Disinfection of a Polyamide Nanofiltration Membrane using Ethanol

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Abstract

It is imperative that nanofiltration membranes are disinfected before they are used for laboratory-scale bacterial adhesion or biofouling experiments, yet currently no suitable disinfection protocol exists. This study aimed to determine if an ethanol treatment at a minimum inhibitory concentration (MIC) could be used to effectively disinfect nanofiltration membranes without altering membrane properties which could affect research. Two strains of bacteria, Pseudomonas fluorescens and Staphylococcus sp., were exposed to a range of ethanol concentrations to determine the MIC required for a $4\log_{10}$ reduction in bacteria. In parallel, ethanol's effects on the filtration, surface and mechanical properties of a Dow Filmtec NF90 membrane were analysed. A 1.5 hour treatment with 40% ethanol was shown to effectively disinfect the membrane without significantly affecting any of the membranes properties tested. This treatment protocol can now be safely used to disinfect the studied membrane prior to bacterial adhesion or biofouling experiments. This study also acts as a guideline for researchers using other membranes to determine a suitable disinfection protocol for their needs.

Keywords

Disinfection, ethanol, pre-treatment, MIC, nanofiltration

1. Introduction

Nanofiltration (NF) is becoming a prevalent process in the production of drinking water [1], in water recycling [2] and may play an increasing role in water desalination [3]. Nanofiltration research in these areas has focused on two main objectives: 1) maximising permeation while achieving a high level of rejection, and 2) maintaining the longevity of filtration properties, primarily through the mitigation of fouling [4, 5].

Of the several types of fouling that occur, the adhesion and proliferation of microorganisms on the membrane known as biofouling is particularly problematic. It is of utmost importance that fundamental research in which bacterial pure cultures are used, for adhesion and biofouling experiments, be conducted on sterile surfaces. Biofouling experiments performed on non-sterile surfaces are at risk of experimental bias, resulting in unspecified multispecies biofilms. Virgin membranes received from manufacturers may be initially clean but are non-sterile, representing a potential contamination source for controlled adhesion and biofouling studies in which pure cultures are utilised.

Sterilisation can be achieved by chemical, thermal, and irradiation means. There is a major emphasis on sterility in microbiological research and yet there is little research into sterilisation techniques for NF and reverse osmosis (RO) membranes. Although a number of different research groups have reported the use of thermal [6, 7] and chemical [8-11] sterilization procedures on membranes, few have considered the damaging effects that such treatments could potentially have. This comes at notable risk considering the available information showing the thermal [12, 13] and chemical sensitivity [14-16] of filtration membranes. These studies show changes to the flux, active layer stability, pore size, pore density and chemical composition of the membrane, all of which could be detrimental to a study involving membrane permeation.

Alcohols are commonly noted to have biocidal properties. They act as cell membrane disruptors and denature proteins, inhibiting growth [17, 18]. With their short carbon chains, ethanol and isopropanol are the most widely used alcohols for this purpose. They are commonly used in the food industry, as a preservative, and in the healthcare sector, where their low volatilities makes them useful for hand sanitizers. Studies in both fields have shown

ethanol to be most potent within a concentration range from 30% to 70% [19, 20]. Even in this range ethanol still remains ineffective against a wide range of fungal and bacterial spores, and so cannot be considered a means of sterilisation, rather as a means of disinfection.

It is important to acknowledge that there is no universally accepted definition of disinfection, an observation made in Seymour Block's "Disinfection, Sterilization and Preservation" [21]. While sterilisation is defined as the removal of all forms of life, disinfection is considered to be the removal of infection, *i.e.* the killing of microorganisms but not necessarily the removal of spores. Accepted levels of microorganism removal vary from 3 to 6 $\log_{10}$ (99.9% to 99.9999%) to qualify as disinfection, with the concept largely dependent on the application.

For the purposes of membrane biofouling research, the application of disinfection is to bring the quantity of microorganisms on the virgin membrane to a level whereby it will have minimal effect on the experiment. According to the European surface test (EN 13697), an effective disinfectant is one capable of reducing the number of sessile cells by 4 $\log_{10}$ (99.99% removal) or more [22]. It is therefore important that a minimum inhibitory concentration (MIC) of ethanol is determined to achieve such a reduction in a membrane application.

To date, the effects of ethanol on water filtration properties of thin film composite (TFC) Nanofiltration membranes are still poorly understood. The majority of research in this field analyses solvent permeability through the membrane rather than using the solvent as a pre-treatment [23, 24]. Shukla et al. [25] expressed their concern that membrane polymers would become damaged upon re-exposure to organic solvents which had previously been used in the membrane's manufacture process. Although membrane surface destabilization seems likely to occur due to the solubility parameters of polysulfone and ethanol, Lencki et al. [26] mentioned that the affinity of ethanol to hydrogen bonding reduces this risk. Earlier studies have shown that exposure of ultrafiltration (UF) and NF membranes to solvents such as ethanol resulted in the swelling of the membrane polyamide and polysulfone layers, subsequently leading to membrane curling [27, 28]. Moreover, a series of papers by Geens, Van der Bruggen and Van der Casteele on the effects of solvents, including ethanol, on nanofiltration membranes showed polymer swelling to alter the pore size and pore density of the membrane, as membrane polymers stretch and pores were forced to contract [29-31].

Furthermore, they showed that solvents can also affect the hydrophobicity of a nanofiltration membrane, and highlight how each solvent reacts uniquely with each polymer.

With most studies focusing purely on solvent-membrane interactions in the context of solvent permeability applications, it is still unclear what bearing their results would have on water permeability. Only two studies were found detailing the interaction of an alcohol treatment on the pure-water flux of a TFC membrane: 1) In their 2006 study Jeżowska et al. compared the pure water flux of a Dow Filmtec NF90 membrane before and after treatment with isopropanol, a chemically similar alcohol to ethanol [32]. Although a small increase in pure water flux was observed, the increase shown is an average of three separate treatments (pressure, alkaline treatment and alcohol treatment). 2) Van der Bruggen et al. in 2002 looked at the effects of solvent exposure on a collection of solvent-stable nanofiltration membranes [33]. In this paper they observe that a hydrophobic membrane exposed to ethanol for 10 days experiences a significant increase in pure water flux despite incurring no mechanical damage visible by scanning electron microscopy. Neither study analysed the other possible membrane changes resulting from treatment such as: changes in membrane surface physico-chemical properties, charge and mechanical properties, all of which play important roles in bacterial adhesion on NF membranes [34]. Thus, it is impossible to conclude the full effect ethanol treatment has in this application.

Due to the lack of sterilising methods of NF membranes and the unclear effect of ethanol on polyamide based TFC NF membranes, this study sought to assess the suitability of ethanol as a means of disinfecting polyamide NF membranes. Our aim was to determine a minimal ethanol concentration and a treatment protocol that could expressly be implemented for bioadhesion and biofouling research on NF and RO membranes. In this study, model Gram-positive and Gram-negative bacterial strains were used to test the disinfection efficacy of different ethanol concentrations. In parallel the filtration, surface and mechanical properties of Dow Filmtec NF90 membranes were characterised following different ethanol treatment regimes.

2. Materials and methods

2.1. **Chemicals**

The water used throughout this study was Grade 1 pure water (18.2 $M\Omega cm^{-1}$) obtained from an Elga Process Water System (Biopure 15 and Purelab flex 2, Veolia, Ireland), hereafter referred to as MilliQ water.

Emsure® absolute ethanol (Merck, Ireland) was used in this study. All ethanol concentrations are given as % vol. /vol. based on the ratio of MilliQ water or Phosphate Buffer Solution (PBS; Sigma-Aldrich, Ireland) and ethanol volumes added together.

