A Comparison between Laboratory and Industrial Fouling of Reverse Osmosis Membranes used to Concentrate Milk

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Abstract

Reverse osmosis with polyamide spiral wound membranes is used to concentrate milk to reduce its volume before transport to processing facilities. The main aim of this work was to identify the cause of low flux through membranes that had been used in an industrial facility. The fluxes of new and used industrial membranes were measured using a flat-sheet cross-flow laboratory system. It was found that the fouling characteristics of laboratory fouled membranes were entirely different from the industrial membrane. The laboratory membranes could be restored to a high flux with a regime of water flushing, caustic and acid. FTIR showed no significant build-up on the membrane. In contrast the industrial membrane could not be cleaned in this manner. FTIR and FT-Raman showed species associated with milk lipids. Cleaning by solvent extraction using a two-phase mixture of water, isopropanol and cyclohexane increased the flux from 1% to almost 50% of the value of a new membrane. Analysis of the solvent-extracted material indicated the presence of phospholipids with a relatively high concentration of sphingomyelin. It was concluded that the laboratory experiments did not mimic industrial processes and an effective industrial cleaning system was not found.

Keywords: reverse osmosis; polyamide; fouling; milk lipids; Raman and Infrared spectroscopy.

1. Introduction

Reverse osmosis is used to concentrate raw milk to reduce its volume before transport to processing facilities, and also within dairy factories to concentrate dairy liquids (Grandison and Lewis, 1996). Short term fouling during each production run, and long term fouling over many months of production, limit the efficiency of the process. During concentration, the rejected salts, proteins, and fat in milk will accumulate at the membrane surface causing increases in

the effective osmotic pressure and/or resistance to flux. Minerals can precipitate on the membrane once the solubility limit of solutes is exceeded. This layer reduces the membrane permeability and at the same time inhibits the back diffusion of salts to the bulk solution (Hoek and Elimelech, 2003). Chemical cleaning is required to remove the deposited layer. Acids such as nitric, phosphoric and citric, are generally used to clean inorganic precipitates from membranes while bases such as sodium hydroxide are used to remove biofilms, proteins and other organic fouling (D'Souza and Mawson, 2005).

The composition and concentration of both dissolved and suspended solids in the feed, and the pH of feed have a significant impact on membrane fouling (Rabiller-Baudry et al., 2009; Bouzid et al., 2012; Kulozik, 1998; Kulozik and Kessler, 1990a, 1990b). Protein, in particular casein, is the main component found in the deposit that forms on membrane surfaces during reverse osmosis of milk solutions (Glover and Brooker, 1974; Skudder et al., 2010). Minerals especially calcium phosphate increase the fouling formation by enhancing the casein cross-linking. Skudder et al. (2010) claimed that the fat globules and phospholipids simply get caught up in the protein deposit and had limited influence on flux reduction. For solutions without casein, calcium-phosphate precipitation appeared to cause strong fouling on the membrane (Hiddink, de Boer and Nooy, 1980; Smith and MacBean, 1978). No literature was found examining the foulant layer on reverse osmosis membranes that had been used for industrial processing of milk.

Membrane flux is defined as the mass flow rate of permeate passing through a unit area of membrane ($g\,m^{-2}\,s^{-1}$). Membrane permeance ($g\,m^{-2}\,bar^{-1}\,s^{-1}$) is defined here as the flux divided by transmembrane pressure, ΔP (bar) . Flux decline can be attributed to the effects of osmotic pressure, reversible fouling such as concentration polarization and gel formation, and irreversible fouling (Bouzid et al., 2012).

The main objective of this study was to determine the cause of fouling, and improve cleaning, of industrial thin-film composite polyamide reverse osmosis membranes by chemical analysis and cleaning. Experiments were conducted to determine if small quantities of mineral, proteins or lipids remained on new membranes after cleaning, and to determine the composition of any residual material on industrial membranes. New membranes (DOW FT30) were fouled and cleaned in a flat-sheet cross-flow laboratory system, with surface analysis using FTIR and

Raman spectroscopy. Then used industrial membranes were tested in the same way, but with an extended set of cleaning regimes.

2. Methods

2.1. Apparatus and operating conditions

A SEPA flat sheet membrane system (Sepa® CF, Osmonics, USA), with a filtration area of 134 cm^2 and with a feed spacer (0.70 mm thick taken from the spiral module specified below), was used to measure the flux, rejection, fouling and cleaning of components of milk. The system was operated at up to 25 bar transmembrane pressure using a Hydra-Cell D/G 03 pump (Wanner Engineering, Inc., USA) with a pulsation dampener that reduced the pressure range to ± 10% of the operating pressure. The cross flow rate was set to 2.3 L min^{-1} giving a cross flow velocity of 0.2 m s^{-1}. The temperature of the feed solution was maintained at 30 ± 1 °C by immersing the feed container in a water bath, the flux was corrected inversely proportionally to the viscosity of water at the feed temperature. Transmembrane pressure was controlled manually using a retentate flow control needle valve with a typical maximum variation of ±1%. The feed temperature, mass of permeate and transmembrane pressure were recorded by a data logging unit. The calculated flux was always accurate to better than ±1%. Permeate and retentate were recycled into the feed. The rejection coefficient, *R*, was calculated using the electrical conductivity, σ, of the bulk feed and permeate, i.e., $R = 1 - \sigma_{permeate}/\sigma_{feed}$ Repeated experiments in earlier work showed that the value of $1 - R$ was repeatable to better than ±10%. For example, if the rejection coefficient was 0.98, then it was repeatable to ±0.002.

