MEMBRANE FOULING IN COLD MICROFILTRATION OF SKIM MILK: MECHANISMS AND CONTROL

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MEMBRANE FOULING IN COLD MICROFILTRATION OF SKIM MILK: MECHANISMS AND CONTROL

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Microfiltration (MF) can effectively remove microorganisms and somatic cells from milk, increasing the safety and shelf life of milk and dairy products. The main challenge in MF is membrane fouling, which leads to a significant decline in permeate flux over time. This work aimed to elucidate the mechanisms of membrane fouling in cold (6°C) MF of skim milk and optimize a CO_2 backpulsing technique that can diminish membrane fouling by physically removing the foulant from the membrane surface.

Using 3 injection ports, a CO_2 injection frequency of 120 s and an injection duration of 1 s, a permeate flux of 30.02 ± 0.48 L/m^{2}h was obtained after 3 h of MF at a cross-flow velocity of 3.8 m/s, as compared to 25.85 ± 0.99 L/m^{2}h for the control. Additionally, a smaller drop in flux was observed for the optimized CO_2 backpulsing process (15.57% after 3 h) as compared to the control (29.83% after 3 h). When MF was conducted at 6 m/s, the permeate flux after 3 h was 82.32 ± 4.42 L/m^{2}h for MF with CO_2 backpulsing, as compared to 71.39 ± 4.21 L/m^{2}h for the control. MF with CO_2 backpulsing also resulted in a higher transmission of total solids and protein than the control MF.

A systematic investigation of the mechanisms of membrane fouling was conducted by identifying the proteins and minerals in four foulant streams: weakly attached external foulants (W_e), weakly attached internal foulants (W_i), strongly attached external foulants (S_e), and

strongly attached internal foulants (S_i). The foulants were evaluated both after MF (without and with CO_2 backpulsing) and after a brief contact between the membrane and milk (adsorption study). The concentration of minerals was very small in all foulant streams, below 2.5 ppm, and likely they do not contribute significantly to membrane fouling in cold MF. Proteomics analysis showed that all major milk proteins were present in all foulant streams. In adsorption trial, α-lactalbumin level in W_e was higher than in milk, which indicates an affinity of this protein for the membrane material. The serum proteins α-lactalbumin and bovine serum albumin (BSA) were found in a higher proportion in the "weakly attached" fractions (W_e and W_i) from the instantaneous adsorption study as compared to the control MF (without CO_2 backpulsing), which suggests that caseins were mainly introduced into the fouling layer when transmembrane pressure was applied. Casein concentration did not increase in the foulants from CO_2 backpulsing MF, despite the localized decrease in pH caused by the contact with CO_2. More significantly, CO_2 backpulsing reduced the total protein concentration in W_e, with 52.98 ± 4.87 μg/mL for CO_2 backpulsing MF as compared to 62.20 ± 10.13 μg/mL for control MF. Overall, the data indicates that CO_2 backpulsing resulted in less weakly attached external foulants and possibly less loosely deposited materials on the membrane surface as compared to control MF, which improved permeation through the membrane.

The knowledge generated in this study could be used to identify solutions to further minimize membrane fouling and increase the efficiency of milk MF. The CO_2 backpulsing technique could be an efficient and affordable solution to mitigate fouling, and is also applicable to other cold membrane filtration processes, such as the MF of juice or beer.

BIOGRAPHICAL SKETCH

Teng Ju Tan was born in Kuala Lumpur and was raised in a far less privileged village called Bagan Hailam in Malaysia. Unlike most of the villagers, Teng Ju's parents always emphasize the importance of knowledge to their children. They had invested a lot of time, energy, and love to ensure their children had a full and enriching education. They instilled in Teng Ju the value of education in empowering the powerless.

In 2001, Teng Ju graduated with a First Class Honours in Bachelor of Science in Bioindustry from Universiti Putra Malaysia (UPM). Following graduation, he worked as a public school teacher in Pandamaran Jaya, Malaysia. He was back to UPM again in 2002 as a Graduate Research Assistant in the Department of Food Science. He worked as a demonstrator for the laboratory of undergraduate food science courses. He also assisted some professors in supervising undergraduate final year projects and products development project. In 2004, he worked as a Research Assistant in the same department. He had successfully published his research papers in the international science journals.

With an ambition to be a professor, Teng Ju decided to pursue a Ph. D. degree in Food Science and Technology at Cornell University in August 2008, under the supervision of Dr. Carmen I. Moraru. Upon completing his doctorate, Teng Ju will be relocating to International Islamic University Malaysia as an assistant professor. He looks forward to being able to give back to the community once he begins his teaching and research career.

For my mother Chong Kwi Eng and my late father Tan Lian See, who have been great sources of encouragement, strength, and blessings to me.

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Most of all, I would like to express my appreciation to my family and friends who have given me unending support throughout my life. To my mother Chong Kwi Eng who supported

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CHAPTER 1

INTRODUCTION

Microfiltration (MF) is a nonthermal processing technology that can remove microorganisms from beverages such as milk and juices, while preserving their organoleptic and nutritional properties. MF is a pressure-driven process and a purely physical technique. It is usually operated at transmembrane pressures less than 1 bar (Bylund, 1995). MF membranes have pore diameters ranging from 0.1 to 10 μm, which can selectively separate micron-sized particles and macromolecules from fluids based on a sieving effect. Typical materials that are retained by MF from fluid food feeds, depending on membrane pore size, are somatic cells, bacteria, fat, mold and yeast cells, but also macromolecules such as starch, casein micelles or hydrocolloids. The advents of high performance membranes and cross-flow filtration have enabled MF to be applied on a commercial scale. Today, cross-flow MF is being employed in the dairy industry for many applications, such as the removal of bacteria from skim milk, fat removal of whey, and concentration of micellar casein for cheesemaking (Saboya and Maubois, 2000).

MF for Bacterial Removal from Skim Milk

Compared to pasteurization and ultra-high-temperature (UHT) processing, MF offers the advantage of the physical removal of bacteria spores and somatic cells from milk. HTST (high temperature short time) pasteurization typically involves heat treatment at 72°C for 15 s and produces a pathogen-free, but non-sterile product. Bacterial spoilage is the main limiting factor

in extending the shelf life of HTST pasteurized fluid milk beyond 14 days at 6°C (Boor, 2001). The ultra high temperature (UHT) process, in which milk is heated in the range of 135 to 150°C for several seconds followed by aseptic packaging, may extend the milk shelf life to 12 months without refrigeration (Lewis and Deeth, 2009). However, the resulting cooked flavor (cabbage or boiled flavor) is not liked by some consumers (Lewis, 2003). Furthermore, UHT induces changes such as age gelation, lactosylation and protein cross-linking between β-lactoglobulin and kappa-casein, with the extent of changes increasing with elevated storage temperature. These changes can have adverse effects on the nutritional and functional properties of milk proteins (Datta and Deeth, 2001; Holland et al., 2011).

