4±0.2	-	-	99.0	-	98.0	-
			-	98.1	98.5	-	98.3	-
RO6	-	99.0±0.2	-	99.2	98.9	99.3	99.4	98.8
			-	-	-	-	98.1	98.7
RO7	-	98.3±0.0	-	-	-	-	-	-

APPENDIX B- PILOT-SCALE CLEANING TRIALS
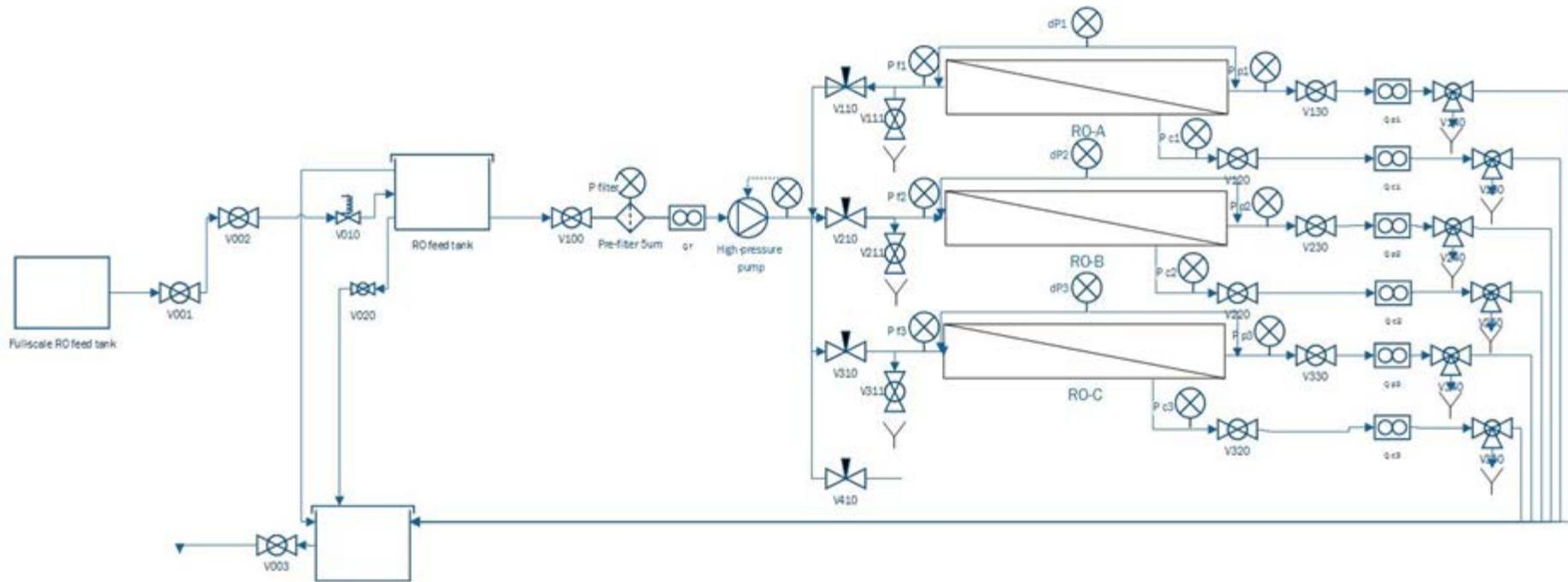
Figure SI 2. Description of the RO pilot-plant.

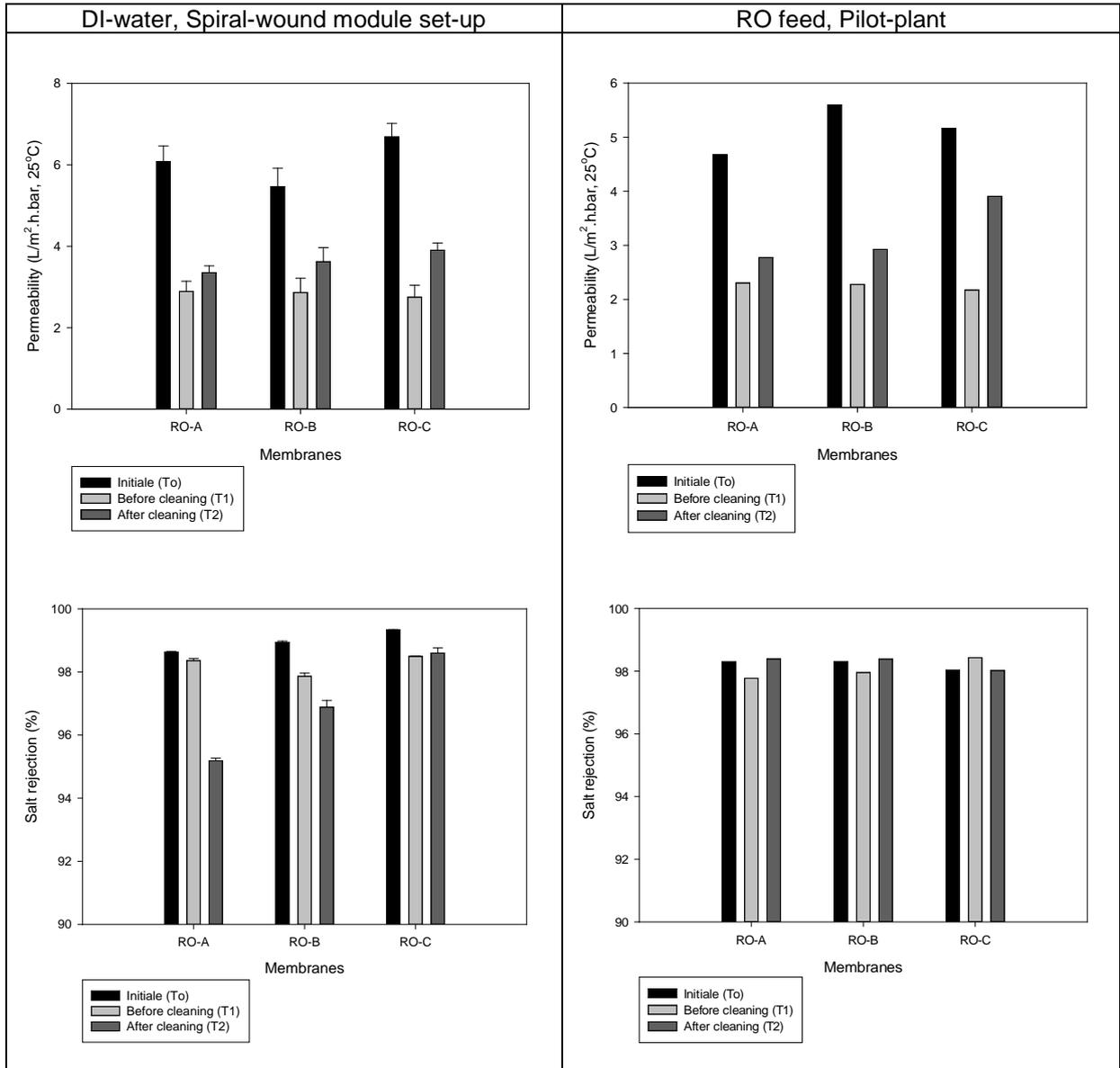Figure SI 3. Cleaning results with each reagent on pilot-scale fouled membrane samples RO-A (50 $mgNO_2^-$-N/L, pH 3.0, 25°C), RO-B (50 $mgNO_2^-$-N/L, pH 3.0, 35°C) and RO-C (NaOH, pH 11.0, 35°C). The membrane performances were measured with DI=water using a spiral-wound module set-up (ex-situ) and RO feed water on the pilot-plant (in-situ). The error bars show the average of three measurements.

APPENDIX C- SHORT-TERM PRESERVATION TESTS

Table SI 4. Hydraulic performances of membranes after short-term preservation tests.