The water used in all experiments was supplied from a Millipore water purification system (Elix-5, Millipore, USA). The membranes used were cut from a new DOW Filmtec FT30 polyamide thin-film composite spiral module (DOW HYPERSHELL^{TM} RO-8038/48) for filtration tests in the laboratory and an FT30 spiral module that has been used for concentrating unpasteurised whole milk in industry for several months at pressures in the range 15 to 25 bar. These membranes were termed “laboratory membranes” and “industrial membranes” respectively. The industrial module had been chemical cleaned at least daily during its operation following industrial practice with an undisclosed regime of caustic, acid and enzyme solutions. It was cleaned after it last processed milk. The cut membranes were kept moist with purified water (for the laboratory membrane) or with a small amount of 1% w/w sodium

metabisulphite solution (for the industrial membranes) in sealed bags at about 4 °C. The industrial membranes had no visible signs of fouling.

2.2. Membrane characterization

The elemental and chemical composition of the membranes were analysed with energy dispersive spectroscopy (EDX), Fourier transform infrared spectroscopy (FTIR) and Fourier transform Raman spectroscopy (FT-Raman). EDX had the potential to identify elements in the foulant that were not present in the virgin membrane. FT-Raman and FTIR are similar techniques that were likely to show the presence of chemical groups in the foulants. The membranes were vacuum dried at 30 °C overnight and were kept in resealable plastic bags. For EDX analysis, the membranes were carbon coated and the images of the membrane surfaces were captured at an accelerating voltage of 10 kV using an EDX system with AZtec analysis software and X-Max^N 50 silicon drift detector (Oxford Instruments, UK). FTIR spectra between 630 and 4000 cm^{-1} with a resolution of 4 cm^{-1} were obtained at 10 different locations on each membrane at room temperature using a Vertex 70 Fourier transform infrared spectrometer with OPUS software (Bruker Optics Gmbh, Ettlingen, Germany) and attenuated total reflectance (ATR) sampling technique. FT-Raman spectra were collected using a MultiRam (Bruker Optics Gmbh, Ettlingen, Germany) equipped with a 1064 nm Nd:YAG laser and a liquid nitrogen cooled Ge-detector. Spectra were collected using Opus 7.2 with a 145 mW power setting (at sample), 4 cm^{-1} resolution and 32 scans per spectrum collected over the spectral region 50 to 3500 cm^{-1}. The FT-Raman spectra were pre-processed using linear baseline correction and standard normal variate over the spectral region 220 - 1800 and 2650 - 3150 cm^{-1} to remove differences associated with the sample focus, such as the baseline and relative intensity of the spectra.

2.3. Filtration test

For each test, a new piece of membrane was thoroughly rinsed with water and left to soak in water for 1 hour to ensure complete hydration. The immersed membrane was rinsed with water again before placement in the SEPA membrane system. The laboratory membranes were pre-treated with 0.8% w/w nitric acid, water rinse, 0.5% w/w sodium hydroxide and water rinse prior to filtration and/or cleaning tests. Each chemical solution was circulated in the system for 10 minutes with no applied transmembrane pressure. Water rinse refers to the thorough system rinsing with at least 3 L of water, each litre being circulated for 5 minutes, until the electrical conductivity dropped below 25 $\mu S\ cm^{-1}$. This pre-treatment ensured removal of any residues

of the manufacturing process, increased the permeance by 12% - 22%, and gave more stable results. These are referred to as pre-treated membranes. The water fluxes before and after pre-treatments were measured and are termed initial water flux and pre-treated water flux respectively. The flux after pre-treatment was used as the reference flux. The water fluxes of a number of the industrial membranes were measured without any pre-treatment.

Reverse osmosis runs were performed for 3 or 5 hours at transmembrane pressure of 24 bar using the laboratory membrane after pre-treatment. The feed solutions that are listed in Table 1 were selected in an attempt to mimic the industrial membrane fouling. Both low fat and full fat milk were used to observe the effect of casein and fat in milk. Anhydrous milkfat (AMF), containing at least 99.8% fat, which is mostly triacylglycerides from milk fat (Fong, Norris, McGibbon, 2007), was used to observe the effect of free fats not contained within fat globules. Beta serum is a side stream of the AMF process which contains phospholipids from the fat globule membranes (Guan et al., 2015) and was tested after high levels of phospholipids were detected on the industrial membranes. Cholesterol was added because it is the most abundant non-saponifiable lipid in milk. Dispersion of AMF was with a stick homogeniser (IKA Yellowline DI 18). After the filtration, the system was water rinsed and the water flux, corresponding to non-flushable fouling, was measured. The membrane was cleaned with a standard cleaning cycle (see next section). The water flux was measured after each rinse and the flux ratio, the ratio between water flux after each chemical exposure and pre-treatment flux, was used to indicate the cleaning effectiveness.