2.2. **Bacteria**

Two strains of bacteria were used: *Pseudomonas fluorescens* NCTC 10038 (Gram-negative) and *Staphylococcus* sp. (Gram-positive). Prior to disinfection experiments, *P. fluorescens* and *S. sp.* strains were separately grown in Tryptic Soy Broth (TSB; Oxoid, Ireland) medium at 30°C and 200 r.p.m. overnight, reaching cell densities of OD_{600} 2.8 and 2.7 respectively.

2.3. **Disinfection**

2.3.1. *Disinfection of cells in suspension*

200 µl sample suspensions of each bacterium (containing 10 $\log_{10}$ cells) were treated with 1.8 ml of aqueous ethanol for 1.5 hours. Treatment concentrations of 0, 10, 20, 30, 40, 50, 60 and 70 % ethanol in PBS were used. Following treatment the suspensions were centrifuged at 7000 rpm for 10 minutes in an Eppendorf Centrifuge 5415C (Eppendorf, Germany), the supernatant discarded and the pellet re-suspended in PBS, this step was repeated twice. Serial dilutions (to 10^{-10}) were plated on Trypticase Soy Agar (TSA; Sigma Aldrich) plates. These were incubated for 18 hours at 30^{o}C. Finally the colony forming units (CFU) were counted and the post-treatment CFU/ml determined.

2.3.2. *Disinfection of spiked membranes*

To test the inhibitory action of a selected range of ethanol concentrations on spiked membranes, a modified version of a disinfection surface test was used [35, 36]. Membrane samples were autoclaved and sterilized at 121^{o}C for 15 minutes. The samples were spiked with 30 ml of an overnight bacterial culture ($OD_{600} \approx 2.7$) for 15 minutes and subsequently dried under laminar flow for one hour. The membrane was cut into 1cm^2 sections and treated with 5 ml of a 0, 10, 20, 30 or 40 % ethanol solution for 1.5 hours. Disinfection experiments of spiked membranes were initially checked against non-autoclaved membrane samples to ensure that autoclaving did not affect disinfection efficiency by the

infiltration of bacterial cells in structurally damaged sites on the membrane following sterilization. Each treatment was performed in duplicate in at least three independent replicates. The treated membranes were rinsed with PBS and sonicated at 44 kHz for 15 minutes in an Ultrawave Ultrasonic bath. Serial dilutions (to 10^{-10}) were plated using the Miles & Misera method. These plates were then incubated for 18 hours at 30^{o}C and the CFU/ml determined.

2.3.3. *Disinfection of virgin membranes*

Samples of membrane were cut and divided into two sections. One half was treated with an ethanol solution (0, 10, 20, 30 or 40% ethanol) while the other half was left untreated to ensure that all samples had a similar level of bacteria initially attached. TSA contact plates were pressed against the front and back of the membrane and incubated for 18 hours at 30^{o}C. The number of post-treatment CFUs was counted. Experiments were performed in at least three independent replicates.

2.4. Membrane, ethanol treatment and filtration protocol

The NF90 nanofiltration membrane (Dow Filmtec) was used as a flat sheet in all the experiments. All membrane samples used were initially rinsed and soaked overnight at 4°C in MilliQ water to remove any preservatives from the surface.

Membrane pure-water flux and salt retention tests were performed in three cross-flow filtration cells operated in parallel in a closed-loop system driven by a high pressure pump (model P200, Hydra-Cell, UK). Details on the filtration cells and the system can be found in a previous work by the current authors [37].

The membrane samples were compacted in the cross flow system with MilliQ water for 22 hours at 16 bar. When first exposed to high pressure these membranes underwent a period of compaction observed as a steady drop in pure-water flux. Six to ten hours of compaction resulted in an almost constant membrane flux. However it was noticed that if the pressure was alleviated and the membrane was left at atmospheric pressure for a few hours, restarting the compaction at 16 bar led to a higher pure water flux compared to the one obtained at the end of the compaction process. This was attributed to reversibility of the incomplete

compaction process [38]. It was hence determined that at least 18 hours of compaction were necessary to avoid reversibility of the compaction process.

Pure water flux was determined by measuring the mass of permeate over a two minute period. This was repeated three times at half hour intervals to ensure that a steady flux had been obtained.

Salt retention tests of the compacted membranes were performed by filtering a 10 mM Sodium Chloride (NaCl; Sigma-Aldrich Ireland) solution at 16 bar. Equilibrium was established after 15 minutes of filtration at which point the conductivities of the feed and permeate were measured for each cross-flow cell using an inoLab Cond Level 2 system with a Tetracon 325 probe (WTW, Germany). The system was rinsed out with MilliQ water and the salt retention of a 5 mM Calcium Chloride ($CaCl_2.2H_2O$; Merck Ireland) solution was performed in the same conditions.

Ethanol treatment was then performed at atmospheric pressure with the cells disconnected from the system, with the membranes still sealed in place. The water on the feed side of the membranes was replaced with ethanol at the designated concentration and left for 1.5 hours. The ethanol concentrations used were 0, 20, 30, 40, 60 and 70 %. Final results are an average of three independent replicates for each concentration.

Once the treatment solution was rinsed from the cross-flow cells with MilliQ water, they were reconnected to the system and the membranes were compacted again for 22 hours in order to obtain a steady flux. Pure water flux and salt retention tests were performed again as previously described.

2.5. Surface properties

2.5.1. *Surface energy*

Membrane samples were pre-soaked in MilliQ water and then compacted at 15 bar for 22 hours in the flow cell system. The flow cells were then disconnected from the system, filled with treatment solution (0% and 70% ethanol respectively) and left for 1.5 hours. The MFS-cells were rinsed with MilliQ water to remove the treatment solution. The membranes were removed from the cells and samples cut and affixed to a glass slide using double-sided tape.

These were left to dry fully. This process was repeated for another set of samples with the addition of a second 22 hour compaction step at 15 bar immediately following treatment.

The Lifshitz-van der Waals (γ^{LW}), electron-donor (γ^{-}) and electron-acceptor (γ^{+}) surface tension components of dehydrated treated NF90 membrane samples (S) were determined by measuring contact angles using the following expression:

$$cos\theta = -1 + 2\left(\gamma_S^{LW}\gamma_L^{LW}\right)^{\frac{1}{2}}/\gamma_L + 2\left(\gamma_S^{+}\gamma_L^{-}\right)^{\frac{1}{2}}/\gamma_L + 2\left(\gamma_S^{-}\gamma_L^{+}\right)^{\frac{1}{2}}/\gamma_L \quad (1)$$

Contact angles (θ) and surface energy measurements (γ^{S}) of dehydrated compacted NF90 membrane were measured at room temperature using a goniometer (OCA 20 from Dataphysics Instruments) with three static pure liquids (L): deionised water, diiodomethane and ethylene glycol.

The Lewis acid-base component was deduced from:

$$\gamma_S^{AB} = 2\sqrt{(\gamma_S^{+}\gamma_S^{-})} \quad (2)$$

And the total surface energy was defined by:

$$\gamma_S = \gamma^{AB} + \gamma^{LW} \quad (3)$$

Contact angle values, and determined surface energy values, represent the mean of at least 6 to 10 measurements per compacted membrane sample.

2.5.2. *Bench treatment*

Membrane samples for surface property analysis were first soaked in MilliQ water and then submerged in the designated treatment solution for 1.5 hours. After treatment they were then rinsed again with MilliQ water to remove all traces of ethanol before experimentation.