Meanwhile, both bacterial spores and somatic cells in milk are not affected by the standard heat treatments used in dairy processing plants. Lewis and Deeth (2009) claimed that heat resistant bacterial spores which can subsequently cause flat sour defect in milk are not destroyed by UHT processing. Ma et al. (2000) reported that high somatic cell count (SCC) milk showed significant sensory defects on pasteurized milk after 21 days. The sensory defects mainly included rancidity and bitterness and were consistent with higher levels of lipolysis and proteolysis (Azzara and Dimick, 1985; Verdi and Barbano, 1988; Ma et al., 2000).

In addition to its use as drinking milk, heat-treated fluid milk is also used in the manufacture of cheese. Both spores and somatic cells may compromise the quality of cheese products (coagulation time, yield, and quality of cheese, particularly the flavor and texture) (Giffel and van der Horst, 2004), especially the spore-forming *Clostridium tyrobutyricum*, which can cause late blowing during the maturation of cheese (Gesan-Guiziou, 2010).

Microfiltration processes using 1.4 μm pores membranes have been shown to be very efficient in removal of bacteria, spores, and somatic cells from skim milk, while allowing almost

complete permeation of other milk components (Saboya and Maubois, 2000; Giffel and van der Horst, 2004; Fritsch & Moraru, 2008). Shelf life of MF milk stored under refrigerated conditions was reported to be 15 days (Saboya and Maubois, 2000). Elwell and Barbano (2006) reported that a combination of MF and HTST pasteurization could significantly extend the shelf life of fluid milk. They successfully obtained a shelf life of 32 days with respect to proteolysis for the microfiltered and pasteurized fluid milk stored at 6.1°C.

Overall, by reducing the bacteria, spores, and somatic cells in skim milk to very low levels, MF has the potential to increase the quality and shelf life of milk and dairy products, either as a replacement for, or in addition to, heat treatment. However, a drawback of MF of milk is that the size ranges of milk fat globules (0.2 – 15 μm) and bacteria (0.2 – 6 μm) overlap (Saboya and Maubois, 2000), such that much of the milk fat would be removed along with the bacteria in the retentate. Therefore, MF for microbial removal can only be applied to skim milk.

Fouling Mechanisms

The major factor limiting the utilization of microfiltration in many applications is membrane fouling, which leads to a significant decline in the permeate flux over time. Membrane fouling is the irreversible change in membrane permeability due to the deposition of retained particles on the membrane surface and/or constriction of internal pore structure by small particles (Guerra et al., 1997). The typical mechanisms of membrane fouling related to microfiltration include pore constriction, pore blocking and gel/ cake formation (Figure 1.1). Membrane fouling might be caused by a combination of different mechanisms as a result of different size distribution of species in feed. Particles with diameters much smaller than the membrane pore may cause pore constriction. Particles with a diameter equal to the membrane

pore may cause pore blocking. If the particles have diameters greater than the membrane pore, they will be retained on the membrane surface and cause cake formation (Fane and Chang, 2009).

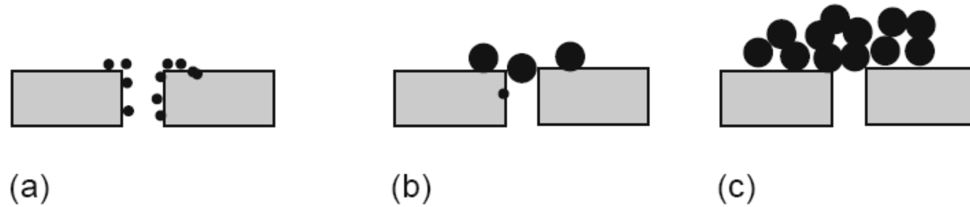

Figure 1.1. Diagrams for different fouling mechanisms. (a) pore constriction, (b) pore blocking, and (c) cake formation. (From Fane and Chang, 2009.)

Fritsch (2006) claimed that the rapid initial flux decline during MF of skim milk is usually attributed to the concentration polarization and the quick formation of fouling deposits. Concentration polarization is the reversible build-up of suspended or dissolved species in the solution phase (Fane and Chang, 2009). Concentration polarization takes place at the beginning of the filtration process, when a concentration gradient of the retained species is formed on or near the membrane surface (Aimar et al., 1988). The main mechanism of this reversible phenomenon is the unbalanced transport of dissolved components between the bulk phase and the membrane surface (Marcelo and Rizvi, 2009). As concentration polarization is due to the bulk mass transfer limitations in the membrane system, its influence on performance varies with different membrane processes. For reverse osmosis and nanofiltration, concentration polarization can cause a large increase in osmostic pressure and reduces the permeate flux. For MF membranes without a fouling layer built on the membrane surface or in the pores, concentration polarization of macromolecules should be insignificant because the relatively large pore size MF membranes are basically not retentive to most macromolecules. Nevertheless, MF membranes can undergo particle polarization due to the retention of colloids and particulates. Both

concentration polarization and particle polarization are associated with the permeation-induced build-up of the concentration profile on the membrane surface without altering the characteristics of the permeability and selectivity of the membranes (Fane and Chang, 2009).

On the contrary, membrane fouling occurs because of specific physical or chemical interactions between the various macrosolutes/ particles and the membrane. The rate and extent of membrane fouling is generally a function of the device fluid mechanics, but fouling usually cannot be eradicated simply by increasing the rate of solute mass transfer (Zydney, 1996). The schematic of concentration polarization and membrane fouling is illustrated in Figure 1.2.