Table 1 Preparation method for feed solutions

Feed solution	Description
Skimmed milk	Commercially available pasteurised Trim milk from Meadow Fresh, Christchurch, NZ, 4 g L^{-1} fat, 37 g L^{-1} protein.
Whole milk	Commercially available homogenised, pasteurised milk from Klondyke Fresh Limited, Christchurch, NZ, 34 g L^{-1} fat, 33 g L^{-1} protein.
Anhydrous milk fat (AMF) enhanced skimmed milk	A solution was prepared by adding 40 g of melted (at 40 °C) AMF (Canary Enterprises Limited, NZ) into 1 L of commercial Trim milk at 30 °C. The mixture was dispersed at 24,000 min^{-1}, 30 °C for 15 minutes and was kept at 4 °C (overnight) before reverse osmosis.
Beta-serum enhanced skimmed milk	A solution was prepared by adding 117.5 g of liquid beta-serum into 1 L of commercial Trim milk.
Cholesterol enhanced skimmed milk	A solution was prepared by dissolving 1 g of cholesterol (*Sigma-Aldrich* Corporation, US) in 39 g of melted (at 40 °C) AMF. The mixture was added into 1 L of commercial Trim milk at 30 °C, dispersed at 24,000 min^{-1} for 15 minutes and was kept at 4 °C (overnight) before reverse osmosis.

2.4. Membrane cleaning

The fluxes and rejection coefficients of a new and an industrial membrane were compared with purified water and 0.2% w/w sodium chloride solution at a fixed transmembrane pressure of 6 bar or at the same flux.

Industrial membranes were cleaned with different cleaning methods; each piece was subjected to only one cleaning sequence. The trials moved from the simplest laboratory cleaning regime to extended cleaning and enzyme cleaning as sometimes recommended by companies selling cleaning chemicals, and finally to a solvent extraction that would be suitable only in a laboratory. The initial water flux and water flux after rinsing were measured at 24 bar.

2.4.1. Chemical cleaning

Membranes were cleaned with several standard cleaning cycles consisting of 0.5% w/w sodium hydroxide, water, 0.8% w/w nitric acid, water, 0.5% w/w sodium hydroxide and water. Each chemical solution was circulated in the system for 10 minutes with no applied transmembrane pressure. The circulation time was intentionally short to enable differences in ease of cleaning to be detected.

An additional procedure suggested by Dow (2016) was used for cleaning of membrane biofouling. It consisted of a 30 minutes circulation of 0.1% w/w sodium hydroxide at 0.2 m s^{-1},

35 °C with no applied transmembrane pressure; 24 hours immersion; a final 30 minutes circulation with the same chemical at 0.3 m s^{-1}, 35 °C with no applied transmembrane pressure; and water rinse.

2.4.2. Enzyme cleaning

An industrial membranes was cleaned sequentially with various enzyme solutions, each followed by water rinse, 0.8% w/w nitric acid, water rinse, 0.5% w/w sodium hydroxide and water rinse. Each enzyme solution was circulated in the system for 30 minutes with no applied transmembrane pressure, while each chemical solution was circulated for 10 minutes at the same operating condition. The enzyme solutions used are listed in Table 2.

Table 2 The pH of enzyme solutions. All the enzymes were supplied by Zymus International Ltd, New Zealand.

Enzyme solution	Effective pH range	Solution pH
0.2% w/w protease (Enzidase® APC)	pH 7.0 – 10.0	8.8*
0.1% w/w cellulase (Enzidase® Cellulase 4000)	pH 3.0 – 6.0	5.8
0.2% w/w amylase (Enzidase® HT 425L)	pH 5.0 – 8.0	6.5
0.1% w/w lipase (Enzidase® Lipase MJ	pH 5.0 – 9.0	6.8

* adjusted with NaOH

In a separate test an industrial membrane was cleaned with a standard cleaning cycle, then 0.1% w/w lipase solution, followed by a standard cleaning cycle. The lipase cleaning consisted of enzyme circulation for 5 hours at 35 °C with no applied transmembrane pressure, membrane immersion in the solution overnight, enzyme circulation for 3 hours and a water rinse.

2.4.3. Lipid extraction with solvent

A pre-treated laboratory membrane and industrial membranes were cleaned with solvent extraction using a two-phase mixture of water, isopropanol and cyclohexane (Manirakiza, Covaci and Schepens, 2001). The membranes were immersed in the mixture of water, isopropanol and cyclohexane (11:8:10 volume basis) for 1 hour, then in 10% v/v isopropanol in cyclohexane for 1 hour, followed by 100% isopropanol for 30 minutes. The solvent extracts were dried under a stream of nitrogen at room temperature. The membrane was flushed with water prior to flux measurement in the SEPA flat sheet system.

The solvent extracts were analysed with liquid chromatography – mass spectrometry (both LC/MS and LC-MS/MS) to identify and quantify the contents of phospholipids in the solvent. The method of analysis for LC-MS/MS is outlined in Norris et al. (2009).

3. Results and discussion

3.1. Properties of industrial reverse osmosis membranes

Table 3 summarizes the fluxes and rejection coefficients of a laboratory membrane and an industrial membrane. The initial permeability of the industrial membrane was very low, about 7.6% of the permeability of a new laboratory membrane after pre-treatment. The rejection coefficient of the industrial membrane for NaCl was lower than a new laboratory membrane when operating at same transmembrane pressure (6 bar), but this was thought to be due to its low flux. Figure 1 shows how flux (changed by altering transmembrane pressure) affected the NaCl rejection of a laboratory membrane. These results are consistent with the model of Kimura and Sourirajan (1967). When both membranes were run at the same water flux (0.35 g $s^{-1}m^{-2}$), the rejection coefficients were similar. This showed that the industrial membranes had rejection properties similar to, or possibly better than, the laboratory membrane. Therefore, there was no evidence of degradation in membrane structure after long-term operation and cleaning.