	Tested preservation solutions	Water permeability (L/m^2.h.bar, 25°C)		Salt rejection (%)	
		Sample 1	Sample 2	Sample 1	Sample 2
ESPA-2	DI water (Baseline)	5.0±0.1	4.4±0.1	96.8±0.4	99.1±0.0
	DI water	3.4±0.1	3.5±0.1	99.3±0.1	99.0±0.1
	DI water, pH 5.0	4.7±0.1	4.7±0.2	98.6±0.1	97.7±0.0
	0.1 mgHNO$_2$-N/L FNA, pH 5.0	5.0±0.1	4.7±0.2	98.1±0.2	98.1±0.0
	1 mgHNO$_2$-N/L FNA, pH 5.0	4.9±0.1	-	98.3±0.2	-
	3 mgHNO$_2$-N/L FNA, pH 5.0	4.8±0.3	-	99.0±0.1	-
	10 mgHNO$_2$-N/L FNA, pH 5.0	4.1±0.1	4.4±0.1	98.8±0.1	99.0±0.0
SWC-5	DI water (Baseline)	1.6±0.0	1.6±0.0	99.1±0.1	98.1±0.8
	DI water	1.6±0.1	1.5±0.1	98.5±0.5	98.9±0.1
	DI water, pH 5.0	1.3±0.1	1.3±0.1	98.6±1.0	99.3±0.4
	0.1 mgHNO$_2$-N/L FNA, pH 5.0	1.5±0.4	1.5±0.0	97.6±0.4	98.6±0.4
	1 mgHNO$_2$-N/L FNA, pH 5.0	1.7±0.1	-	98.3±0.5	-
	3 mgHNO$_2$-N/L FNA, pH 5.0	1.6±0.1	-	98.8±0.4	-
	10 mgHNO$_2$-N/L FNA, pH 5.0	1.5±0.5	1.7±0.0	98.5±1.0	96.7±0.6
RO2	DI water (Baseline)	4.5±0.1	3.7±0.1	94.2±1.6	96.7±1.4
	DI water	-	5.6±0.2	-	97.7±0.3
	DI water, pH 5.0	n.a	n.a	n.a	n.a
	0.1 mgHNO$_2$-N/L FNA, pH 5.0	n.a	5.6±0.2	n.a	96.1±0.2
	1 mgHNO$_2$-N/L FNA, pH 5.0	-	-	-	-
	3 mgHNO$_2$-N/L FNA, pH 5.0	-	-	-	-
	10 mgHNO$_2$-N/L FNA, pH 5.0	5.4±0.1	n.a	96.1±0.2	n.a
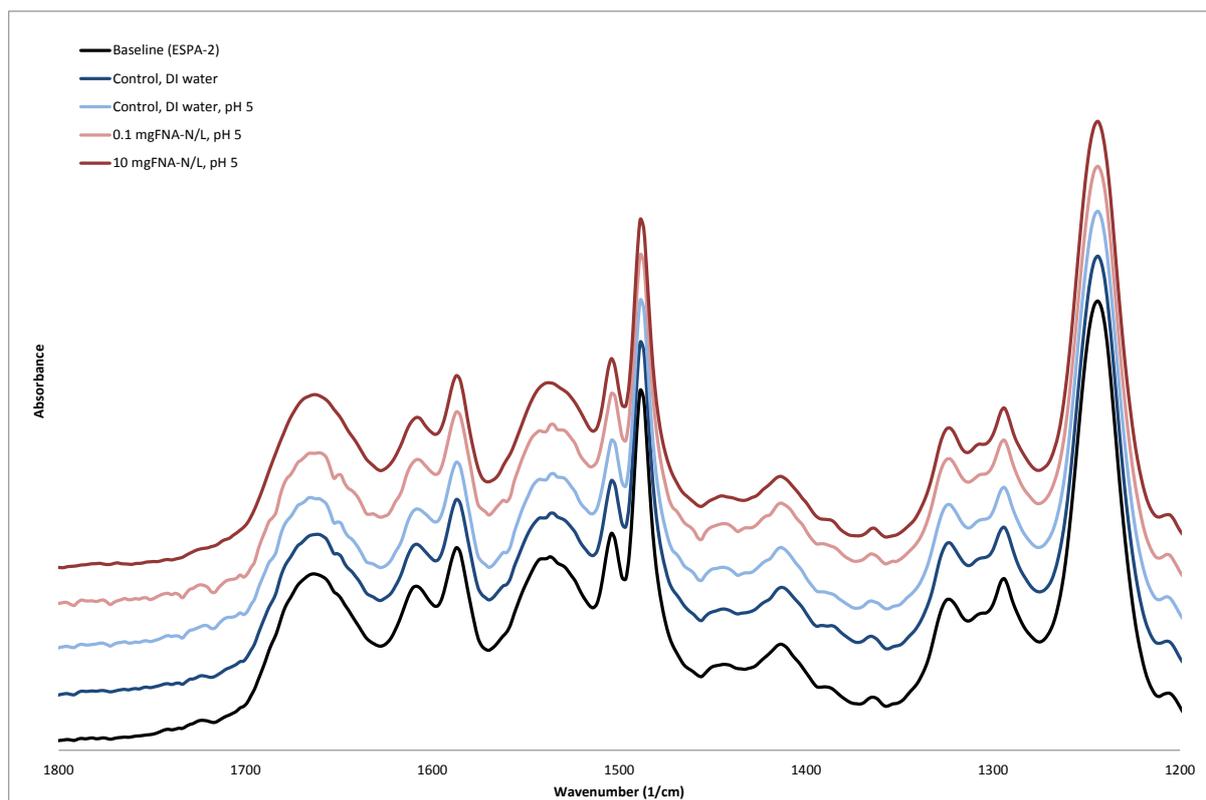
a.
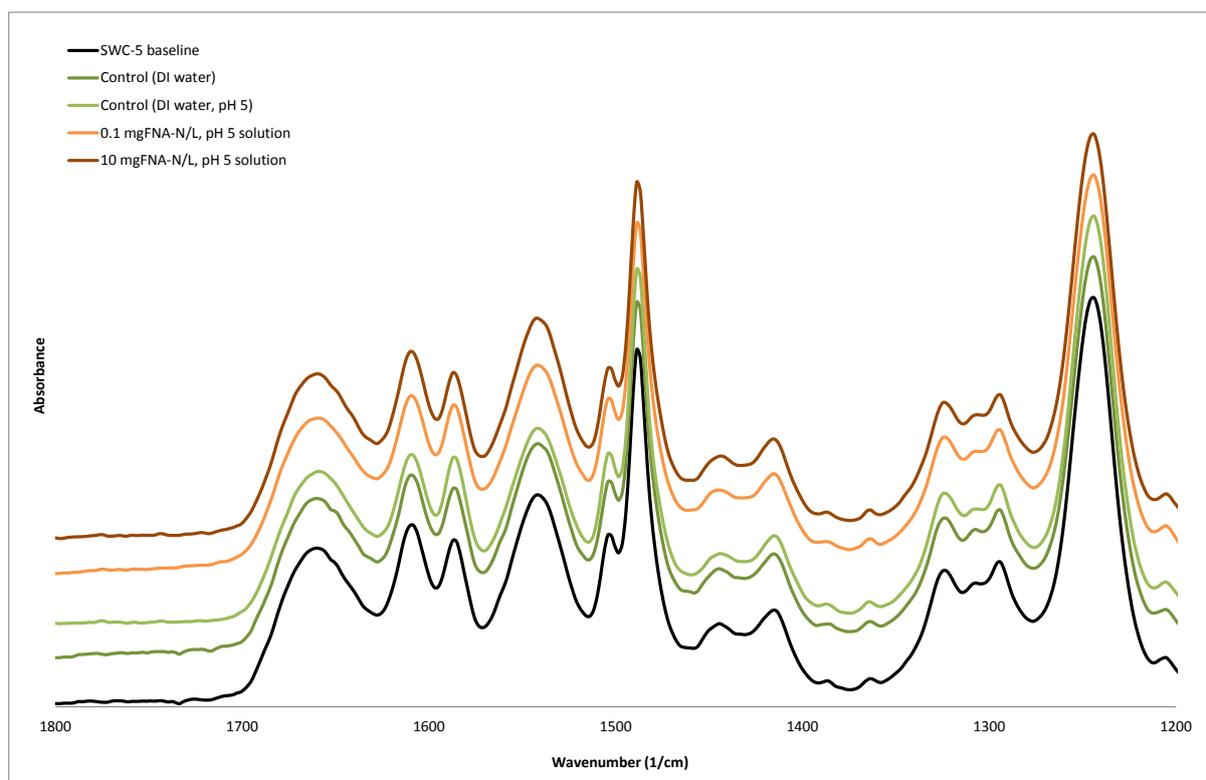
b.