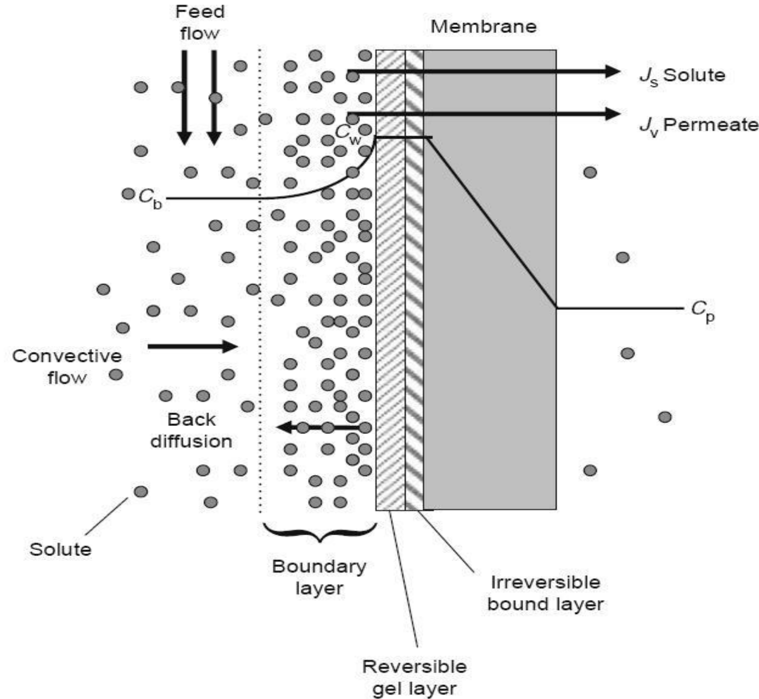

Figure 1.2. Diagram of concentration polarization and fouling at the membrane surface. (From Goosen et al., 2004.)

Factors that Influence Membrane Fouling in MF of Milk

The main factors that influence the occurrence of membrane fouling are the process parameters of the MF system, the physicochemical properties of the membrane, and the feed solution (Huisman et al., 2000).

Effect of Process Parameters

Process parameters of MF such as transmembrane pressure, cross-flow velocity, and temperature have significant effects on membrane fouling. The influences of these process parameters on the MF performance are discussed below.

Effect of transmembrane pressure (TMP). As MF is a pressure-driven process, higher transmembrane pressure (TMP) usually gives higher permeate flux. However, pressure higher than a critical pressure was found to accelerate membrane fouling (Brans et al., 2004; Fritsch and Moraru, 2008). According to the critical flux theory, three regimes can be differentiated for membrane filtration as shown in Figure 1.3. In regime I, which is also called sub-critical flux operation, permeate flux has a positive linear relationship with TMP, where flux increased with increasing TMP. In regime II, the TMP is above the critical pressure and permeate flux is equal to the limiting flux, where the transport of particles towards the membrane is in equilibrium with the back diffusion towards the cross-flow. In regime III, where TMP is far above critical pressure, the flux decreases with increasing TMP because of cake formation and compaction (Brans et al., 2004).

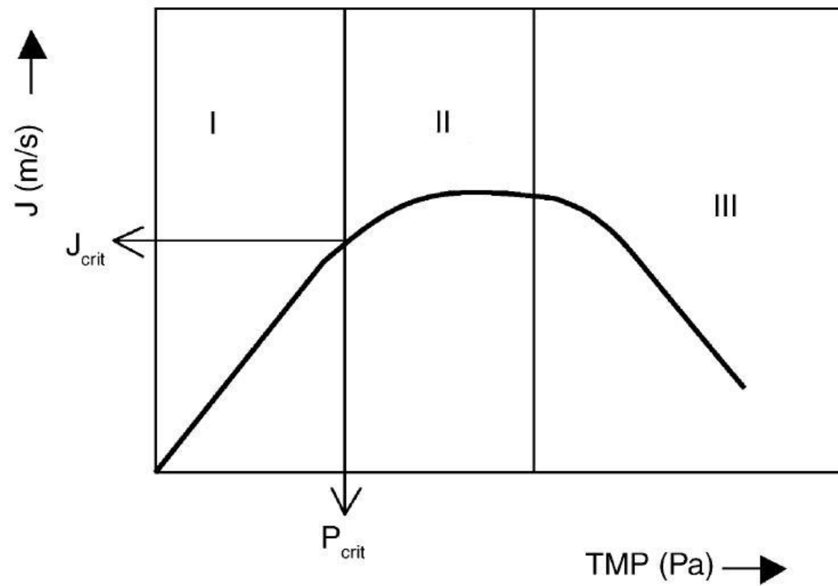

Figure 1.3. Diagram of permeate flux (J) vs. transmembrane pressure (TMP). (From Brans et al., 2004.)

Mourouzidis-Mourouzis and Karabelas (2008) studied the microfiltration of whey protein using large pore-size (0.8µm) ceramic membrane under the pressure range of 5 – 17.5 psi, at a low cross-flow velocity of 1.2 m/s. They found that permeate flux tends to increase with increasing TMP up to a value of 10 psi; an increment of pressure beyond 10 psi decreased the flux. This is attributed to the interplay of cross-flow with transmembrane pressure. Beyond a certain pressure, benefits of cross-flow might be gradually eliminated by the TMP, which leads to a thicker protein layer formation on the membrane due to increasing compressive stresses. The thickened protein layer thus increases the fouling resistance (Mourouzidis-Mourouzis and Karabelas, 2008).

Effect of high cross-flow with uniform transmembrane pressure (UTMP). The hydrodynamic conditions in high cross-flow filtration may lessen fouling because, while the permeate flux drags particles toward the membrane, the cross-flow induces particle back

diffusion into the bulk phase. In the MF of skim milk, membrane fouling is often controlled by the utilization of a high cross-flow velocity with low uniform transmembrane pressure (UTMP) concept. Cross-flow microfiltration can be carried out in conventional or non-uniform transmembrane pressure (non-UTMP) and the UTMP modes. The pressure profiles of these two modes are illustrated in Figure 1.4. In the conventional mode, the high cross-flow velocity (≥ 7 ms^{-1}) results in a relatively large pressure drop over the cross-flow channel, which causes a decreasing transmembrane pressure over the length of the tube. This leads to rapid membrane fouling at the inlet and severe flux decline. In the UTMP mode of operation, a constant transmembrane pressure is maintained over the length of the filter by applying a cross-flow at the permeate side (Saboya and Maubois, 2000). The UTMP mode operates at a lower and uniform transmembrane pressure, while simultaneously maintaining a high cross-flow velocity, which discourages fouling and cake build-up. This provides the advantages of more constant flux, better utilization of available filtration area and lower flux decline than the conventional mode (Marshall and Daufin, 1995).