Table 3 Fluxes and rejection coefficient of a laboratory membrane and an industrial membrane that was measured using water and 0.2% w/w sodium chloride solution at a fixed transmembrane pressure of 6 bar or at 1.67 bar to give the same flux as reported for the industrial membrane. ± values represent uncertainties (2 SD) determined in numerous similar tests.

Membranes	Water flux ($g\ s^{-1}\ m^{-2}$)		Rejection coefficient
	Water	0.2% NaCl	
Laboratory pre-treated membrane (6 bar)	7.42	5.33	97.4% ±0.3%
Industrial membrane (6 bar)	0.565	0.355	86.9% ±1.3%
Laboratory pre-treated membrane (1.67 bar)		0.352	85.1% ±1.5%

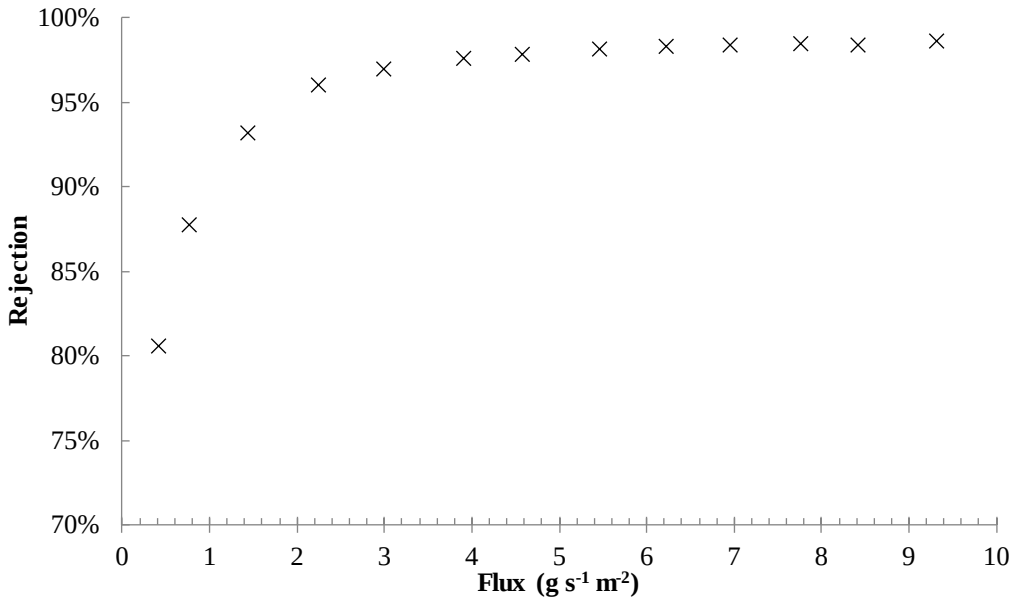

Figure 1. The effect of flux on the NaCl rejection of a new membrane. Data points correspond to transmembrane pressure from 4 to 16 bar in steps of 1 bar using 0.6% NaCl as the feed solution.

In Figure 2 the FTIR spectrum of an industrial membrane is compared with the spectrum of a laboratory membrane after pre-treatment (without any exposure to milk). Both membranes had similar vibration bands for the polyamide active layer and polysulfone support layer. The industrial membrane had strong vibration bands at 2954 cm^{-1} and 2921 cm^{-1}, and at 2852 cm^{-1} for asymmetric and symmetric CH_2 stretching vibration of methyl and methylene groups (Bassbasi et al., 2014); at 1741 cm^{-1} for C=O rotation and stretching vibration of ester groups in glycerides or of carboxylic acid groups (Bassbasi et al., 2014; Moros et al., 2006); at 1465 cm^{-1} for CH_2 scissoring vibration (McMullen, Lewis and McElhaney, 1994); and at 966 cm^{-1} for C=C in trans fatty acids (Mossoba et al., 2009). These distinctive bands showed that the fouling of the industrial membrane might be attributed to the lipid-based components in milk solution. The presence of milk protein and lactose on the industrial membrane was unlikely as the absorbance of amide I band at 1666 cm^{-1}, amide II band at 1540 cm^{-1} (Delaunay et al., 2008), and C-O vibration band for lactose at 1064 cm^{-1} (Rabiller-Baudry et al., 2002) were similar to those obtained for a laboratory membrane. Bacteria and extracellular polymeric substances from biofilms were also considered to be unlikely because of the lack of indicative peaks (Omoike and Chorover, 2004, Suci et al., 1998).