Figure SI 4. FTIR spectrum of (a) ESPA-2 and (b) SWC-5 membranes before and after one-month preservation tests. Normalisation of the spectra has been made on maximum peak near 1250 cm^{-1}, associated with the C-O-C asymmetric stretching vibration from Aryl-O-aryl group.

APPENDIX D- LONG-TERM PRESERVATION TESTS

Figure SI 5. Temperature evolution during the long-term preservation trials.

Table SI 5. Permeability and salt rejection results (Permeability (L/m^2.h.bar, 25°C)/ Salt rejection (%)) of the long-term preserva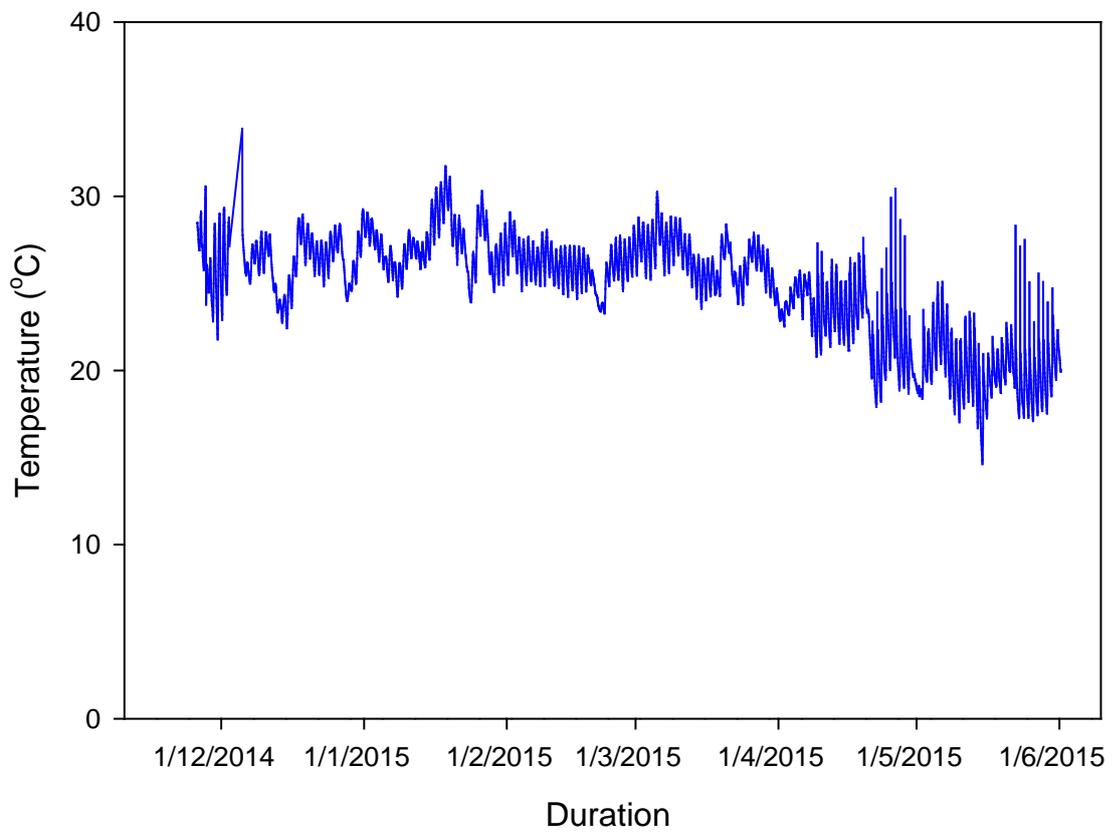tion tests. Standard conditions: 4-inch RO modules (ESPA-2, Hydranautics) stored in vacuum sealed bag to simulated "Preservation out of the plant".

Tested preservation solutions	Preservation duration	Water permeability (L/m^2.h.bar, 25°C)		Salt rejection (%)	
		Sample 1	Sample 2	Sample 1	Sample 2
DI water, pH 5.0 (control)	DI water (Baseline)	4.6±0.6	5.5±0.4	99.0±0.0	99.2±0.0
	2 months	4.8±0.1	5.2±0.5	97.6±0.0	98.7±0.1
	DI water (Baseline	6.5±0.2	5.8±0.8	98.8±0.1	99.0±0.1
	4 months	4.3±0.0	3.9±0.0	97.3±0.2	98.2±1.4
	DI water (Baseline	5.1±0.4	6.4±1.4	98.9±0.3	99.0±0.0
	6 months	6.7±0.4	5.8±0.5	99.1±0.0	99.3±0.0
10 mgHNO$_2$-N/L FNA, pH 5.0	DI water (Baseline)	6.0±0.9	5.9±0.6	99.1±0.0	99.2±0.1
	2 months	5.8±0.2	4.9±0.2	97.8±0.1	98.3±0.0
	DI water (Baseline	6.8±0.7	7.4±0.7	99.2±0.0	98.9±0.0
	4 months	4.7±0.2	5.1±0.1	98.7±0.1	96.5±0.0
	DI water (Baseline	7.1±0.7	4.9±0.5	99.0±0.0	99.1±0.0
	6 months	7.2±0.8	8.1±0.2	98.9±0.0	98.9±0.1
1% SMBS	DI water (Baseline)	4.7±0.7	4.2±0.4	98.4±0.8	98.9±0.0
	2 months	5.5±0.2	4.8±0.2	99.0±0.0	97.2±0.1
	DI water (Baseline	5.1±0.4	6.0±0.4	98.8±0.0	99.1±0.0
	4 months	4.2±0.2	4.2±0.0	98.7±0.1	97.6±0.1
	DI water (Baseline	6.9±0.8	5.9±0.5	99.0±0.0	99.0±0.0
	6 months	7.4±0.4	6.0±0.6	98.9±0.1	98.9±0.0

Table SI 6. pH, nitrite concentration and FNA concentration data of the preservation during the long-term preservation trials. Standard conditions: 4-inch RO modules (ESPA-2, Hydranautics) stored in vacuum sealed bag to simulated "Preservation out of the plant".

Parameters	Preservation solutions	Preservation duration (months)			
		0 (n=2)	2 (n=2)	4 (n=2)	6 (n=2)
pH	DI water, pH 5.0 (control)	5.8±0.0	4.0±0.1	4.1±0.3	4.0±0.3
	10 mgHNO$_2$-N/L FNA, pH 5.0	5.4±0.0	7.0±0.2	7.1±0.0	7.1±0.0
	1% SMBS	3.8±0.0	3.2±0.2	2.4±0.1	2.7±0.2
Nitrite concentration (mgNO$_2^-$-N/L)	DI water, pH 5.0 (control)	-	-	-	-
	10 mgHNO$_2$-N/L FNA, pH 5.0	376±0	331±7	295±1	301±3
	1% SMBS	-	-	-	-
FNA concentration (mgHNO$_2$-N/L)	DI water, pH 5.0 (control)	-	-	-	-
	10 mgHNO$_2$-N/L FNA, pH 5.0	3.63	0.08	0.06	0.06
	1% SMBS	-	-	-	-

Table SI 7. Permeability and salt rejection results (Permeability (L/m^2.h.bar, 25°C)/ Salt rejection (%)) of the long-term preservation tests. Standard conditions: 8-inch RO modules (TML20, Toray) stored in PVC tubes to simulated "Preservation within membrane pressure vessels".