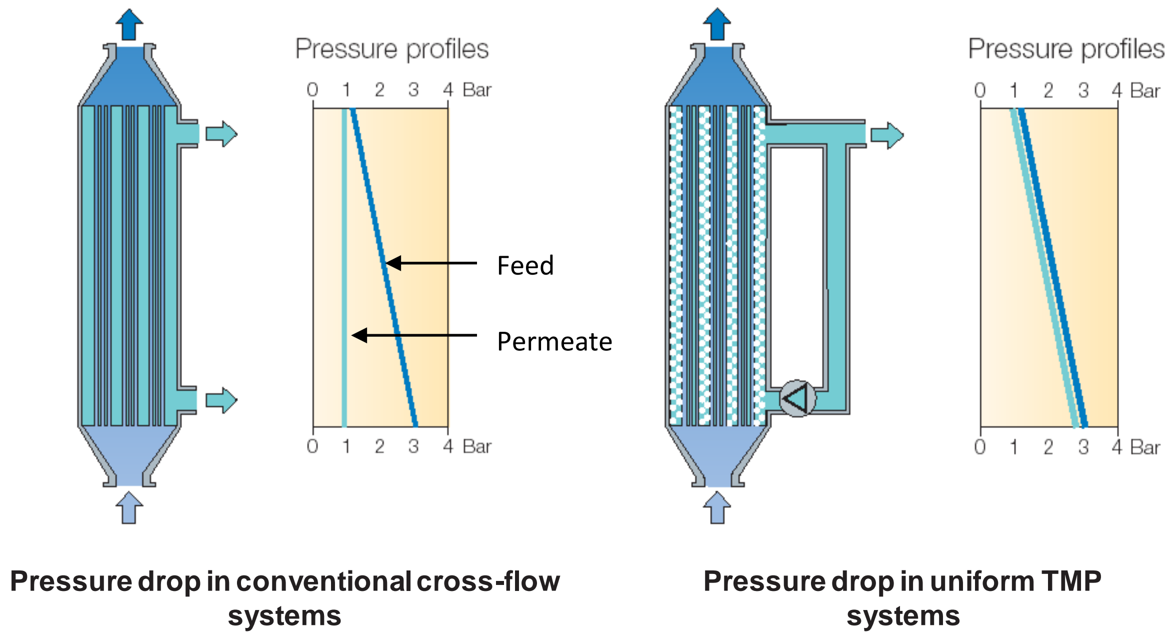

Figure 1.4. MF pressure profiles in conventional and UTMP systems. (From Bylund, 1995.)

Vadi and Rizvi (2001) carried out a study to compare the effectiveness of UTMP and non-UTMP modes in the separation of casein micelles from skim milk by cross-flow microfiltration at temperature of 50°C. The authors found that UTMP mode shows a lower initial flux than the corresponding non-UTMP mode but enabling the concentration of suspensions to higher concentration factor due to its flux decline with time is significantly lower. In addition, the authors observed that cake resistance increased for non-UTMP mode and decreased for UTMP mode. This suggests that the cake formed in non-UTMP mode was more compact and more difficult to erode than in UTMP mode. Thus, the extent of fouling and cake formation was less in the UTMP mode, which would reduce the cost of cleaning and maintaining the membrane.

A drawback of high cross-flow velocity with UTMP concept is the high energy demand due to the cross-flow at both sides of the membrane (Brans *et al.*, 2004). This would increase the operating costs and require higher investment.

Alternatively, Isoflux and Membralox GP membranes have been employed to achieve the same effect as the UTMP concept. Isoflux membranes from TAMI Industries are based on continuous variation of the membrane layer thickness. Membralox GP membranes from Pall Corporation are based on a longitudinal permeability gradient built into the support structure without modification of the filtration layer. These membranes have a decreasing membrane resistance over length of the tube, which has the same effect as UTMP but with no pressure control in different sections at permeate side. However, these membranes must be used for well defined applications, the porosity gradient of the membrane support and the thickness gradient of the membrane being built both for precise flux and selectivity (Saboya and Maubois, 2000).

Effect of temperature on MF. Microfiltration of milk is normally conducted at a temperature ranging from 50 to 55°C, to achieve the highest possible flux by having lowest possible permeate viscosity, while avoiding the protein denaturation. However, for milk to maintain its raw status according to the Pasteurized Milk Ordinance, the microfiltration must be performed at temperatures lower than 45°F (6.7^{o}C). Furthermore, there might be advantages in performing MF at low temperatures, such as preventing the growth of thermophilic bacteria and germination of spores inside the membrane system (Fritsch and Moraru, 2008). An expected consequence of performing that MF at low temperatures is a reduction in flux, due to the increased viscosity of the fluid streams. According to Fritsch and Moraru (2008), the fluxes in low temperature MF were much lower than when the process was conducted at 50°C, and the difference in flux could not be explained based on the difference in temperature alone. This suggested that fouling mechanisms may be different in cold vs. warm MF of skim milk.

It is known that the casein micelle structure undergoes some changes at temperatures below 10°C, such as an increase in micelle voluminosity as a result of weakened hydrophobic

bonds within the micelles (Walstra, 1990). This subsequently allows some β-caseins to dissociate from the casein micelles and move into the serum phase of the milk (Van Hekken and Holsinger, 2000; Holland et al, 2011). This might produce a different composition of the fouling layers between cold and warm MF.

Effect of Membrane Materials

Different types of membrane materials have different physical and surface chemistry properties. Thus the nature of adsorption fouling by milk components will be different with different types of membrane materials. Van der Horst (1995) reported that the adsorption of individual whey proteins to a hydrophobic polysulfone surface is higher than to a hydrophilic silica surface. There is a direct relation between the protein adsorption, the increase in membrane resistance and protein rejection. Polymeric membranes such as polyether sulfone (PES) and polyvinylidene fluoride (PVDF), which are more hydrophobic than ceramic membranes, tend to absorb more protein. Zulewska et al. (2009) found a serum protein removal factor of 0.99 for a ceramic uniform transmembrane pressure system and 0.66 for a polymeric spiral wound system. However, the influence of the factors mentioned above was not well defined by the authors.