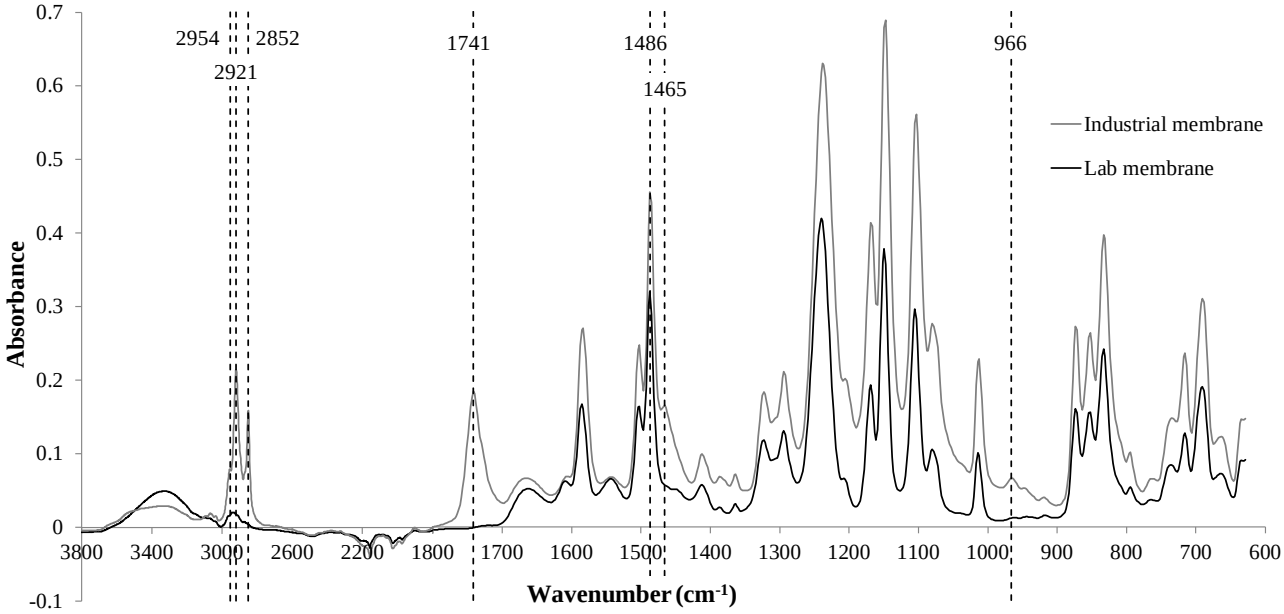

Figure 2 The FTIR spectra of an industrial membrane and a laboratory pre-treated membrane. Different x-axis scale was used for the region 600 – 1800 cm^{-1} and 1800 – 3800 cm^{-1}.

Different samples cut from the industrial module were found to vary considerably as measured by permeance and FTIR absorption, so these two measurements were compared. After permeance was measured, the membrane sample was dried and the FTIR spectra obtained. The relative concentrations of milk lipids that were detected were quantified by using the absorbance ratio of the vibration band at 1741 cm^{-1} (ester groups) to the vibration band of the membrane after baseline correction (Delauny et al., 2008; Diagne, Rabiller-Baudry and Paugam, 2013). The baseline between 3750 cm^{-1} and 4000 cm^{-1} was chosen as no vibrational bands were registered in this region. The band at 1486 cm^{-1} corresponds to the C-C stretching vibration of aromatic in-plane ring for polysulfone (Tang, Kwon and Leckie, 2009). The ratio of absorbances, *A*, is defined to represent the relative amount of lipid compared to polysulfone:

$$Absorbance\ ratio = \frac{A_{1741} - A_{3750\ \text{to}\ 4000}}{A_{1486} - A_{3750\ \text{to}\ 4000}} \qquad (1)$$

A linear correlation with an R^2 value of 0.85 was obtained for water permeance and the absorbance ratio of the FTIR band of industrial membranes (Figure 3). Generally, the membrane permeability decreased with increasing absorbance ratio. This suggested that the permeability of the industrial membrane was largely dependent on the concentration of the foulant components which contain an ester group, probably milk lipids, that remained attached to the industrial membrane after long-term operation and cleaning. Along with flux recovery,

the FTIR absorbance ratio can be used as an indication for fouling severity and cleaning efficiency. The high standard deviation in absorbance ratio for some industrial membranes indicates the uneven distribution of the foulant components on the membrane surface. The different degree of localized irreversible fouling was also reported by Rabiller-Baudry et al. (2014) for a spiral wound polyethersulfone ultrafiltration membrane after 3-hour skimmed milk run at 50 °C and inlet transmembrane pressure of 3.7 bar (1.5 bar outlet), and was attributed to the effects of the velocity profile. Further insight might have been gained if the position of the membrane samples within the spiral module had been recorded.

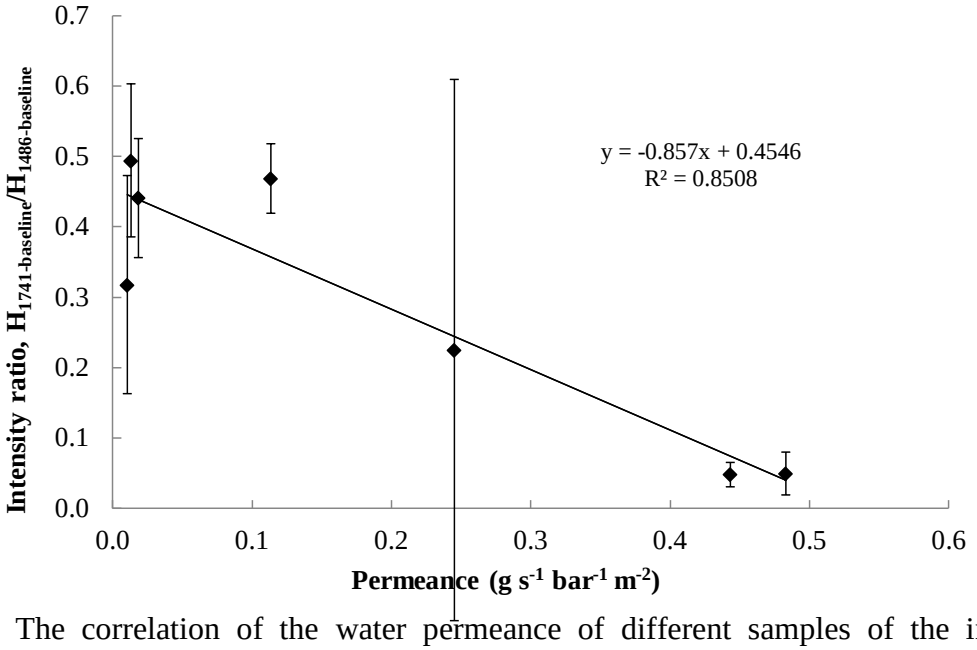

Figure 3 The correlation of the water permeance of different samples of the industrial membrane measured at 24 bar and 30 °C and the average FTIR absorbance ratio defined by Equation (1) The error bars represent ± 2 standard deviations of 10 different measurement locations.