2.5.3. *Fourier transform infrared (FTIR) spectroscopy*

Samples for FTIR spectroscopy were dried in air after bench treatment. Three absorption spectrums were recorded for each membrane sample, using an Agilent Cary 670 FTIR air-bearing spectrometer, and an average taken.

2.5.4. *Zeta potential*

Zeta potential measurements were performed using a ZetaCAD® system (CAD Instruments, France). Bench treated samples were suspended in the buffer solution overnight (0.1M NaCl, pH 7) to equilibrate with the salt solution prior to analysis. Zeta potential values were determined by streaming the buffer solution across each sample, and measuring the resultant voltage difference. Measurements were taken for a range of flowrates, alternating flow direction between measurements, each sample was analysed three times.

2.5.5. *Atomic force microscopy (AFM)*

Submerged contact mode AFM images were obtained for 10 x10 μm^2 scan areas, for membranes bench treated with 0, 30 and 70% ethanol, with a JPK AFM system (JPK Instruments, Germany) using a Silicone (DNP) cantilever manufactured by Bruker (UK).The cantilever spring constant was 0.32 $N.m^{-1}$ with a nominal tip radius of 10 nm and the line scan rate was 0.7 Hz. Prior to contact mode imaging of the treated membranes, it was demonstrated that no difference in R_{rms} values was calculated for contact or tapping mode images for the NF90.

The scanned images were flattened using Gwyddion SPM image analysis software by fitting a second-order polynomial into the data in the scanned region and subtraction of the resulting best fit from the image. Flattening was done to remove curvature and slope from the images. After flattening, root-mean-squared roughness (R_{rms}) was calculated using Equation 4, where $\bar{z}$ is the average of the z values within the given area, z_n is the current z value, and N is the number of data points within the given area. Three R_{rms} values were calculated for each membrane sample and the average taken.

$$R_{rms} = \sqrt{\frac{\sum_{n=1}^{N}(z_n - \bar{z})^2}{N-1}} \quad (4)$$

2.6. **Physical properties**

2.6.1. *Tension tests*

Samples for tension-failure analysis were cut using a dog-bone punch (3.3 mm wide in the testing region) prior to. Six samples were cut for each bench treatment (0, 30 and 70 % ethanol). The polyester support layer was removed from three of these, leaving only the polyamide and polysulfone layers. A Zwick/Roell tensile testing machine (Zwick, Germany) exerted an increasing tensile load on the samples while a VideoXtens camera unit (Zwick, Germany) recorded the extension of the testing region. Each sample was tested to failure.

3. Results and discussion

3.1. Disinfection efficacy of ethanol

High concentrations of ethanol (60-70%) may be the most potent for disinfection but they are also more likely to damage a nanofiltration membrane. This study attempted to determine the minimum inhibitory concentration (MIC) required to achieve a 4 $\log_{10}$ reduction of the chosen bacterial strains, based on European surface test (EN 13697). Preliminary tests (not shown) performed to determine a suitable exposure time, to achieve maximum efficacy with minimum concentration, showed insignificant difference in $\log_{10}$ reduction between a 1.5 hour and 24 hour treatment time.

3.1.1. *The inhibitory action of different ethanol concentrations on planktonic cells*

The inhibitory action of different ethanol concentration was determined using planktonic Gram-positive and Gram-negative model bacterial cells. This study enabled to help define the minimum inhibitory ethanol concentration required to reduce the cultivable bacterial population in suspension by approximately 4 $\log_{10}$ cfu/mL.

Results showed that following a treatment time of 1.5 hours, 40% ethanol was sufficient to reduce the bacterial population of planktonic *Pseudomonas fluorescens* and *Staphylococcus* sp. cells by 5.7 $\log_{10}$ and 6.5 $\log_{10}$ cfu/mL respectively (Figure 1). This MIC is within the effective range of ethanol (30 – 70%) on similar bacterial strains discussed by numerous sources [19-21]. Considering that disinfection efficacy assays are usually performed using a treatment period from 5 to 60 minutes [21], the long exposure time of 1.5 hours revealed that lower inhibitory concentrations can be employed to achieve acceptable levels of $\log_{10}$ reductions. Lower concentrations of ethanol with the required inhibitory action are preferable

in this application as they reduce the risk of potentially damaging or altering the membranes properties.

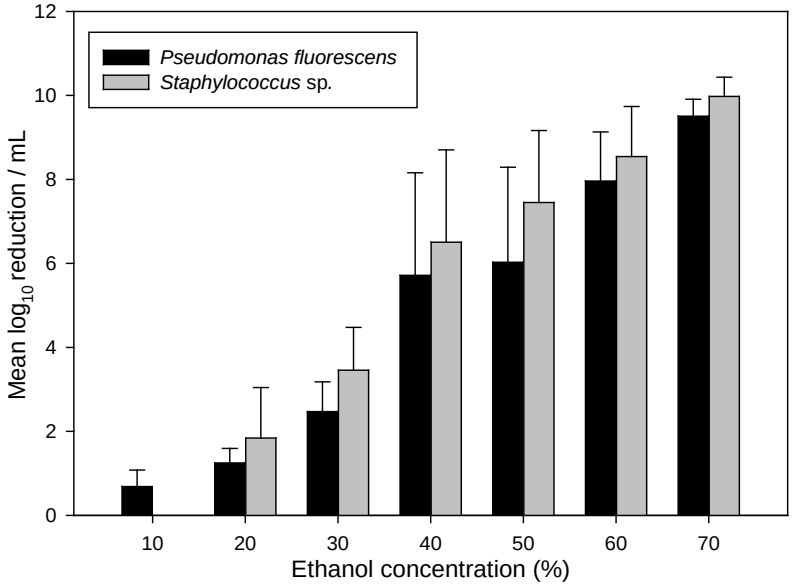


Figure 1: The inihibitory action following a 1.5 hour exposure period of ethanol at different concentrations (10-70% vol/vol ethanol in PBS) on Pseudomonas fluorescens and Staphylococcus sp. planktonic cell suspensions. Data points are averages of three independent replicates with error bars displaying standard deviations.

3.1.2. *Disinfection of spiked membranes*

Based on the results obtained from the suspension test, the inhibitory action of ethanol on spiked membranes was investigated using 1.5 hour treatments with 10, 20, 30 and 40% ethanol. To ensure that membranes were free from organisms before spiking with either *Pseudomonas fluorescens* or *Staphyloccus* sp. model strains, membranes were autoclaved at 121°C for 15 minutes. Although sterilising the membranes may have led to their physical damage, as described elsewhere [13], it did not affect the cell loading and susceptibility to ethanol exposure (results not shown).

Membranes spiked with *Staphylococcus* sp. tended to be less susceptible following 1.5 hour exposure to low ethanol concentration 10% and 20% compared to *Pseudomonas fluorescens* spiked membranes (Figure 2). For both spiked membranes, 1.5 hour exposure to 30% and 40% ethanol led to at least a 4 $\log_{10}$ reduction. *Staphylococcus* sp. spiked membranes

revealed the highest log reduction with values exceeding 5 $\log_{10}$ following exposure to 30% and 40% ethanol concentrations.

An equal MIC for adhered and planktonic cells for a specific exposure time is contrary to what is reported by Chambers et al. [20] who observed a rise in tolerance to 70% ethanol for plastic-adhered bacteria (including *Staphylococcus* sp.). They discussed that the material properties may influence the bacteria adhesion, and that the material may grant the cells protection by reducing the exposed cell surface area. The porosity of the membrane in our study however may not act in the same way as the plastics described, as the bacteria are in contact with the ethanol treatment solution absorbed within the membrane active layer. It is therefore understandable that an MIC for cells adhered to the membrane could equal to that of planktonic cells.