In addition, membrane surface roughness also could be a significant factor of absorption fouling of microfiltration membranes. Elimelech et al (1997) reported that fouling rate of colloids on the membrane surface was determined by the membrane surface roughness. In their study, surface images obtained by atomic force microscopy (AFM) and scanning electron microscopy (SEM) showed that the thin-film composite membrane has large-scale surface roughness of ridge-and valley structure, while the cellulose acetate membrane surface is relatively smooth. A higher fouling rate was observed for the thin-film composite membrane compared to that for the cellulose acetate membrane (Elimelech et al., 1997).

Membrane surface modifications, such as alteration of membrane surface charge and hydrophilicity, have been carried out to reduce adsorption fouling in polymeric membranes. In studies in which membranes were modified by coating with polymers of non-ionic, cationic and anionic origin, it was found that in all cases the non-ionic polymers reduced the adsorption of β-lactoglobulin, independent of pH. Absorption of protein to membranes coated with ionic polymers was depended on pH. The non-ionic, hydrophilic coatings methylcellulose and polyvinylmethylether gave the best results in decreasing protein adsorption by prevention of electrostatic interactions, hydrogen-bridge formation and hydrophobic interactions (Van der Horst, 1995).

A new type of membranes called microsieves, which are very thin and smooth membranes with uniform pores made by silicon micromachining has been developed at a laboratory scale (Kuiper et al., 2002). They consist of a thin micro-perforated silicon nitride membrane attached to a macro-perforated silicon support. The membrane thickness is of the order of the pore size, thus allowing high fluxes and relatively simple cleaning procedures. Moreover, the membrane is flat and smooth, with a surface roughness typically <10 nm, which hinders adsorption of foulants. Furthermore, the pores are uniform in size and distribution, which may be important for quality control of membrane selectivity. Microfiltration of whole milk with silicon microsieves has been carried out by Fuente et al. (2010). Permeate fluxes in the range from 5000 up to 27,000 L/m^2h were found. These results are remarkable in comparison with those obtained using conventional MF membranes, higher by a minimum of one order of magnitude (Fuente et al., 2010). Fuente et al. (2010) also found that the physical properties of whole milk (viscosity and particle size distribution) were not affected by these high permeation rates. Although very

promising, the manufacturing of microsieves membranes is still in the experimental stage and scaling up for industry application is required.

Effects of Milk Components on Fouling in Membrane Filtration of Milk

In the membrane separation of dairy streams, milk proteins are typically involved in fouling. Using a polysulfone membrane with molecular weight cutoff of 10,000, Tong et al. (1988) found that flux decline is severe in the early stages of whole milk ultrafiltration (UF) at 49°C and is associated with irreversible adsorption of milk proteins onto the membrane surface. Their results showed that whey proteins (β-lactoglobulin and α-lactalbumin) represented 95% of the membrane foulants, and very little casein was identified as a membrane foulant. Membrane fouling during MF and UF of skim milk was also studied by James et al. (2003) using scanning electron microscopy (SEM), atomic force microscopy (AFM) and X-ray photoelectron spectroscopy. By examining the cross-sections of used polymeric membranes (MF membrane with pore size of 3 μm and UF membrane with a molecular weight cutoff of 3500), James et al. (2003) found that the protein particles interacted with the pore walls of the membrane (caused by protein-polymer interactions), and formed agglomerates as a result of protein-protein interactions, leading to narrowing and ultimately blocking of the pores. This mechanism tallies with the initial sharp decline in permeate flux. However, they did not identify the milk proteins that caused the fouling.

Using dynamic light scattering (DLS) measurements, Mourouzidis-Mourouzis and Karabelas (2006) reported that whey protein aggregates were responsible for membrane fouling in MF of whey protein isolate solution with ceramic membranes of 0.8 μm pore size. Wang (2008) also evaluated the external fouling in cold microfiltration of skim milk with the aid of DLS and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The results

of particle size and SDS-PAGE analyses suggested that milk proteins, especially casein micelles, were the major components of the fouling layer on the membrane surface. One limitation of electrophoresis analysis of milk proteins is that κ-casein is difficult to be identified by SDS-PAGE since its carbohydrate moiety prevents it from taking up stain (Wake and Baldwin, 1961). Thus, electrophoresis analyses might lead to an underestimation of κ-casein as a foulant, and hence the results were not deemed conclusive. Moreover, Wang (2008) focused on foulants attached to the surface of the membrane, which were removed by means of a nylon brush, and did not collect the internal foulants. The specific protein compositions of both external and internal fouling need to be identified in order to fully explain the fouling mechanisms.

Mineral precipitation and complexation of minerals with proteins also contribute considerably to membrane fouling. Adsorbed minerals may act as salt bridges between the protein and the membrane that exacerbates the fouling (Marcelo and Rizvi, 2009). Membrane fouling in the filtration of milk or whey is severe under physicochemical conditions that promote calcium phosphate precipitation or calcium-protein complexation. However, the severity of fouling of membrane filtration of milk is less as compared to that in whey filtration, due to the stabilizing effect of the casein micelles in the former (Marshall and Daufin, 1995).

Microbiological fouling may also contribute to flux decline in membrane processes in the dairy industry. Microfiltration of milk is usually operated under warm conditions, at temperatures ranging from 50 to 55°C. This favors the growth of thermophilic bacteria and germination of spores inside the membrane and the recirculation loop of the MF system. Deposition and growth of bacteria on the stainless steel, and consequent biofilm formation are particularly favored by low shear stress conditions (Azevedo et al., 2006). Growth of biofilms

has been observed in UF and reverse osmosis (RO) membranes used for whey concentration (Tang et al, 2009; Hassan et al., 2010; Anand et al., 2012).

Brans et al. (2004) proposed that one of the fouling mechanisms in the MF of skim for bacteria removal is complete pore blocking by bacteria and spores. However, Fritsch (2006) reported that bacteria and spores were not observed in SEM images of fouled ceramic membranes (pore size of 1.4 µm) after MF of skim milk, as depicted in Figure 1.5. This might be due to the high cross-flow velocity (7 m/s) employed in the MF system that successfully prevented the deposition of microorganisms on the membrane. Nevertheless, in a study to investigate fouling mechanism relevant to adhesion of *Bacillus cereus* spores on a 0.45 µm tubular ceramic membrane, isolated or scattered spores and some clusters comprising dozens of cells were found adhered to the membrane surface after filtering the spore suspension at a cross-flow velocity of 4 m/s, as revealed in the Figure 1.6 (Blanpain-Avet et al., 2011).