The FT-Raman spectra of a laboratory membrane after pre-treatment and an industrial membrane are shown in Figure 4. The spectra of the industrial membrane shows a significant difference from the pre-treated membrane. The band at 1440 cm^{-1} has been attributed to saturated fatty acid $\delta(CH_2)$ scissoring in cholesterol, phosphatidylcholine (PC), phosphatidylinositol (PI) and phosphatidylserine (PS), but also in fatty acids (Czamara et al., 2015) while 2853 cm^{-1}, 2890 cm^{-1}, 2906 cm^{-1}, 2935 cm^{-1} and 2957 cm^{-1} are for the acyl chain stretching (Gallier et al., 2011; McGoverin et al., 2010). The FT-Raman spectra confirmed the FTIR results, showing that the industrial membrane had some milk lipids remaining on it

despite frequent cleaning in industry, but it did not add new information. Neither FTIR nor FT-Raman was able to identify any specific lipids.

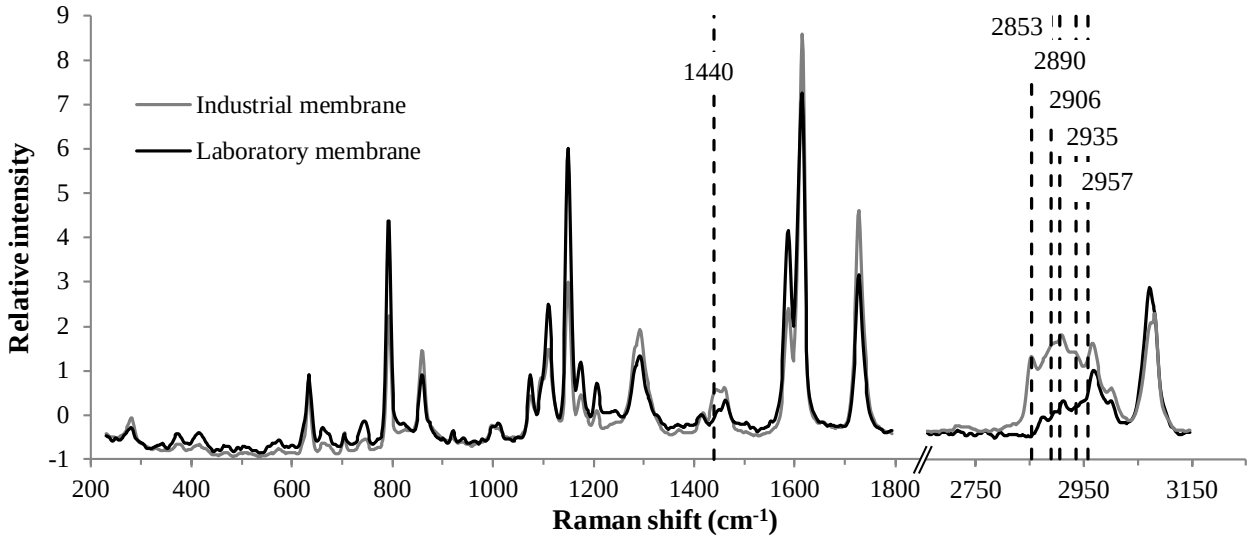

Figure 4 The FT-Raman spectra of a laboratory membrane after pre-treatment and an industrial membrane.

Table 4 summarizes the elemental composition of a laboratory pre-treated membrane and several industrial membranes from EDX analysis. All the industrial membranes had a large amount of oxygen and sulphur which was attributed to the membrane structure. The oxygen could also be attributed to lipids or other organic molecules. No inorganic components were observed apart from chlorine and sodium (at low concentrations) which were likely to be the residue from raw milk and/or cleaning chemicals. This suggested that the severe performance deterioration of industrial membranes was unlikely to be caused by inorganic fouling. Other inorganic components that are normally associated with fouling, such as calcium, were not detected.

Table 4 Elemental composition of a laboratory membrane after pre-treatment and industrial membranes analyzed with EDX. The standard deviation of two scans is given. nd indicates not detected.

	Mass fraction of elements				
Component	Lab membrane	Industrial membrane 1	Industrial membrane 3	Industrial membrane 5	Industrial membrane 9
	52.6±0.6%	70.6±0.2%	71.2±0.1%	71.1±0.5%	59.0±0.4%
S	47.2±0.3%	25.8±0.3%	26.4±0.2%	26.6±0.3%	39.1±0.5%
Cl	nd	2.4±0.4%	2.5±0.1%	2.3±0.1%	2.1±0.1%
Na	nd	1.3±0.1%	nd	nd	nd

FTIR and FT-Raman analysis consistently showed species associated with milk lipids on the industrial membranes. The milk lipids were not evenly distributed across the polyamide surface of the industrial membranes. It is hypothesized that some of the milk lipids are able to bind to the polyamide surface of the membrane during reverse osmosis and are not being removed by the chemical cleaning used in industry.