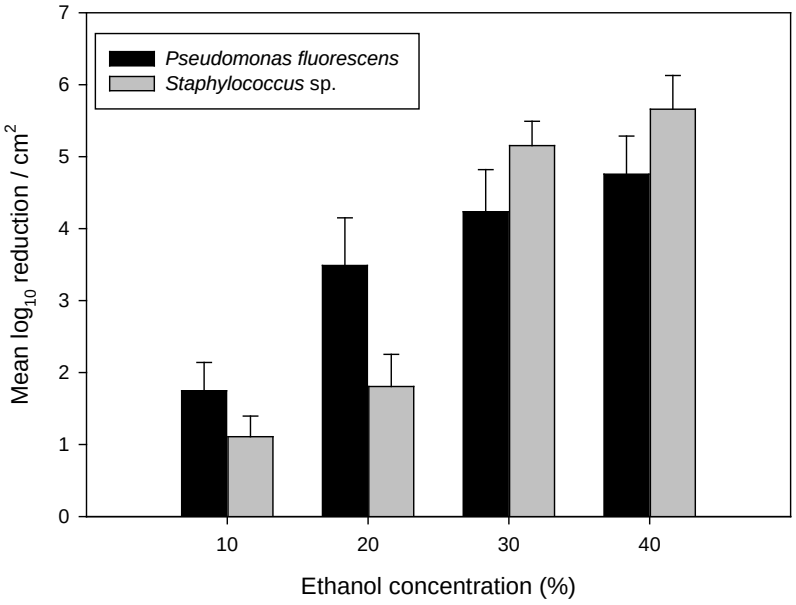


Figure 2: The inihibitory action following 1.5 hour exposure to ethanol at different concentrations (10-40% vol/vol ethanol in PBS) on Pseudomonas fluorescens and Staphylococcus sp. spiked NF90 membranes. Data points are averages of three independent replicates with error bars displaying standard deviations.

3.1.3. *Disinfection of virgin membranes*

In the previous sections it was shown that an MIC of 40% is necessary for a 4$\log_{10}$ reduction of two sample strains. In practice the treatment will be applied to virgin membranes which

will host many wild strains of bacteria, each with a different tolerance to ethanol. To determine the efficacy of ethanol treatment on wild strains, samples of virgin membrane were treated with a range of ethanol concentrations and contacted with agar plates to quantify the amount of bacteria that had survived the treatment (Figure 3).

Each of the plates contacted with an untreated membrane showed excessive growth highlighting the importance of disinfecting membranes before conducting any form of bacterial adhesion of biofouling studies with them. There is a notable difference between the quantity and types of bacteria on the front (active layer) of the membrane and back (support layer) of the membrane. More growth was observed for plates which had contacted the back of the membrane, possibly due to the higher porosity and roughness of the support layer incurring a higher level of bacterial adhesion.

There is an expected decreasing trend of bacterial growth with increasing ethanol concentration, with almost no colonies detected on samples treated with concentrations of 30% or higher. Spore growth could be seen after incubation, however, on plates treated with concentrations as high as 70% ethanol (not shown) concurrent with the understanding that ethanol can be used for disinfection but not sterilisation [21].

The MIC of 40% discussed previously was high enough to remove almost all microorganisms from the three membranes tested at this concentration (7 CFU were counted on a single plate contacted with the back of one of the membranes).

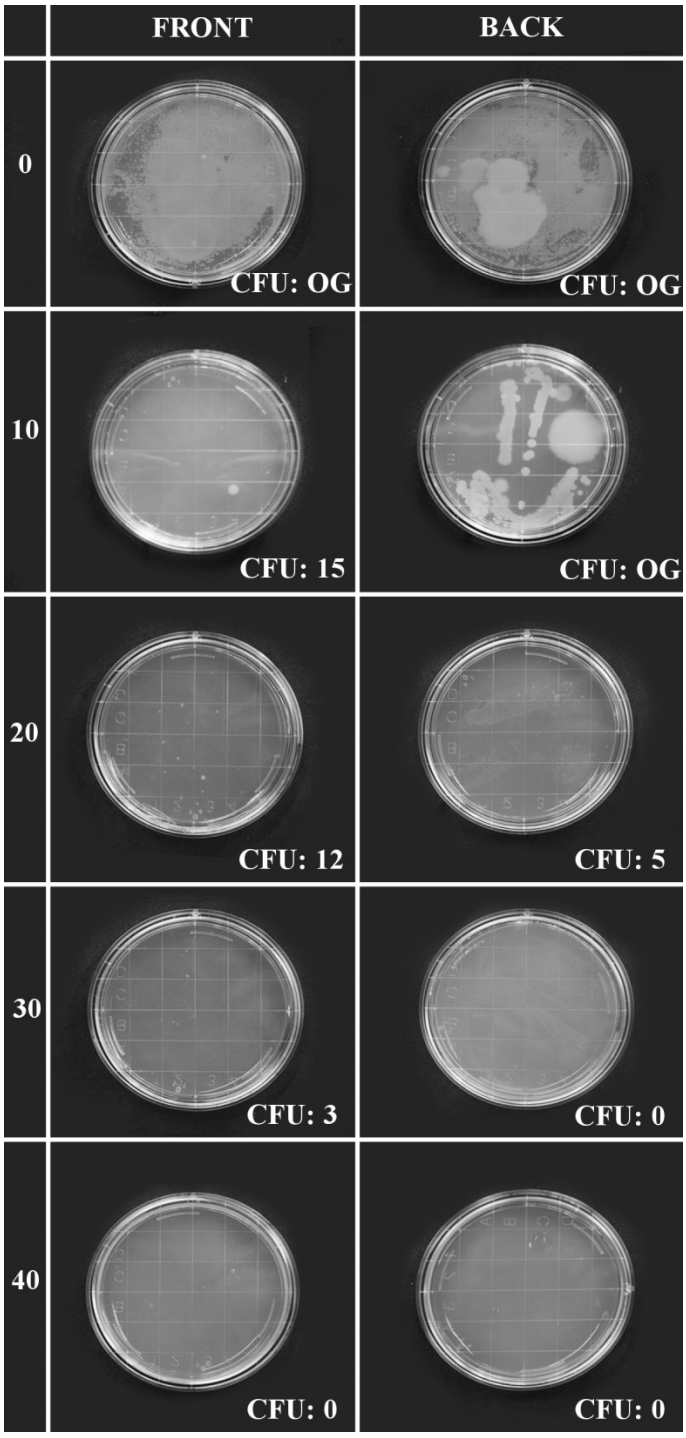


Figure 3: TSA plates, contacted with the front (active layer) or back (support layer) of an NF90 membrane pre-treated with 0, 10, 20, 30 or 40% ethanol after 18 hours of incubation. Plates with a cell density too high to be counted are marked as having overgrowth (OG). Images shown are representative of the three repetitions.

3.2. Ethanol's effects on membrane performance

406 It has been shown in Section 3.1 that ethanol can be used as a very effective means of
407 membrane disinfection, capable of at least a 4 $\log_{10}$ reduction of both planktonic and sessile
408 bacterial populations. Before it can be considered a suitable disinfection method for
409 polyamide NF membranes however, it is vital to understand how ethanol affects membrane
410 performance as well as its physical and chemical properties.

411 Two parameters, pure-water flux and salt retention, were considered in order to quantify
412 ethanol's effects on the NF membrane's filtration performance. The change in pure water flux
413 before and after ethanol exposure was initially used to assess if ethanol had any effect in the
414 membrane structure, such as pore size or porosity.