Tested preservation solutions	Preservation duration	Water permeability (L/m^2.h.bar, 25°C)		Salt rejection (%)	
		Sample 1	Sample 2	Sample 1	Sample 2
DI water, pH 5.0 (control)	DI water (Baseline)	4.2±0.1	4.8±0.1	99.6±0.0	99.3±0.1
	2 months	4.8±0.0	5.2±0.0	99.3±0.0	99.2±0.0
	DI water (Baseline	5.2±0.0	5.4±0.1	99.4±0.0	99.3±0.0
	4 months	5.1±0.1	5.6±0.1	99.3±0.0	99.2±0.0
	DI water (Baseline	5.2±0.1	5.6±0.0	99.3±0.1	99.3±0.0
	6 months	5.5±0.1	5.3±0.1	99.0±0.1	99.1±0.1
10 mgHNO$_2$-N/L FNA, pH 5.0	DI water (Baseline)	4.4±0.0	4.5±0.1	99.5±0.0	99.5±0.0
	2 months	4.8±0.0	5.4±0.1	99.4±0.0	99.2±0.0
	DI water (Baseline	5.1±0.1	4.1±0.1	99.3±0.1	99.6±0.0
	4 months	5.3±0.1	4.4±0.1	99.3±0.0	99.5±0.0
	DI water (Baseline	4.5±0.1	5.3±0.1	99.5±0.0	99.2±0.1
	6 months	5.1±0.1	5.6±0.1	99.1±0.1	99.0±0.1
1% SMBS	DI water (Baseline)	4.9±0.0	5.2±0.1	99.3±0.0	99.3±0.1
	2 months	4.5±0.1	5.3±0.1	99.5±0.0	99.3±0.0
	DI water (Baseline	5.2±0.1	4.9±0.1	99.3±0.0	99.4±0.0
	4 months	5.0±0.1	5.0±0.1	99.5±0.0	99.3±0.0
	DI water (Baseline	5.4±0.1	4.2±0.1	99.2±0.0	99.6±0.0
	6 months	5.4±0.1	4.2±0.1	99.2±0.1	99.4±0.0

Table SI 8. pH, nitrite concentration and FNA concentration data of the preservation during the long-term preservation trials. Standard conditions: 8-inch RO modules (TML-20, Toray) stored in PVC tubes to simulated "Preservation within the pressure vessels".

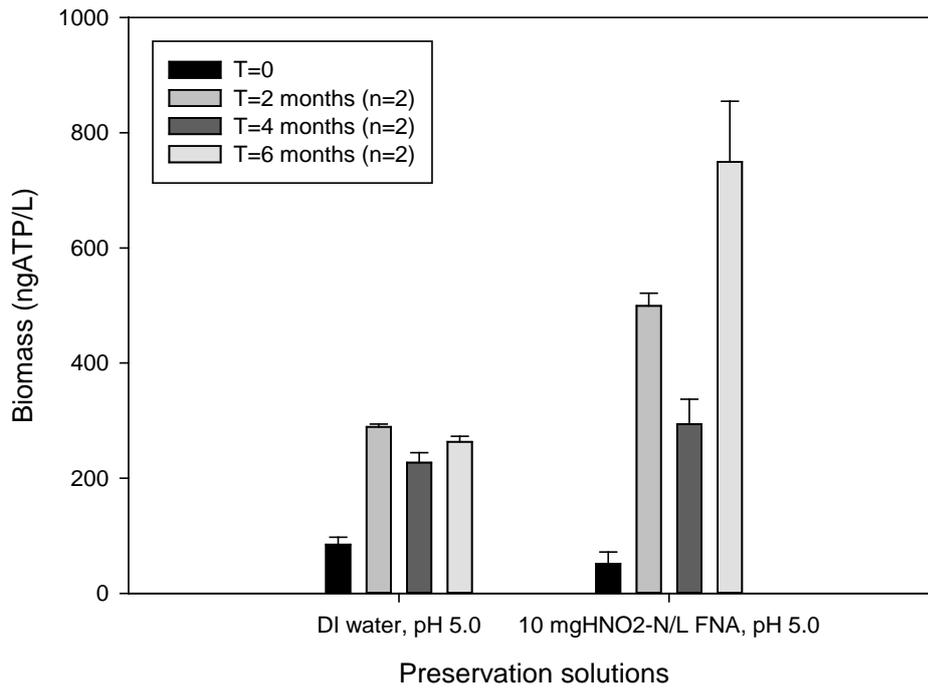| Parameters	Preservation solutions	Preservation duration (months)						
		0 (n=6)	1 (n=6)	2 (n=6)	3 (n=4)	4 (n=4)	5 (n=2)	6 (n=2)
pH	DI water, pH 5.0 (control)	5.4 ±0.0	6.1 ±0.2	5.8 ±0.2	5.6 ±0.2	5.7 ±0.3	5.3 ±0.4	5.2 ±0.3
	10 mgHNO$_2$-N/L FNA, pH 5.0	5.0 ±0.0	5.7 ±0.1	6.2 ±0.2	6.2 ±0.2	6.4 ±0.2	6.7 ±0.0	6.6 ±0.0
	1% SMBS	4.2 ±0.0	3.4 ±0.1	3.5 ±0.2	3.7 ±0.0	3.5 ±0.1	3.6 ±0.0	3.6 ±0.0
Nitrite concentration (mgNO$_2^-$-N/L)	DI water, pH 5.0 (control)	-	-	-	-	-	-	-
	10 mgHNO$_2$-N/L FNA, pH 5.0	422 ±0	381 ±6	400 ±10	386 ±9	380 ±4	366 ±2	405 ±4
	1% SMBS	-	-	-	-	-	-	-
FNA concentration (mgHNO$_2$-N/L)	DI water, pH 5.0 (control)	-	-	-	-	-	-	-
	10 mgHNO$_2$-N/L FNA, pH 5.0	10.07	1.85	0.62	0.59	0.37	0.18	0.25
	1% SMBS	-	-	-	-	-	-	-

Figure SI 6. ATP content in different preservation solutions after 2, 4 and 6-month storage. The errors bars show the standard errors of six measurements.
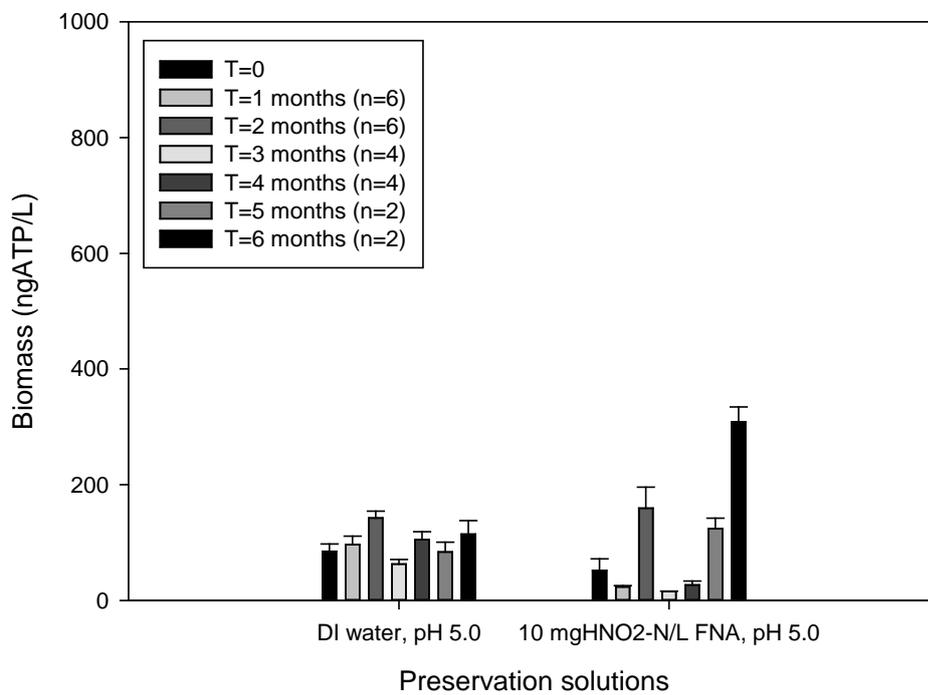
Figure SI 7. ATP content in different preservation solutions after 1, 2, 3, 4, 5 and 6-month storage. The errors bars show the standard errors of 3xn measurements.

APPENDIX E- FNA DECAY STUDY.