3.2. Filtration test in the laboratory

The flux ratios for 3-hour runs (except whole milk which was a 5-hour run) for the range of milk solutions used in the laboratory are listed in Table 5. The first row for water refers to the flux ratio of a laboratory pre-treated membrane after water fluxing for 1 hour and after each step of chemical cleaning.

Table 5 Effect of lipid components on flux recovery for reverse osmosis runs and cleaning with various milk solutions at 24 bar for 3 hours (except whole milk which was for 5 hours).

Feed solutions	Flux ratio = Water flux/pre-treated water flux				
	End of filtration	After water rinse	After NaOH	After HNO_3	After NaOH
Water	0.968 ± 0.006	-	0.989 ± 0.002	1.09 ± 0.01	1.03 ± 0.01
Skimmed milk	0.081 ± 0.002	0.446 ± 0.002	0.851 ± 0.008	0.982 ± 0.005	0.969 ± 0.005
Whole milk (5 h)	0.077	0.451	-	-	0.970
AMF enhanced skimmed milk	0.086	0.504	0.872	0.951	0.957
Beta-serum enhanced skimmed milk	0.087	0.524	0.844	0.935	0.939
Cholesterol enhanced skimmed milk	0.076	0.479	0.872	0.947	0.932

Note: ± indicates the range of measurement over 2 runs

Considering the second row for the skimmed milk runs, it can be seen that the flux while operating with milk was about 8.1% of the pre-treated water flux. After a water rinse, the water flux ratio increased to 44.6% showing that much of the flux reduction is due to the feed osmotic pressure and the temporary build-up of a concentration polarization layer. After the first sodium

hydroxide clean the water flux ratio increased to 85.1% showing that sodium hydroxide is not sufficient to remove all the foulant. After the subsequent nitric acid and sodium hydroxide steps the flux ratio increased to about 96.9%. This needs to be compared to 103% for cleaning of a clean membrane (first row) and it shows that cleaning is not complete. Additional cleaning cycles recovered the membrane permeability further.

The runs with whole milk and lipid enhanced skimmed milk had a higher flux ratio after water flushing than the unmodified skimmed milk run. This is probably because of the higher fat content in the cake layer which decreased the cake layer firmness and hardness, and increased its permeability.

The laboratory fouled membranes could be restored to a high flux with a regime of water flushing and several standard chemical cleaning cycles. The addition of milk lipids slightly increased the cleaning difficulty as the recoveries of the membrane permeability after cleaning with a standard chemical cleaning cycle for these modified skimmed milk runs were slightly lower than those reported for the unmodified skimmed milk run. The FTIR spectra obtained for the laboratory membrane cleaned after fouling showed no measureable build-up of lipids (results not shown). These results when combined with those from the industrial membranes suggested the possibility of an undetectable but small amount of lipid adsorption during each run leading to long-term fouling.

3.3. Cleaning of the industrial membranes

Table 6 summarizes the permeance ratio and FTIR absorbance ratio of the industrial membranes for different cleaning methods. The industrial membranes had a large variation in flux and the fluxes were low compared with a new membrane. Because each industrial membrane was different, the uncertainty in the permeance ratio was estimated from previous results such as those in Table 5 to be better than ±1% of the ratio. "Lab membrane – two standard cleaning cycles" refers to a run with whole milk for 5 hours at transmembrane pressure of 24 bar followed by two standard chemical cleaning cycles using a new pre-treated laboratory membrane. Solvent cleaning was used to extract lipids, but it is noted that such cleaning could not be carried in industry because of prohibitive costs to ensure process safety and food safety. The initial and cleaned permeances are the water permeance for the new membrane after pre-treatment and after cleaning, respectively.

Table 6 Effect of cleaning methods on the permeance ratio and FTIR absorbance ratio for used industrial reverse osmosis membranes.

Cleaning method	Permeance ($g\ s^{-1}\ bar^{-1}\ m^{-2}$)		Permeance ratio	FTIR absorbance ratio Average ± 1 s.d.	
	Initial	Cleaned		Initial	Cleaned
Lab membrane – two standard cleaning cycles	1.07	1.13	1.05	-	-
Four standard cleaning cycles	0.013	0.037	2.87	0.49 ±0.05	0.49 ±0.04
Enzyme cleaning	0.113	0.168	1.48	0.47 ±0.03	0.30 ±0.12
Lipase cleaning	0.051	0.100	1.96	0.23 ±0.16	0.17 ±0.16
0.1% w/w NaOH immersion	0.081	0.084	1.04	-	-
Lipid extraction with solvent	0.010	0.488	46.7	0.32 ±0.08	0.031 ±0.008

Note: Permeance ratio = permeance of cleaned membrane/initial permeance; FTIR absorbance from Equation (1).

Cleaning of the industrial membrane with cleaning regimes such as chemicals (sodium hydroxide and/or nitric acid), and enzyme (protease, lipase, cellulase and amylase) had little or no improvement in membrane permeability (Table 6). No significant change in the FTIR spectra was observed for the industrial membranes after membrane cleaning with these methods. This showed that the industrial membrane is probably fouled with specific milk lipids that were difficult to remove by normal chemical and/or enzyme cleaning.