415

416 No significant correlation between increasing ethanol concentration and flux change was
417 observed (Figure 4). As pure water flux is predominantly dependant on pore size and porosity
418 (when all filtration conditions are the same) it appears that ethanol exposure within the tested
419 range followed by MilliQ water compaction did not significantly damage the NF90
420 membrane structure. The observed variance in water flux values over the range of ethanol
421 treatment concentrations is more likely attributable to the heterogeneity of the membrane
422 sheet. Membrane samples, although cut from the same sheet, had a range of initial pure-water
423 fluxes (5.03 ± 0.95 L/ hr bar m^2).
424

425 A small decrease in flux after treatment was seen for the majority of samples measured. Since
426 this decrease is also present for each of the samples treated with 0% ethanol, the experimental
427 process rather than exposure to ethanol was concluded to be the cause. Despite the steady
428 flux achieved during the compaction period, a rise in pure-water flux (up to 28% of the initial
429 flux) was measured immediately after treatment: evidence that the membrane expanded
430 during this time. Over the second compaction period the flux decreased once again to a
431 steady state, lower than the initial steady state in most cases. The decrease is therefore
432 determined to be associated with slight changes in the polymer configuration following the
433 expansion and re-compaction of the membrane due to the changing pressure conditions over
434 the course of the experiment.

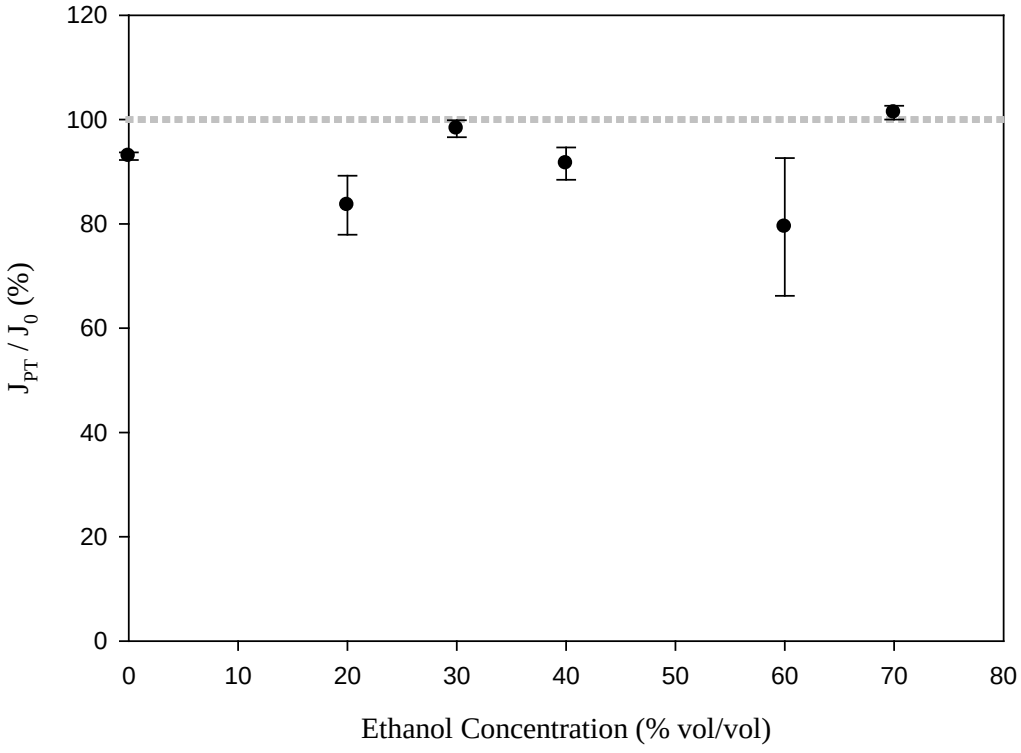
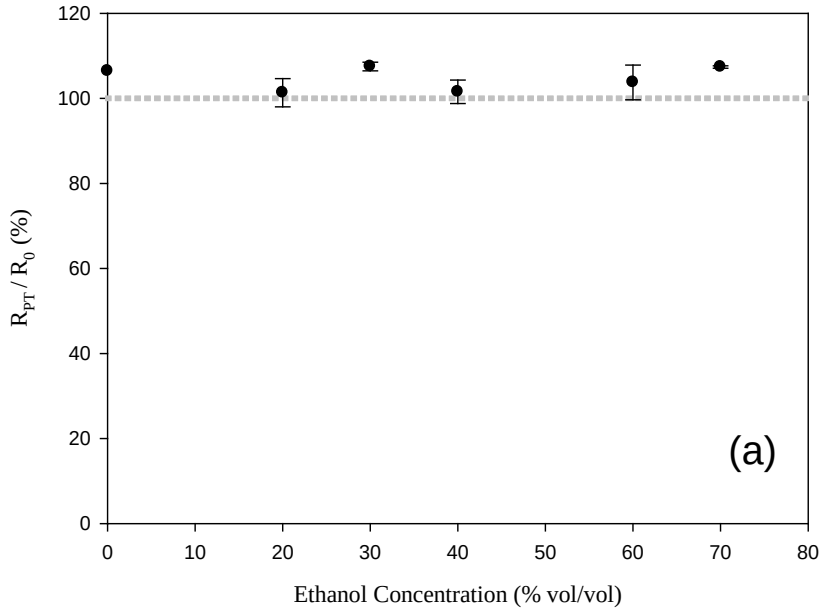


Figure 4: Pure water flux of NF90 membrane samples, after a 1.5 hour treatment (J_{PT}) with 0, 20, 30, 40, 60 or 70 % vol/vol ethanol in MilliQ water, as a percentage of initial pure water flux (J_0). The dotted horizontal line represents no change in flux after treatment. Data points are averages of three independent replicates with error bars displaying standard deviations. Test conditions employed were: Temperature = 22^{o}C, Pressure = 16 bar, and crossflow velocity = 2.2 L/min.

Changes to the Donnan charge and steric exclusion of the membrane can be quantified by analysing the rejection ability of the membrane to charged particles of different sizes such as monovalent and divalent ions, obtained from the dissolution of NaCl and $CaCl_2$ respectively. Salt retention was determined by measuring the conductivity of the feed (C_f) and permeate (C_p) samples and employing Equation 5.

$$R = 100 * \left(1 - \frac{C_p}{C_f}\right) \quad (5)$$



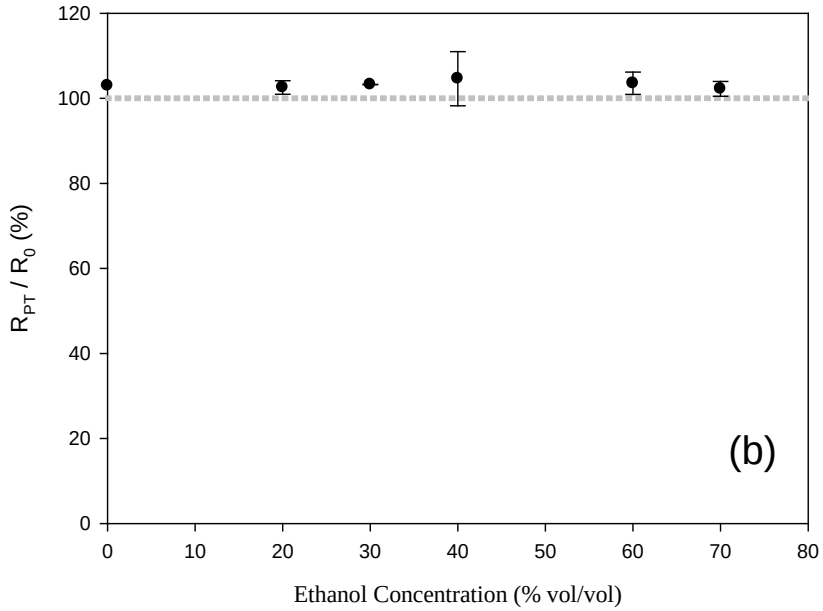


Figure 5: NaCl (10mM; a), and $CaCl_2$ (5mM; b) retentions of NF90 membrane samples after a 1.5 hour treatment (R_{PT}) with 0, 20, 30, 40, 60 or 70 % vol/vol ethanol in MilliQ water, as a percentage of initial salt retention (R_0). The dotted horizontal line represents no change in retention after treatment. Data points are averages of three experiments with error bars displaying standard deviations. Test conditions employed were: Temperature = 22^oC, Pressure = 16 bar, and crossflow velocity = 2.2 m/min.