A FNA decay study was conducted at pH 2.0 and an initial FNA concentration of 100 mgHNO$_2$-N/L (i.e., 100 mgNO$_2^-$-N/L). The aim was to determine the FNA stability at high concentration and calculate how often the ageing solution needs to be replaced to maintain the correct concentration.
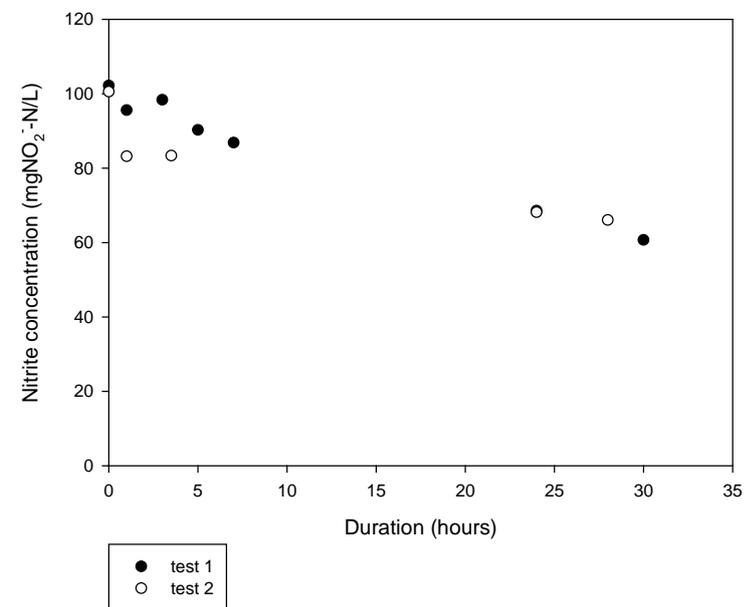
a.
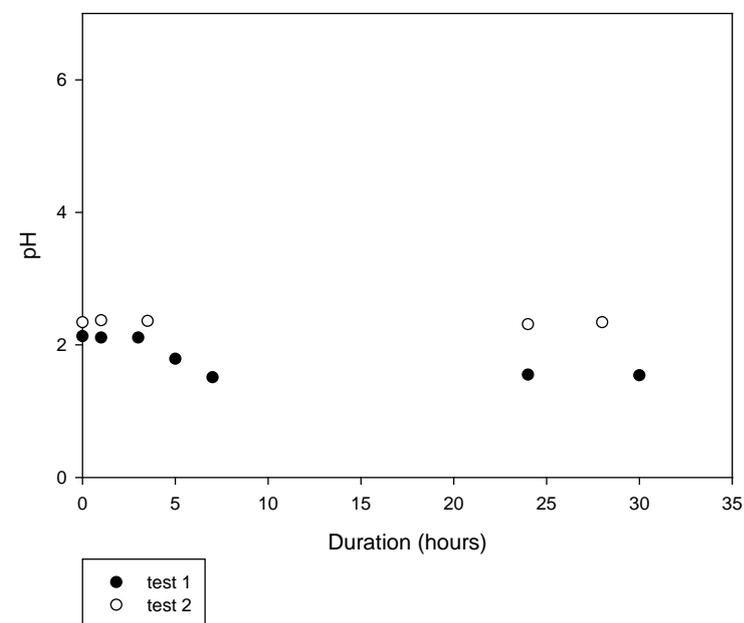
b.

Figure SI 8. (a) Nitrite concentration and (b) pH decay during 30 hours. Initial conditions: pH 2.0, initial FNA concentration of 100 mgHNO$_2$-N/L (i.e., 100 mgNO$_2^-$-N/L).

APPENDIX F- STATIC AGEING TESTS

Table SI 9. Permeability and salt rejection results (Permeability (L/m^2.h.bar, 25°C)/ Salt rejection (%)) of the accelerated ageing test in static conditions.

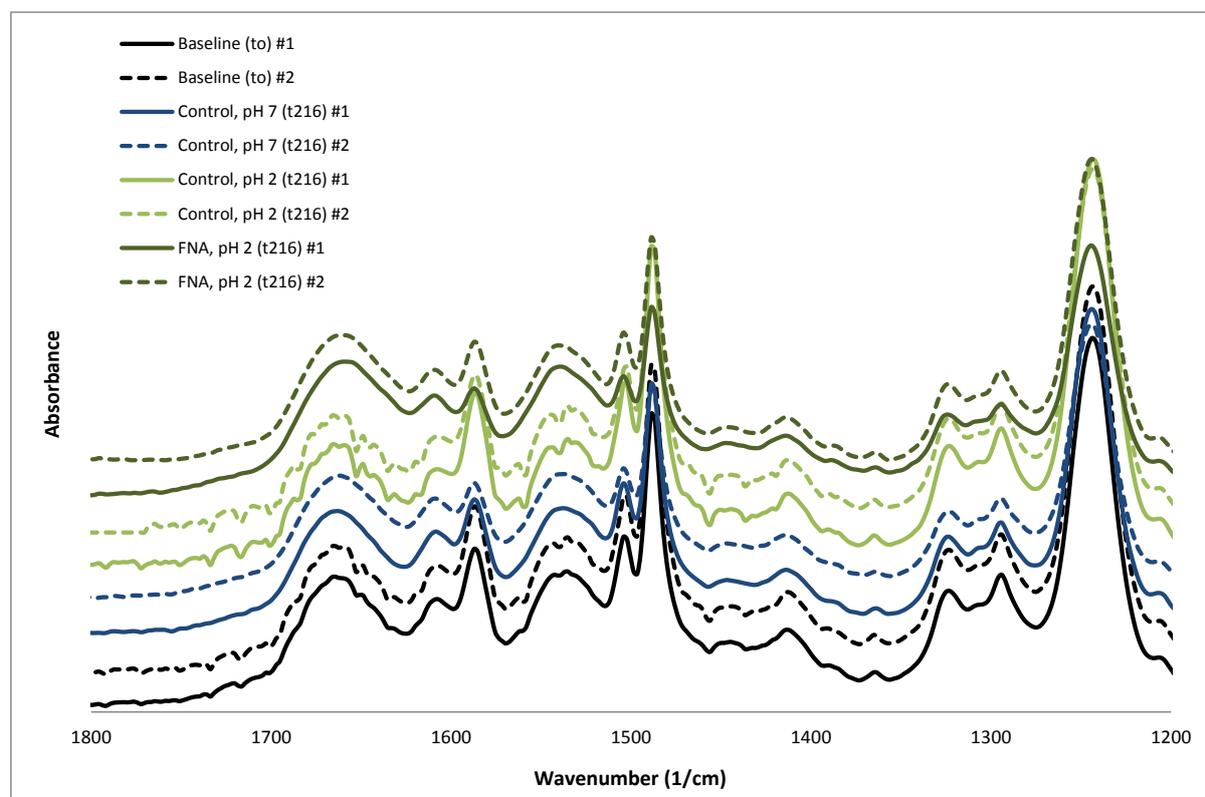|	Exposure time (h)	Water permeability (L/m^2.h.bar, 25°C)		Salt rejection (%)	
		Sample 1	Sample 2	Sample 1	Sample 2
New membrane (Baseline)	0	3.1	3.0	98.3	98.6
Control, pH 7	5	3.2	3.2	98.8	98.7
	216	3.1	3.3	98.7	98.6
Control, pH 2	5	3.0	3.0	97.9	97.6
	24	3.2	3.1	97.7	97.6
	120	2.7	2.6	97.6	98.9
	168	3.3	3.2	98.3	98.2
	216	3.0	3.0	96.7	98.7
100 mgHNO$_2$-N/L FNA, pH 2	5	2.8	2.7	96.7	95.7
	24	2.8	2.7	95.8	96.1
	120	2.3	2.4	93.2	95.9
	168	3.2	3.0	95.9	97.3
	216	2.5	2.7	97.6	95.7

Figure SI 9. FTIR spectrum of ESPA-2 membrane aged in static conditions with (a) 100 mgHNO$_2$-N/L FNA at pH 2.0 and (b) with DI-water at pH 2.0 and 7.0 (control solutions) for 216 hours. Normalisation of the spectra has been made on maximum peak near 1250 cm^{-1}, associated with the C-O-C asymmetric stretching vibration from Aryl-O-aryl group.

APPENDIX G- DYNAMIC AGEING TESTS

Table SI 10. Permeability and salt rejection results (Saline solution permeability (L/m^2.h.bar, 25°C)/ Salt rejection (%)) of the accelerated ageing test in dynamic conditions.