After cleaning by solvent extraction, the industrial membrane gave a significant flux improvement (last row in Table 6) and the rejection improved from 60% to 92% probably because of the higher water flux. Similarly, the FTIR spectra showed a clear removal of the lipids from the industrial membrane where the absorbance ratio of the vibration bands at 1741 cm^{-1} reducing by about 90% after cleaning by solvent extraction. To confirm that the changes were not due to solvent damage a laboratory membrane was treated with solvent in the same way. The water flux of a laboratory membrane treated with solvent increased by about 10% above the pre-treated water flux, while the rejection coefficient reduced slightly from 97.8% to 96.7%. The FTIR spectra of the pre-treated membrane before and after solvent extraction were similar to each other (spectra not shown).

3.4. Analysis of solvent extract

An LC-MS analysis of the material extracted by the solvent from the industrial membrane showed a mixture of triacylglycerols (TAGs), diacylglycerols (DAGs), monoacylglycerols, free fatty acids, phospholipids and cholesterol (results not shown), but the species were not

clearly identified or quantified. All of these except cholesterol are considered to be saponifiable and hence it was expected that they would be removed by caustic cleaners that were used on a daily basis in industry and tested in the laboratory.

Analysis of the solvent extracted material with LC-MS/MS showed the presence of phospholipids with a relatively higher concentration of sphingomyelin. Table 7 summarizes the amount of phospholipids, expressed as mass per unit membrane area, that was extracted from the industrial membranes. While the mass was quite different for the two membranes, the relative proportions of each component were consistent to with ±2% of the total. Contarini and Povolo (2013) reported that the phospholipids in liquid milk include phosphatidylethanolamine (PE) and phosphatidylcholine (PC) with about 30% each, 20% sphingomyelin (SM), 5% - 10% each of phosphatidylinositol (PI) and phosphatidylserine (PS), and other minor compounds. Table 7 shows that about 67% - 70% of the lipid extracted from the industrial membranes was sphingomyelin (SM), followed by phosphatidylethanolamine (PE) and phosphatidylcholine (PC) at about 12% each. This suggests that sphingomyelin (SM) preferentially binds to the reverse osmosis membrane compared to other phospholipids. The preferential binding of sphingomyelin (SM) to the polyamide membrane might be due to the hydrogen accepting groups (phosphate and carbonyl groups) and, most importantly, the additional hydrogen donor groups (the amide N-H group and the hydroxyl –OH group) which could form strong hydrogen bonds with the polyamide active layer. This would leave the fatty acid tails free making the surface more hydrophobic. The surface density of phospholipids for Membrane 12 of 283 µg m^{-2} compares to 1800 µg m^{-2} for a monolayer of phosphatidylcholine (of similar size) on $Si(Ti)O_2$ given by Csúcs and Ramsden (1998). This density is sufficiently high for there to be a measurable effect from the hydrophobic fatty acid tails. Unfortunately, sphingomyelin is very difficult to separate and too expensive to carry out fouling tests at meaningful scale.

Table 7 The amount of phospholipids, per unit area of used reverse osmosis membrane, and their relative fractions in the solvent used for lipid extraction for two of the industrial membranes.

Samples		Total phospholipids in a unit area of membrane						
		PI	PE	LPE	PC	PS	SM	LPC
Membrane 12	μg m^{-2}	ND	35	8.3	35	2.8	191	11
	%		12.4%	2.9%	12.4%	1.0%	67.7%	3.9%
Membrane 14	μg m^{-2}	ND	7.8	4.1	12	ND	64	4.1
	%		8.5%	4.5%	13.0%	0%	69.6%	4.5%

Note: PI - phosphatidylinositol, PE - phosphatidylethanolamine,
LPE - lysophosphatidylethanolamine, PC - phosphatidylcholine,
PS - phosphatidylserine, SM - sphingomyelin, and LPC - lysophosphatidylcholines.
ND - not detected.

4. Conclusions

The characteristics of laboratory fouled membranes were entirely different from the industrial membrane. The industrial membranes had a significantly lower permeability than a new membrane, but their rejection coefficients were similar when compared at the same flux. EDX analysis indicated that mineral fouling was unlikely. The FTIR and FT-Raman spectra of the industrial membrane showed species associated with milk lipids. The absorbance ratios of the FTIR band at 1741 cm^{-1} (associated with lipids) to 1486 cm^{-1} ($A_{1741} - A_{baseline}/A_{1486} - A_{baseline}$) gave a good indication on the fouling severity of the industrial membranes and were linearly correlated to the water flux.

Some of the species on the industrial membrane were removed by solvent extraction using a two-phase mixture of water, isopropanol and cyclohexane.. Analysis of the solvent extract with LC-MS/MS indicated the presence of phospholipids with a relatively higher concentration of sphingomyelin. The laboratory fouled membrane could be cleaned by acid and caustic while the industrial membrane could not be effectively cleaned in this manner. Alternative cleaning regimes such as chemicals (sodium hydroxide and/or nitric acid), and enzyme (protease, lipase, cellulase and amylase) were not successful in recovering the permeability of the industrial membrane back to the level expected for a clean membrane. No effective cleaning regime was found that could be used in industry to recover the membrane flux. The solvent-based cleaning would almost certainly not be a realistic option for dairy processing. It is suspected that small

amounts of phospholipids bind to the polyamide in a manner that is not readily reversible, and over time the accumulation causes a significant reduction in flux.

Given that lipids are the likely source of the foulant, separation of cream from milk before filtration is likely to reduce the rate of flux reduction of the industrial membranes. However, this would require with extra capital and operating costs which might exceed the cost of early membrane replacement.

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