A small increase in the membrane's retention for each salt solution (maximum of 7.5% for NaCl and 4.6% for $CaCl_2$) was observed after all treatments, even for samples treated with

0% ethanol. This could be associated with the minor drop in post-treatment flux seen previously in Figure 4. A reduction in porosity of the membrane's active layer due to a polymer rearrangement during the membrane expansion and recompaction explains both the membranes drop in pure water flux and this increased rentention.

Ethanol exposure did not affect the membrane performance in terms of salt retention (Figure 5) and pure water flux (Figure 4), and therefore appears not to have affected the membrane's pore-size or charge exclusion capacity. These results are contrary to those found by Geens, Van der Bruggen and Van der Casteele who showed that membrane swelling in ethanol lead to a higher pure-water flux for a hydrophobic membrane [33]. The differences in results most probably arise from the different active layer polymers of the membranes studied, and the different treatment times used: 10 days (to see the maximum damage wrought by the solvent) versus 1.5 hours (to incur a minimal amount of damage by the solvent).

3.3. **Ethanol's effects on membrane surface properties**

Filtration performance is not the only factor that could be affected by exposure to ethanol. An important prerequisite for undertaking membrane fouling and biofouling research is the characterisation of the membrane surface properties including hydrophobicity, surface chemical groups and roughness [39]. Alterations to membrane surface physic-chemical properties could potentially lead to experimental biases during dynamic biofouling studies. It was therefore necessary to assess the membrane surface properties following ethanol treatment.

3.3.1. *Visual observations*

During the treatment process some changes in the physical shape and colour of the membrane were observed. Exposure to ethanol solutions of 20-70% led to a deviation from the membranes natural curl (originating from the flat sheet having been rolled up by the supplier). Figure 6 shows how increasing ethanol concentration causes the membrane samples to become flat and then to bend against the natural curl. Once rinsed with MilliQ water, these membranes reverted to their original shape.

Likewise, a visible loss in opacity was observed on membranes treated with high ethanol concentrations. The NF90 membrane has a natural cream colour which becomes more

transparent after exposure to higher ethanol concentrations (>30%). This, however, does not revert to its original state after rinsing. These results have been combined in Table 1.

Table 1: Visual effects of ethanol treatment on the NF90 membrane samples.

Ethanol (% vol/vol)	0	10	20	30	40	50	60	70
Curvature	Roll curve	Flat				Polyamide expanding (Curling)		
Colour	No Colour Change				Discolouring/transparency			

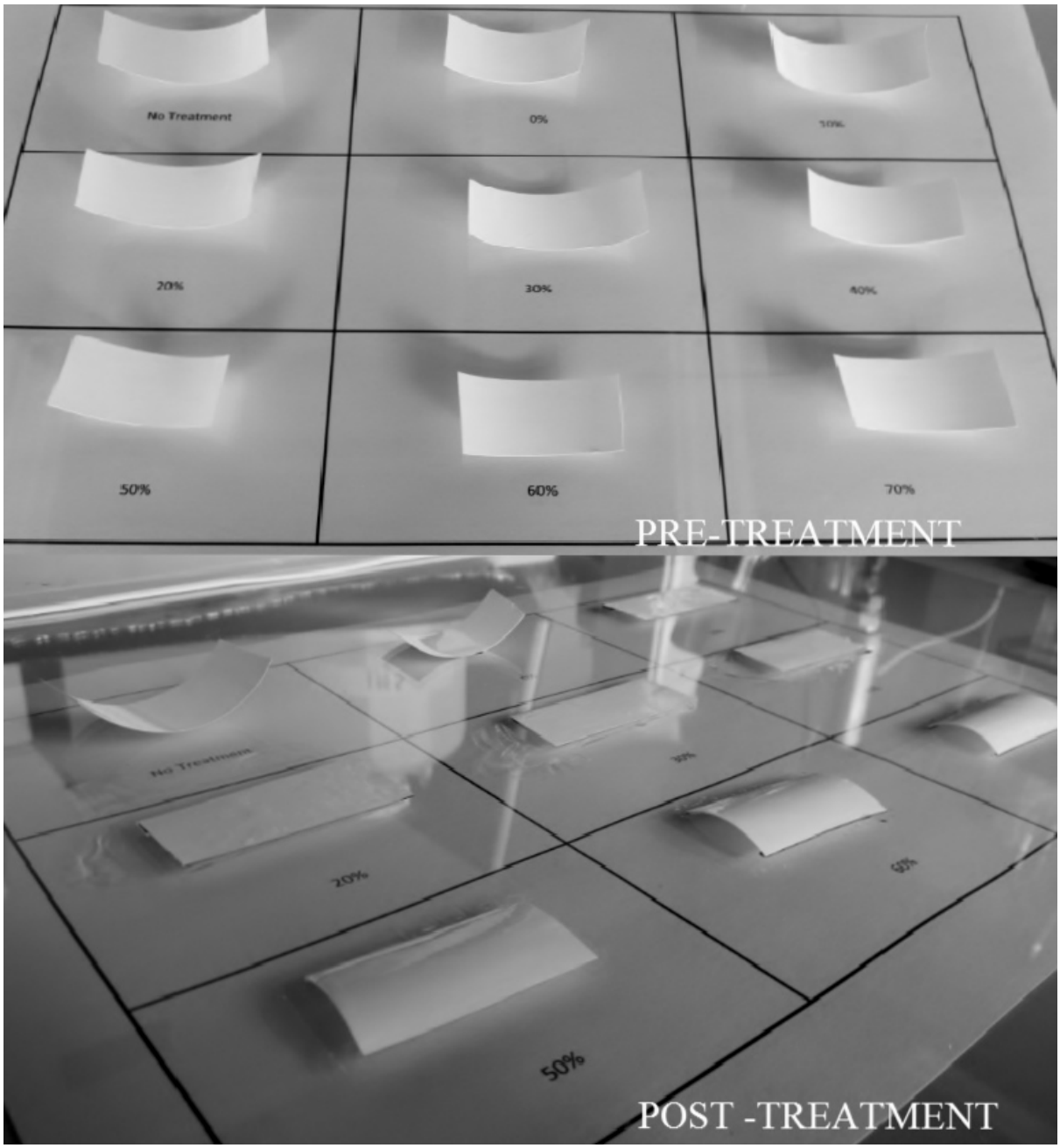


Figure 6: NF90 membrane samples before and after treatment. Treatments shown: No treatment, 0%, 10%, 20%, 30%, 40%, 50%, 60%, 70% ethanol. Rinsing with MilliQ water returned the samples to their original shape.