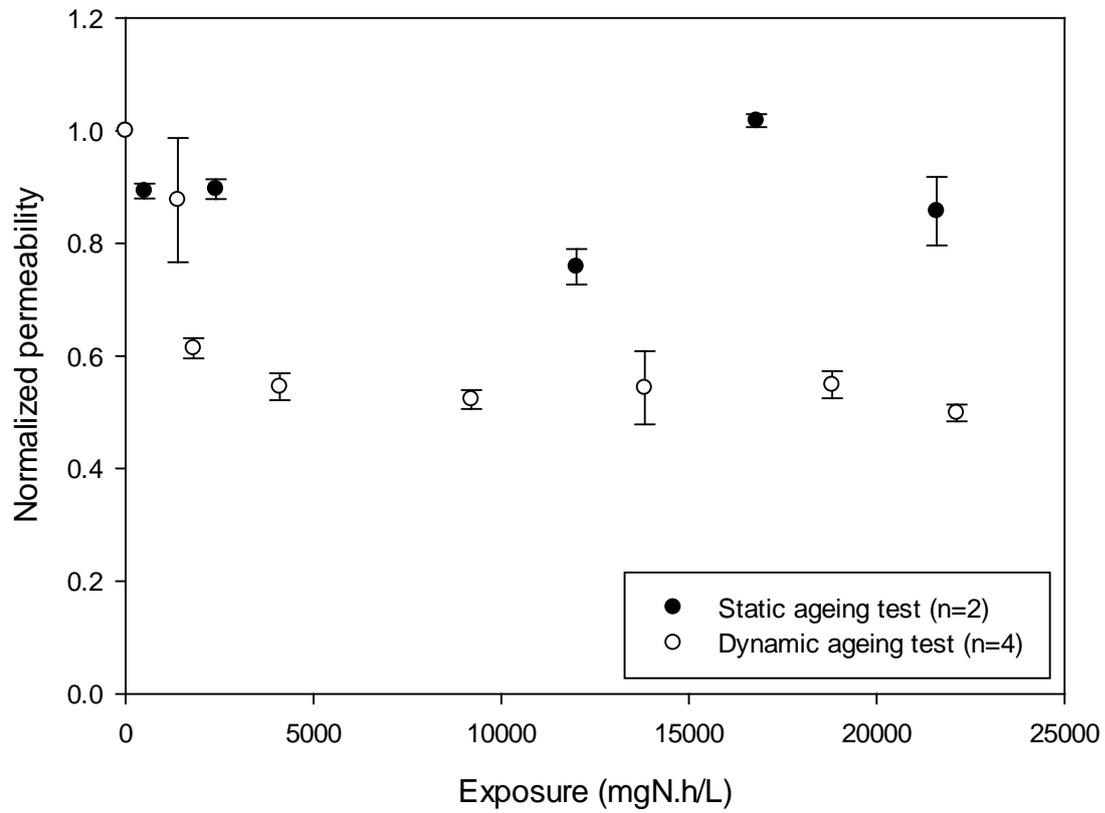|	Exposure time h	Saline solution permeability (Water permeability) L/m^2.h.bar, 25°C		Salt rejection %	
		Sample 1	Sample 2	Sample 1	Sample 2
New membrane (Baseline)	0	3.1 (3.7)	3.0 (3.5)	98.2	99.0
Control, pH 2 (1.5 g/L NaCl)	0	2.8	2.9	89.7	91.6
	24	2.3	2.2	91.9	93.4
	120	1.9	1.9	91.8	93.8
	168	1.6	1.7	91.2	93.2
	216	1.6	1.6	91.2	93.3
New membrane (Baseline)	0	4.0 (5.9)	3.9 (5.7)	98.6	98.3
Control, pH 4 (1.5 g/L NaCl)	0	3.6	3.4	97.5	97.2
	24	2.8	2.6	98.7	98.4
	120	2.1	2.0	98.9	97.8
	168	2.1	2.0	99.0	97.5
	216	2.0	2.0	99.0	98.1
New membrane (Baseline)	0	3.7 (5.8)	3.6 (5.2)	98.7	99.0
100 mgHNO$_2$-N/L FNA, pH 2	0	2.8	2.9	82.1	82.1
	24	2.2	2.3	89.3	89.5
	120	2.0	1.9	88.6	88.6
	168	1.9	1.9	87.9	88.1
	216	1.8	1.8	88.2	88.2
New membrane (Baseline)	0	3.0 (4.3)	2.7 (4.0)	98.6	98.8
100 mgHNO$_2$-N/L FNA, pH 2	0	2.6	2.7	79.5	80.2
	24	1.6	1.5	79.1	81.4
	120	1.7	1.6	79.9	81.8
	168	1.6	1.5	80.0	82.0
	216	1.5	1.4	79.9	81.4
New membrane (Baseline)	0	3.6 (5.0)	3.5 (4.8)	98.6	98.8
100 mgHNO$_2$-N/L FNA, pH 4	0	3.1	3.0	58.3	57.4
	24	2.8	2.6	59.9	59.9
	120	2.4	2.6	56.3	54.8
	168	2.3	2.2	57.7	57.5
	216	2.1	1.9	58.2	58.8
New membrane (Baseline)	0	3.1 (4.7)	2.8 (4.4)	99.1	98.8
100 mgHNO$_2$-N/L FNA 150 mg/L H$_2$O$_2$, pH 4	24	2.7	2.6	58.6	59.2
	120	2.4	2.4	58.4	58.2
	168	2.3	2.3	59.7	60.3
	216	2.3	2.3	61.1	61.1