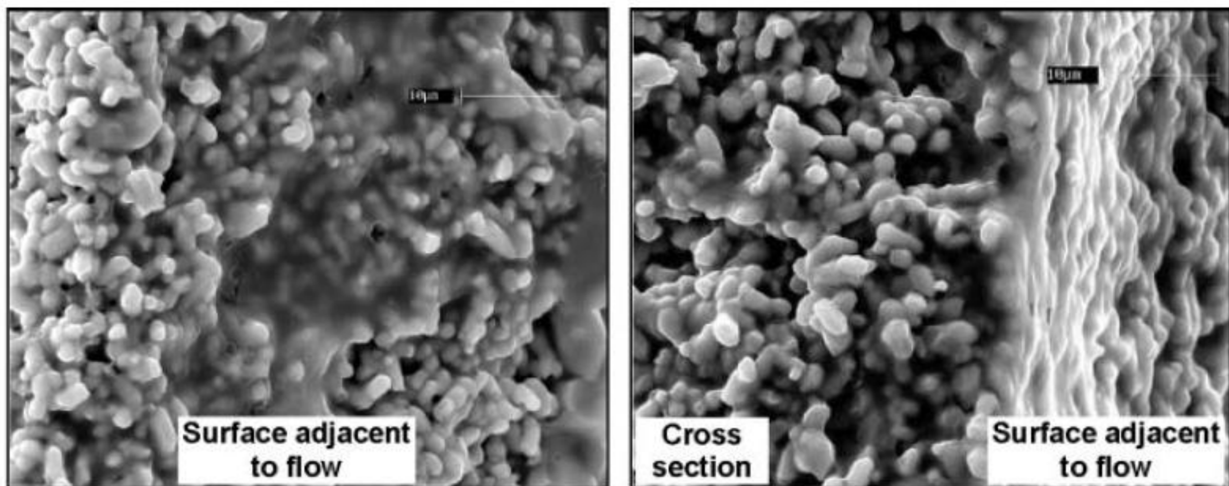

Figure 1.5. SEM images of fouled membranes that show the fouling layer. (From Fritsch and Moraru, 2008.)

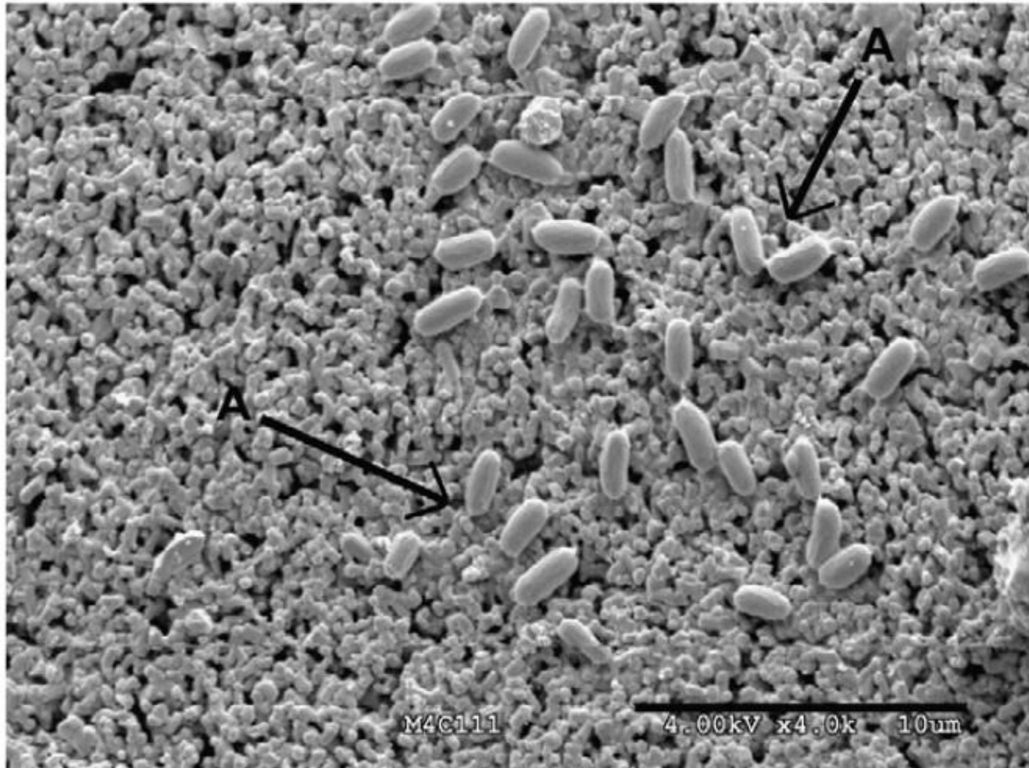

Figure 1.6. SEM image of fouled membrane that shows the *B. cereus* spores on the membrane surface. Arrow A: adhered *B. cereus* cells. (From Blanpain-Avet et al., 2011.)

To date, no study has been conducted to fully understand the fouling mechanism of ceramic membranes in microfiltration of skim milk for microbial removal. Further investigations are therefore necessary in order to elucidate both the fouling mechanism and its impact on the performance of the membrane separation process, which in turn will provide insight into ways to control fouling.

Fouling Control

Fouling control is fundamental for a correct and efficient membrane separation process development. In addition to improving the permeate flux, control of fouling also results in easier

cleaning of membranes. This may avoid the need for severe cleaning regimes, and prolong the lifetime of membranes, particularly polymeric membranes.

The common strategies used to minimize membrane fouling include chemical (e.g. membrane surface modification) and physical (e.g. high cross-flow velocity with uniform transmembrane pressure, turbulence promoters, backpulsing, electric fields and ultrasound) methods (Fane and Chang, 2009). The method of choice for fouling control must be technically and economically feasible, scalable to commercial size, and well suited for cleaning in place. The backpulsing strategy, which was employed in this research project, is discussed below.

Backpulsing Method

Backpulsing and comparable techniques, such as backwashing, backflushing and backshocking are effective means of fouling control that allow the use of low cross-flow velocity and therefore involve low energy costs. These are the *in situ* methods of membrane cleaning by periodically reversing the transmembrane pressure. Depending on the experiment design, either part of permeate or the clean water flows back into the cross-flow channel. As depicted in Figure 1.7, the fouling deposits which accumulate on the membrane surface during forward filtration are lifted and taken up by the cross-flow during reverse filtration (Redkar and Davis, 1995). The effectiveness of backpulsing depends on the frequency, duration and the pressure profile, and is highly dependent on the feed composition. High frequency backpulses might prevent the formation of a fouling layer or remove the foulants shortly after they are deposited on the membrane (Davis, 2001). Short duration pulses might reduce the loss of permeate that occurs during the backpulsing period.