Membrane curvature and transparency changes have been reported in previous studies involving similar membranes and solvents [27, 28]. Water molecules linked to the hydrogen bonds within the polyamide structure are reportedly replaced by ethanol molecules for the higher concentration treatments. This causes the polyamide layer to swell slightly stretching

505 the top layer, causing the membrane to curl. When the membrane is immersed in water this
506 effect is reversed due to water's higher affinity to hydrogen bonding.

507 3.3.2. *Surface energy*

508 Surface energy plays a leading role in bacterial adhesion, as shown in a study by Lee et al.
509 whereby an increasing concentration of adhered bacteria was linearly correlated to increasing
510 hydrophobicity [39]. Changes to membrane surface energy would therefore negatively impact
511 on biofouling fundamental research.
512
513 Surface energy experiments were performed on membranes that had been compacted once
514 (prior to treatment with 0 and 70% ethanol) with MilliQ water, and on membranes that had
515 been compacted twice (before and after treatment with 0 and 70% ethanol). In this way the
516 effects of both compaction and ethanol on membrane hydrophobicity could be analysed in
517 parallel. The results are presented in Table 2.
518
519 The results show membranes that had been compacted a single time to have a hydrophobic
520 tendency with a contact angle (θ) in the range 106-110^{o} while those that had been compacted
521 twice had a hydrophilic nature with a contact angle in the range 80-86^{o}. Contact angle
522 measurements of membranes are often difficult to perform as the porous surface of the active
523 layer absorbs the droplets: the contact angle has been found to vary with time [40]. This issue
524 was alleviated somewhat for the compacted membranes: the droplets retained their shape for
525 longer and the results were more reproducible for each sample than ones measured previously
526 with non-compacted membranes (results not shown). While ethanol had no significant effect
527 on the membrane's surface energy, compaction seems to have affected its hydrophobicity,
528 possibly linked to the decrease in porosity of the active layer shown previously in section 3.2,
529 hence the differences in absorption of the water droplets on the membrane surface. This could
530 be an interesting area for future research.
531
532 The values obtained for the other components of surface energy show a large variance for the
533 membrane samples studied probably linked to the heterogeneous nature of the membrane's
534 active layer.
535

Table 2: Surface energy components of NF90 membrane samples treated with 0 and 70% ethanol: contact angle (θ), electron-donor (γ^-), electron-acceptor (γ^+), Lewis acid-base (γ^{AB}), Lifshitz-van der Waals (γ^{LW}), total surface free energy (γ^S).

Sample Treatment	**θ**	**γ^-**	**γ^+**	**γ^{LW}**	**γ^{AB}**	**γ^S**
0% 1 compaction	106.9 ± 0.69	40.15 ± 0.28	0.67 ± 0.14	2.42 ± 0.38	10.17 ± 1.2	12.60 ± 1.49
70% 1 compaction	108.3 ± 1.23	41.80 ± 0.36	0.99 ± 0.35	4.06 ± 0.969	12.10 ± 2.26	16.16 ± 3.08
0% 2 compactions	81.28 ± 1.41	37.60 ± 0.33	1.39 ± 0.68	14.19 ± 3.58	12.01 ± 4.13	26.21 ± 7.65
70% 2 compactions	85.74 ± 2.19	41.02 ± 0.18	0.47 ± 0.27	6.17 ± 2.61	7.16 ± 2.57	13.34 ± 5.1

3.3.3. *Surface chemistry*

FTIR spectrums of the active layer chemistry of membrane samples treated with various ethanol concentrations are shown in Figure 7. The results show no peak straying and minimal change in peak area. The largest changes in peak area were around wavenumbers 800, 1100, 1220 and 1480. The largest deviations in each case were for 10 and 40 % ethanol while those treated with intermittent concentrations remained unaffected. Furthermore, these wavenumbers are commonly associated to ‘C-H bend’ and ‘C-O stretch’ molecular motions; these bonds are abundant in the polyamide structure. As there is no peak change around wavenumbers 3300-3400 (associated with ‘O-H stretch’ motions) it is unlikely that these peaks are associated to any alterations of the membrane’s surface chemistry by ethanol.

These results show that ethanol was not responsible for the observed surface chemistry variations. They are merely further evidence of the heterogeneous nature of the polyamide structure and surface chemistry of the membrane.

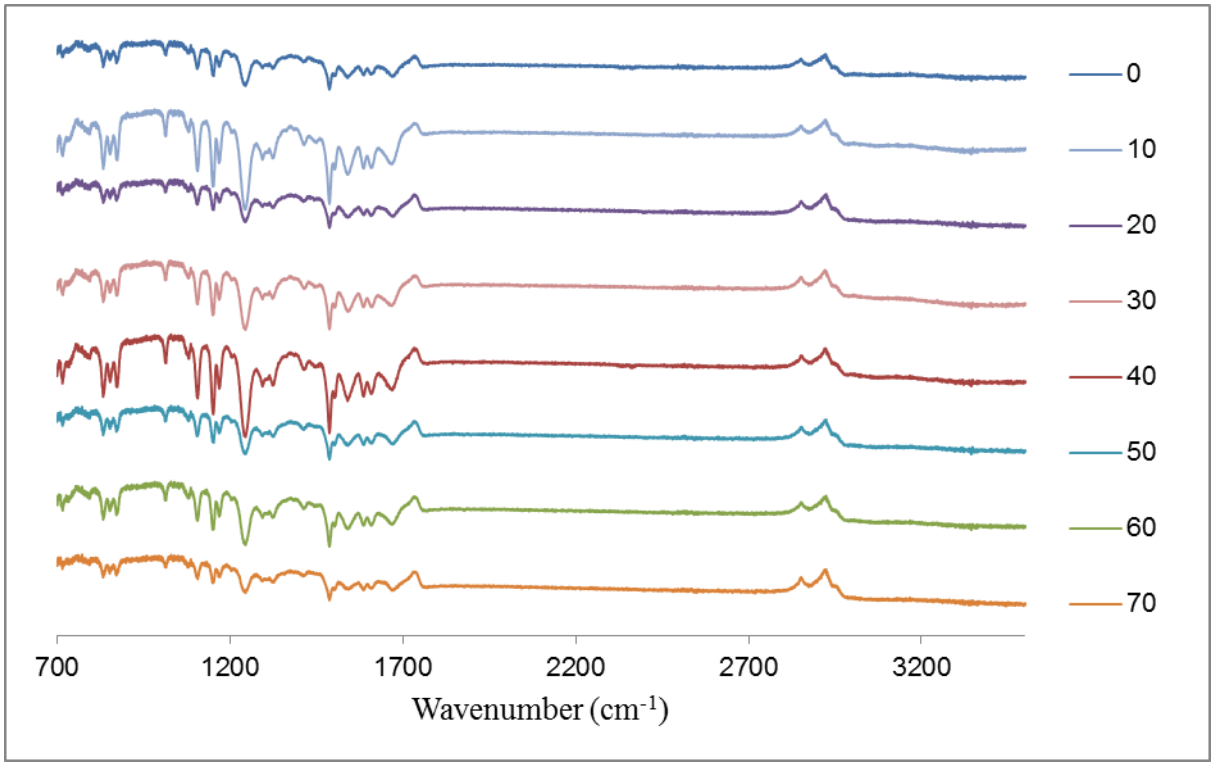


Figure 7: FTIR spectrums of NF90 membrane samples treated with 0, 10, 20, 30, 40, 50, 60, or 70% vol/vol ethanol. Each spectrum shown is an average of readings done in triplicate.

3.3.4. *Zeta potential*

Zeta potential is another factor which is important to characterise due to the role it plays in colloidal deposition and bio-adhesion on NF membranes [41]. If ethanol exposure has had a significant effect on the membrane's surface charge it could affect the membrane's biofouling outcome.