Figure SI 10. Comparison static versus dynamic ageing test results for 100 mgHNO$_2$-N/L FNA at pH 2.0 (normalized permeability versus exposure as mgN.h/L).
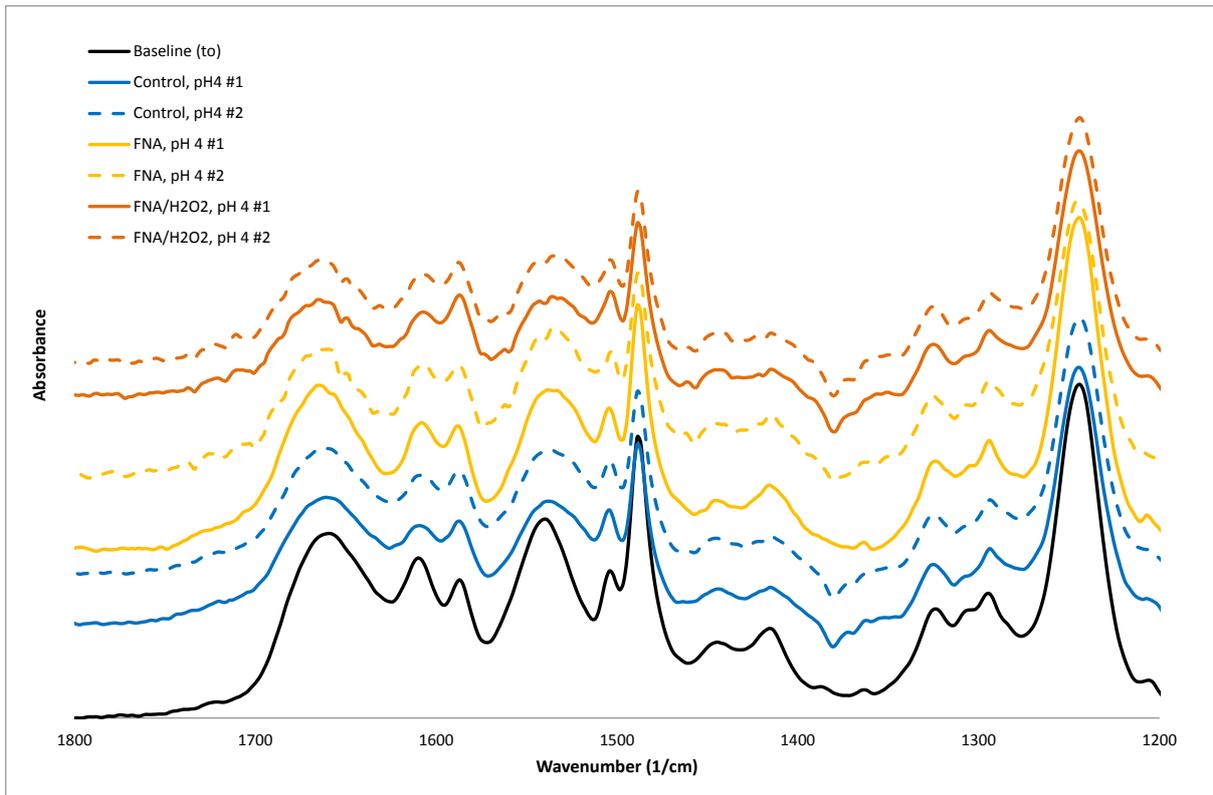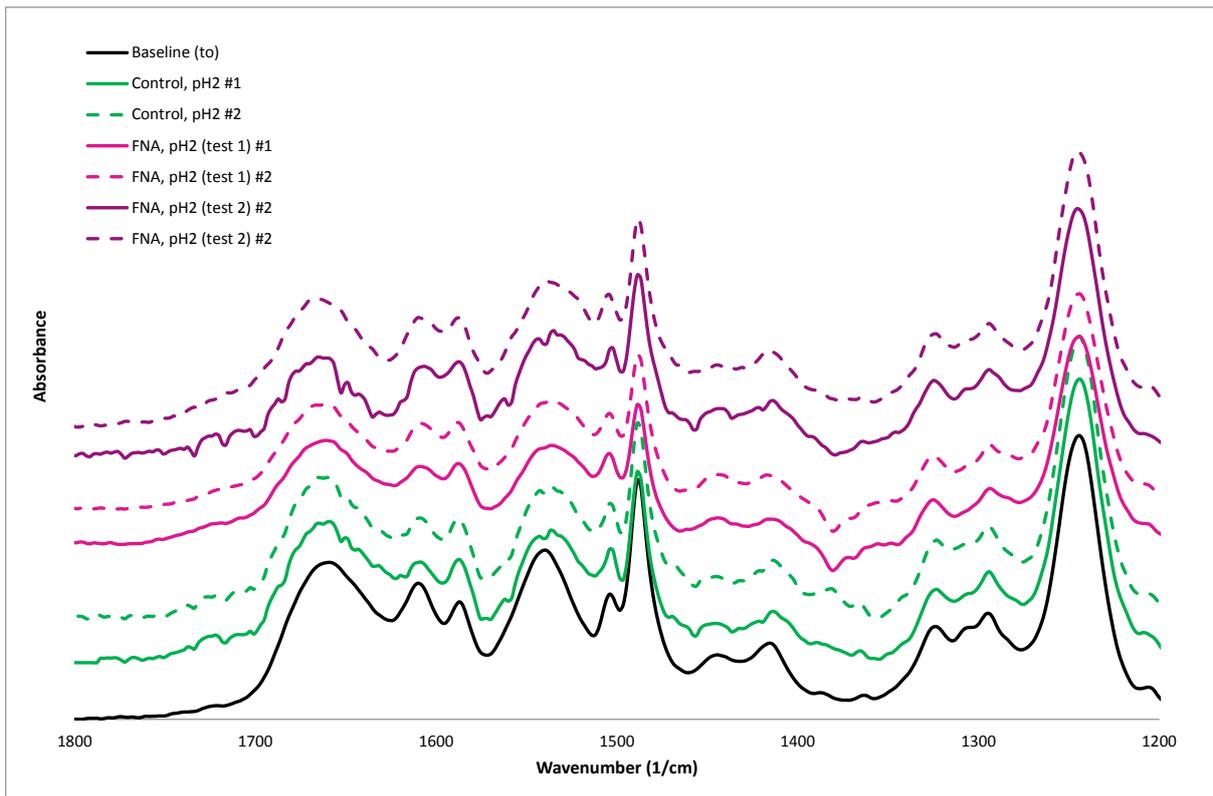
Figure SI 11. FTIR spectrum of ESPA-2 membrane aged in dynamic conditions with (a) ageing solution at pH 2.0 and (b) at pH 4.0 for 9 days. Standard test conditions: FNA (100 mgHNO2-N/L). Normalisation of the spectra has been made on maximum peak near 1250 cm-1, associated with the C-O-C asymmetric stretching vibration from Aryl-O-aryl group.

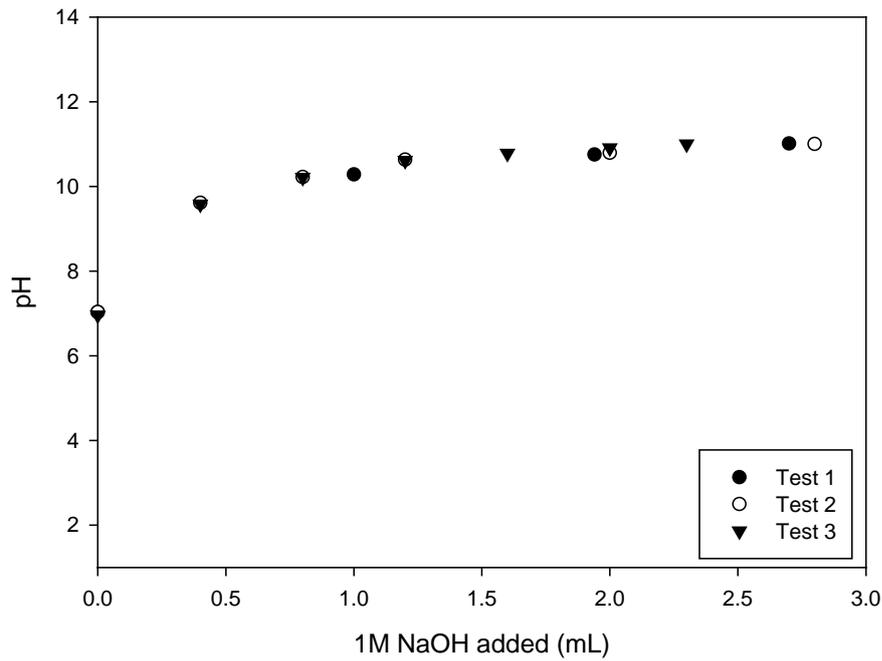# APPENDIX H- COST CALCULATION

Figure SI 12. The cleaning solution titration curve using sodium hydroxide (1M). Test conditions: RO permeate from a full-scale plant, 1 L volume, No. of analysis = 3.