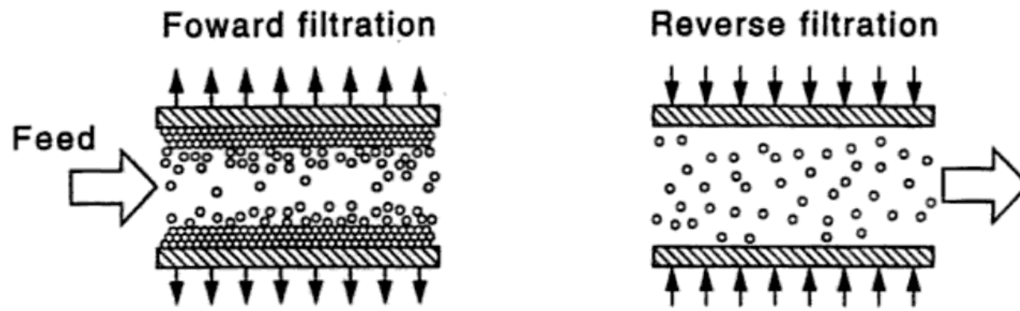

Figure 1.7. Schematic of forward and reverse filtration. (From Redkar and Davis, 1995.)

Mourouzidis-Mourouzis & Karabelas (2008) studied the microfiltration of whey proteins using large pore-size (0.8μm) ceramic membranes. They targeted to achieve a very high microbial reduction, while maintaining the highest possible protein transmission through the membrane. Fouling was controlled using a relatively small cross-flow velocity and periodic backwashing to minimize energy expenditure. A series of three successive protein filtration cycles of 30 minutes duration each, were performed, with backwashing for 5 min using distilled water with NaN_3 in-between each cycle. They also investigated the effect of transmembrane pressures (TMP) ranging from 5 to 17.5 psi. They found that backwashing counteracted reversible fouling and could maintain a stable operation for a long operating period; on the other hand, they reported that irreversible fouling takes place only in the first cycle, and reduced the flux by no more than approximately 20%, with the exception of the 17.5 psi treatment. Protein transmission was high (> 93%) in the tested TMP range. The disadvantage of this system was the downtime during backwashing using distilled water with NaN_3.

A novel carbon dioxide (CO_2) backpulsing system using a single injection port has been developed by Fritsch and Moraru (2008) to lessen the flux decline in cold (6°C) MF of skim milk. The backpulsing of CO_2 every minute for a duration of 10 s at a pressure equal to that of

inlet pressure of feed (138 kPa) led to a 20% increase in permeate flux. The backpulsing system also resulted in a 13% increase in total amount of permeate collected after 3 hours of processing. The CO_2 backpulsing method was believed to physically disrupt the fouling layer in the outer membrane channel thus yielding higher and steadier permeate fluxes (Fritsch and Moraru, 2008).

Further study is required to achieve a maximum possible increase in permeate flux, which can be achieved by using additional CO_2 injection ports along the membrane housing, in combination with optimization of the CO_2 backpulsing parameters (frequency and duration). Moreover, the effect of CO_2 backpulsing on membrane fouling has yet to be evaluated. Thus the goals of this dissertation were to elucidate the underlying mechanisms of membrane fouling and maximize the flux improvement by optimizing the CO_2 backpulsing system in cold microfiltration of skim milk.

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CHAPTER 2

RESEARCH OBJECTIVES

Recent debate about using food preservation techniques that have a minimal impact on food quality resulted in the development of new, non-heat based methods of preserving foods in a way that is both safe but also preserves the intrinsic nutritional and sensory qualities of foods. Microfiltration (MF), a pressure driven membrane separation method that is able to of physically remove bacteria, spores, and somatic cells from milk, has shown potential commercial applications for improving the quality and extending the shelf life of milk and other dairy products.

The main challenge in MF is membrane fouling, which decreases membrane permeability and its separation efficiency. To date, the mechanisms of membrane fouling in large pore MF of skim milk have not been elucidated. With regard to the important implication of membrane fouling in MF, there is a definite need to understand the fouling mechanisms, as this could help develop more effective solutions that will control or minimize fouling, thus improving the performance of the MF process.

This work aimed to elucidate the mechanisms of membrane fouling in cold microfiltration of skim milk for microbial removal, and to develop a method able to diminish membrane fouling. The specific objectives were to:

1. Optimize a CO_2 backpulsing system with multiple injection ports to achieve a maximum increase in permeate flux in cold MF of skim milk.

2. Investigate the mechanisms of membrane fouling in cold milk MF by identifying and quantifying the foulants involved in external and internal fouling, with a focus on milk proteins and minerals.
    2.1. Determine the fouling mechanism in adsorption (static state).
    2.2. Examine the fouling mechanism in microfiltration process (dynamic state).
3. Evaluate the effect of CO_2 backpulsing on the external and internal fouling in cold MF of skim milk.

CHAPTER 3

OPTIMIZATION OF A CO_2 BACKPULSING METHOD FOR INCREASING THE PERMEATE FLUX IN COLD MICROFILTRATION OF SKIM MILK

ABSTRACT

The major challenge associated with cold microfiltration (MF) of skim milk is the decrease in permeate flux, caused by fouling. To address this, a CO_2 backpulsing technique capable to counteract membrane fouling was developed and optimized. Five portable CO_2 backpulsing ports were attached to the membrane housing. Five different combinations of CO_2 backpulsing frequencies and durations were tested. The MF experiments were performed at a temperature of 6 ± 1°C, a cross-flow velocity of 3.8 m/s and a transmembrane pressure of 83 kPa. A series of 45 min experiments were first performed. The combination of 3 ports at 120s frequency with 1s duration (120s/1s) produced the highest flux: 46.09 L/m^{2}h at 45 min. In a series of three hour MF runs, permeate flux from the treatments with 3 ports at 120s/1s and 5 ports at 180s/1s was significantly higher than for the control. After 3 h, the flux for the control was 25.85 ± 0.99 L/m^{2}h, while for the optimized CO_2 backpulsing experiment, a flux of 30.02 ± 0.48 L/m^{2}h was obtained. In addition, a smaller drop in flux was observed for the optimized CO_2 backpulsing run (15.57% after 3 h) as compared to the control (29.83% after 3 h). Furthermore, the protein transmission into permeate was also the highest in treatment of the 3 ports at 120s/1s. Another study was conducted to evaluate the performances of optimized MF and control at a higher cross-flow velocity (6 m/s). The permeate flux after 3 h for the optimized MF was 82.32 ± 4.42

L/m^2h while for control was 71.39 ± 4.21 L/m^2h. The developed method and can be used to effectively increase the flux in microfiltration applications.