The results of the study performed for the range of ethanol treatments can be seen in Figure 8. The zeta potential of the membrane samples was relatively constant (-10 ± 1.5mV) for the majority of the ethanol concentrations tested, concurring with the previous salt retention analysis (*Figure 5*). The samples which were exposed to 10% and 20% ethanol however each had a notably lower zeta potential, -14.4mV and -15.7mV respectively. No correlation could be found for these two concentrations with the salt retention results in Section 3.2.

Pasmore et al. [41] showed in their experiments on the role of zeta potential in bacterial adhesion that a positive (greater than 0) or highly negative (lower than -20) zeta potential can have a significant effect on bacterial adhesion. In this context the range of results obtained in this experiment is not enough to impact on the bacterial adhesion potential of the NF90

membrane. Therefore ethanol's effects on this membrane's zeta potential can be concluded to be insignificant.

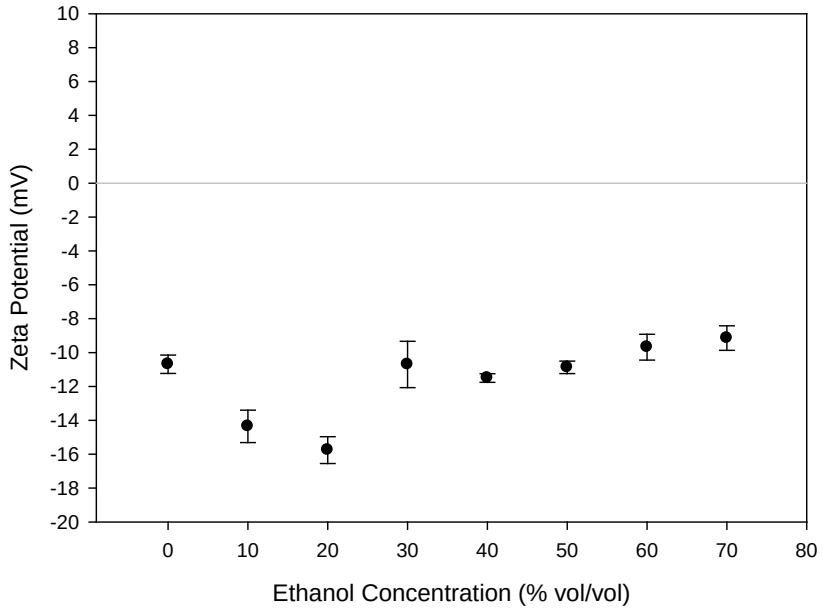


Figure 8: Zeta Potential of NF90 samples after bench treatment with 0,10,20,30,40,50,60, or 70% ethanol. Data points are averages of three experiments with error bars displaying standard deviations. Test conditions employed were: Temperature = 20^{o}C, Test solution = 0.1M NaCl and PH = 7.

3.3.5. *Membrane roughness*

Membrane roughness has been linked in previous studies as a leading factor in the adhesion of bacteria onto a membrane [42]. The roughness values presented in Table 3 show no correlating effect with increasing ethanol concentration on the roughness of the NF90 membrane. There is however a minor decrease (11%) in average roughness for the membranes treated with 30% ethanol. When seen in the context of Subramani's analysis of bacterial adhesion onto membranes with a range of roughness averages (4-108 nm) [34], however, it is obvious that this minor difference would not significantly affect bacterial adhesion experiments. This decrease in roughness is most likely an error due to the small sample size (3 images) taken, and the heterogeneity of the membrane.

Table 3: Roughness values for three NF90 membrane samples treated with 0, 30 or 70% ethanol. R_{rms} values shown are an average three 10x10µm^{2}images for each sample; the standard deviation of these values is shown.

Ethanol Concentration (vol/vol)	R_{rms} Image 1 (nm)	R_{rms} Image 2 (nm)	R_{rms} Image 3 (nm)	**Average Roughness (R_{rms}) (nm)**
0%	90.96	99.52	100.00	**96.83 ± 5.09**
30%	91.64	83.70	83.47	**86.27 ± 4.65**
70%	96.35	103.00	89.97	**96.44 ± 6.52**

3.4. Ethanol's effects on membrane mechanical properties

3.4.1. *Tensile strength*

The polyamide layer of the membrane consists of many long-chain polymers which may be arranged in a crystalline or amorphous way. If ethanol has affected this structure physically, through the swelling behaviour described previously in the visual results or otherwise, it could lead to a weakening of the active layer that may be undetectable via the methods described previously. As these membranes operate at high pressure, changes in strength may elevate the risk of membrane failure.

Table 4 shows the tensile strength properties that were determined by testing samples of the NF90 membrane, with and without the polyester support layer, to failure. The role of the support layer is obvious from the large difference in tensile strength between the membrane samples with (226 ± 25 MPa), and those without (76.4 ± 17 MPa) the support layer. For each case the membrane samples exhibited characteristic stress/strain curves with similar slopes (Young's modulus), yield stresses and sharp declines upon failure indicative of the brittle nature of the polymer material. These values are comparable to those found by Chung et al.[43] in their 2011 study of chlorine-induced mechanical deterioration of a polyamide RO membrane; their results showed the polyamide layer to have a Young's modulus in the range of 1GPa with an ultimate tensile strength of 67MPa. Furthermore, their study showed no significant increase in Young's modulus after a short exposure to chlorine but a four times increase in Young's modulus after a long exposure time.

Ethanol treatment for such a short exposure time had no significant effect on tensile strength, with results from samples treated with each concentration within the margin of error. The error in this experiment stemmed from the tiny cross-sectional area due to the membrane's ultrathin nature.

Table 4: Tensile strength and elastic properties of an NF90 membrane after treatment with various concentrations of ethanol.

	Entire Membrane			Polyamide/Polysulfone		
Ethanol Concentration (% vol/vol)	Young's Modulus (GPa)	Yield Stress (MPa)	Ultimate Tensile Strength (MPa)	Young's Modulus (GPa)	Yield Stress (MPa)	Ultimate Tensile Strength (MPa)
0%	8.14±0.36	62.4±0.37	236±16	3.1±0.20	33.7±3.4	74.1±24
30%	7.76±0.68	58.9±1.19	209±39	3.26±0.13	31.8±8.0	83.3±17
70%	8.26±0.97	66.6±1.11	232±12	3.27±0.58	30.9±3.6	71.8±13

4. Conclusion

This study has shown a 1.5 hour treatment of 40% ethanol to be a suitable and effective method of disinfecting Dow Filmtec NF90 membranes. Researchers conducting bacterial adhesion and biofouling studies with NF90 membranes can now use this treatment to remove competing microorganisms from their samples without affecting membrane properties.

It is important to note that each membrane has a unique active-layer polymeric structure, and thus has a specific chemical interaction with ethanol. While this treatment has been shown to be suitable for an NF90 membrane this may not be true for all other polyamide RO and NF membranes. It is therefore imperative that researchers are aware of the chemical sensitivity of these membranes and that they evaluate a disinfectant's effects on their studied membrane prior to experimental use. Further investigation is required to assess the suitability of this disinfection protocol for other polyamide membranes.

While this study focused on the application of ethanol as a means of disinfecting a membrane prior to biological fouling, the results (showing bacterial removal without damaging membrane properties) may be of interest as a means of removing biological fouling. For this purpose further study would also be required on the repeated treatment of these membranes with ethanol.

The effects of membrane compaction and swelling were seen in a number of the experiments in this study. Further research in this area is required to fully understand reversible compaction and the way in which the polyamide layer restructures itself upon re-compaction.

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