Figure SI 13. The cleaning solution titration curve using hydrochloric acid (1M). Test conditions: RO permeate from a full-scale plant, 50 mgNO_2^--N/L, 1 L volume, No. of analysis = 2.
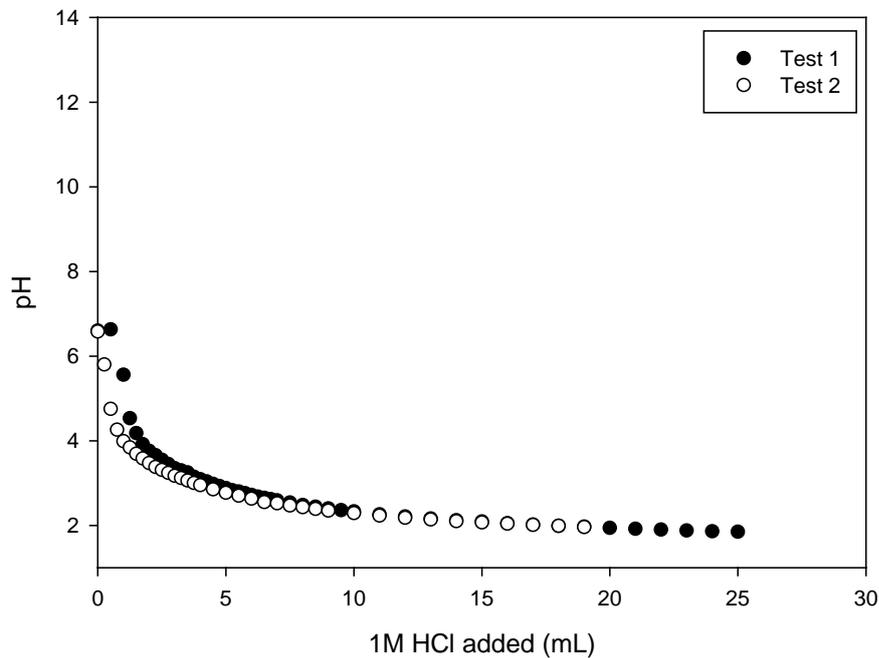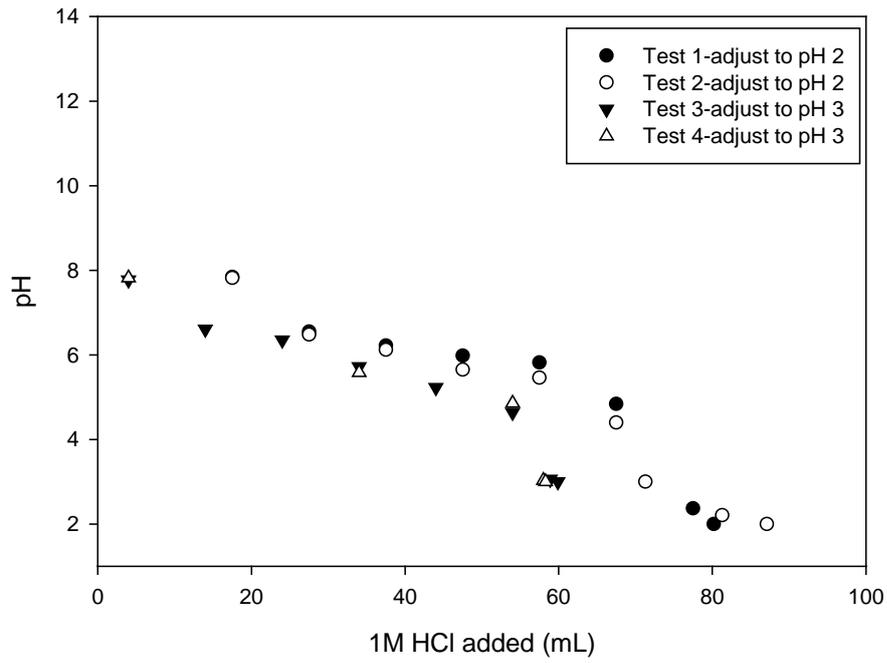
Figure SI 14. The cleaning solution titration curve using hydrochloric acid (1M). Test conditions: RO permeate from a full-scale plant, 50 $mgNO_2^--N/L$, 1.36 $gCaCO_3/L$ (based on 2.17 $mgCa/cm^2$), 1 L volume, No. of analysis = 2 for each pH conditions.

Appendix I - MSDS

SODIUM NITRITE SOLUTION 40%

HAZARDOUS SUBSTANCE. DANGEROUS GOODS. According to NOHSC Criteria, and ADG Code.

HAZARD RATINGS

Flammability				
Toxicity				
Body Contact				
Reactivity				
Chronic				
SCALE:	Min/Nil=0	Low=1	Moderate=2	High=3	Extreme=4
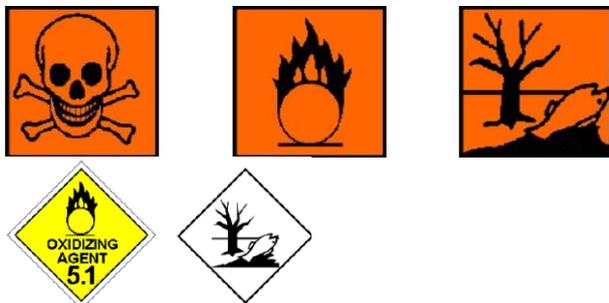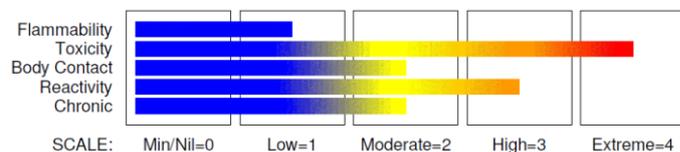
HYDROCHLORIC ACID 33%

HAZARDOUS SUBSTANCE. DANGEROUS GOODS. According to the Criteria of NOHSC, and the ADG Code.

HAZARD RATINGS
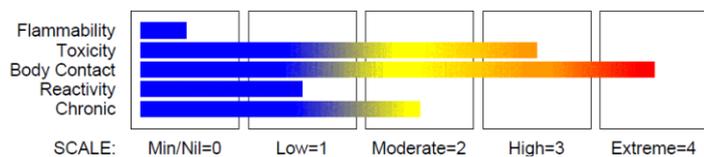
CAUSTIC SODA 46-50%

DANGEROUS GOODS. According to the ADG Code.

APPENDIX J- LIST OF NITROSAMINES

Table SI 11. Structure and cancer risk levels in drinking water for seven nitrosamines reported as possibly human carcinogenic compounds [83] and the standards for quality of recycled water supplied to augment a supply of drinking water [84].

Nitrosamine	Structure	10^{-6} cancer risk level (ng/L) [83]	Guideline value for recycled water in Queensland (Australia) (ng/L) [84]
N-nitrosodi-n-butylamine (NDnBA)-$C_8H_{18}N_2O$		6	
N-nitrosomorpholine (NMOR)-$C_4H_8N_2O_2$		0.8	1
N-nitrosopiperidine (NPIP)-$C_5H_{10}N_2O$		0.8	
N-nitrosodimethylamine (NDMA)-$C_2H_6N_2O$		0.7	10
N-nitrosodiethylamine (NDEA)-$C_4H_{10}N_2O$		0.2	10
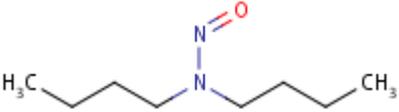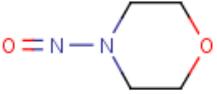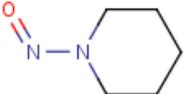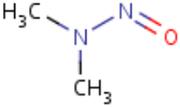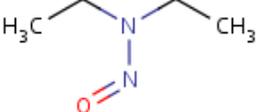
APPENDIX K- LIFE-CYCLE IMPACT ASSESSMENT (LCIA) CATEGORIES

Table SI 12. Description of the life cycle impact assessment categories used in this study [85]

Indicator		Proxy for…	Unit*
Freshwater Extraction Stress		Ecosystem impacts from disruptions to the hydrological cycle	L H_2O
Eutrophication		Ecosystem impacts from nutrient enrichment and oxygen depletion in waterways	kg O_2 eq
Ecotoxicity	- Marine	Environmental impact of chemical emissions to marine systems	kg 1,4-DB eq
	- Freshwater	Environmental impact of chemical emissions to freshwater systems	kg 1,4-DB eq
	- Terrestrial	Environmental impact of chemical emissions to land	kg 1,4-DB eq
Terrestrial Acidification		Chemicals added to the soil which change its acidity	kg SO_2 eq
Global Warming		Contribution to the greenhouse effect, referred to as CO_2e (carbon dioxide equivalent)	kg CO_2 eq
Ozone Depletion		Impact on the ozone layer	kg CFC-11 eq
Resource Depletion	- Fossil Fuels Depletion	Resource availability for future generations	kg oil eq
	- Minerals Depletion		kg Sb eq
Human Health	- Human health Toxicity	Human health impact of chemical emissions	kg 1,4-DB eq
	- Photochemical Oxidants	Contribution to air pollution in the form of smog	kg NMVOC
	- Particulate Matter	Contribution to air pollution which can have respiratory effects	kg PM10 eq

Liter of water (L H_2O); Kilogram(s) of dioxygen equivalent (kg O_2 eq); Kilogram(s) of 1,4-dichlorobenzene equivalents (kg 1,4-DB eq); Kilogram(s) of sulfur dioxide equivalent (kg SO_2 eq); Kilogram(s) of carbon dioxide equivalent (kg CO_2 eq); Kilogram(s) of chlorofluorocarbon-11 equivalent (kg CFC-11 eq); Kilogram(s) of oil equivalent (kg oil eq); Kilogram(s) of antimony equivalent (kg Sb eq); Kilogram(s) of non-methane volatile organic compounds (kg NMVOC); Kilogram(s) of particulate matter (particles with a size of 10 μm) equivalent (kg PM10 eq).

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