INTRODUCTION

Microfiltration (MF) is a well-established technique for the removal of microorganisms - both vegetative cells and spores, which helps improve the safety and extend the shelf life of the filtered fluids. MF has gained significant interest for removal of microorganisms from milk in recent years, particularly for long shelf life products. Heat treatments such as HTST pasteurization and ultra-high-temperature (UHT) sterilization are the processing methods primarily used to inactivate pathogens in milk. The drawback of HTST treatment is that it cannot inactivate bacterial spores, which can compromise the quality and shelf life of milk and other dairy products (Fernández García et al., 2013). While sterilization treatments can inactivate spores, they also induce some undesirable changes in milk, such as cooked flavor and age gelation (Datta and Deeth, 2001; Lewis, 2003). At the same time, heat treatments do not have any effect on somatic cells in milk, whereas they can be removed by MF. High somatic cell counts can lead to increased proteolytic and lipolytic activity in milk, thus compromising the quality and shelf life of dairy foods (Azzara and Dimick, 1985; Verdi and Barbano, 1988).

Microfiltration using 1.4 μm pore membranes has been shown to be very efficient in removal of bacteria, spores, and somatic cells from skim milk, while allowing almost complete permeation of other milk components (Pafylias et al., 1996; Saboya and Maubois, 2000; Te Giffel and Van der Horst, 2004; Fritsch and Moraru, 2008; Skrzypek and Burger, 2010). This can be achieved using both ceramic (alumina, zirconia) and polymeric membranes (made of

polyamides and polysulfones). Ceramic membranes can withstand high temperatures and a wide range of pH, from 0.5 to 13.5, and thus provide significant advantages in terms of chemical, thermal and mechanical stability over the polymeric membranes (Baruah et al., 2006). Nonetheless, polymeric membranes, particularly spiral wound membranes, are still widely used in the dairy industry, due to their lower capital cost compared to ceramic membranes.

The main problem in microfiltration is membrane fouling, which leads to a significant decline in the permeate flux. Therefore, controlling fouling is fundamental for a correct and efficient process development. The complete prevention of fouling is probably not possible, but its impact can be limited by a variety of methods. One solution explored by researchers is the development of new membranes, less prone to fouling. For example, Brito-de la Fuente et al. (2010) carried out microfiltration of whole milk with silicon microsieves and reported permeate fluxes were ranging from 5000 up to 27,000 L/m^2h. These results are remarkable in comparison with conventional MF systems, higher by about two orders of magnitude (Brito-de la Fuente et al., 2010). Although very promising, the manufacturing of microsieves membranes is still in the experimental stage and scaling up for industry application is required.

Other common strategies used to minimize membrane fouling include chemical (e.g. membrane surface modification) and physical (e.g. high cross-flow velocity with uniform transmembrane pressure, turbulence promoters, backpulsing, electric fields and ultrasound) methods (Fane and Chang, 2009). The method of choice for fouling control must be technically and economically feasible, scalable to commercial size, and well suited for cleaning in place.

In an attempt to mitigate the flux decline in cold MF of skim milk, a carbon dioxide (CO_2) backpulsing system has been developed by Fritsch and Moraru (2008). Backpulsing of CO_2 through a single port located on the permeate side close to the feed inlet led to a 20% increase in

the permeate flux, and a less pronounced flux decline after 3 h of processing. This CO_2 backpulsing system is technically simple, does not require additional energetic costs and offers *in situ* control of fouling while processing. CO_2 has generally recognized as safe (GRAS) status, is easily available and inexpensive. Moreover, added CO_2 may bring additional benefits to the microbiological quality of milk, due to the bacteriostatic effect of CO_2, which can further enhance the shelf life of fluid milk. At the same time, it is also possible to remove the CO_2 from the MF milk by applying vacuum, if necessary.

The objective of this study was to evaluate the effectiveness of multiple CO_2 backpulsing ports in the cold microfiltration of skim milk and to optimize the CO_2 backpulsing parameters in order to achieve a maximum possible increase in permeate flux. The developed and optimized CO_2 backpulsing technique could be an efficient and affordable solution to mitigate fouling, and could also be applied to other cold microfiltration processes, including filtration of juice, beer, or heat sensitive fluids that require cold processing.

MATERIALS AND METHODS

MF Apparatus and Procedures

The pilot-scale experimental MF unit consisted of a 190 L feed tank connected to a variable-speed centrifugal pump, a tubular heat exchanger and a tubular ceramic membrane of Tami design (GEA Fitration, WI) placed inside a stainless steel housing (Figure 3.1). The membrane had a nominal pore size of 1.4 μm, length of 1,200 mm, outside diameter of 25 mm, 23 internal channels each with a hydraulic diameter of 3.5 mm, and total membrane area of 0.35

m^2. Two identical membrane elements were used in this work, which will be referred to as "membrane 1" and "membrane 2". Five portable stainless steel backpulsing ports were installed on the membrane housing, and connected to a beverage grade CO_2 gas tank. A data acquisition port was used for collecting of the temperature and pressure data. The permeate flux data was obtained gravimetrically using an electronic scale that also connected to the data acquisition system.

The transmembrane pressure (TMP) was calculated as follows:

$$TMP = \frac{(P_1 + P_4)}{2} - P_p \qquad (1)$$

where P_1 is the feed inlet pressure, P_4 is the retentate outlet pressure and P_p is the permeate pressure (see Figure 3.1).

The permeate flux, J (L/m^2h) was calculated according to the equation:

$$J = \frac{M}{A * t * \rho} \qquad (2)$$

where M is the permeate weight (kg), A is the membrane surface area (m^2), t is time (h), and ρ is the permeate density at the operating temperature (kg/m^3).

The drop in flux over time was expressed as the relative permeate flux, or J/J_0 (%), where J_0 is the initial milk flux. J_0 value was selected at the first 5 min of MF, in order to allow for initial flux stabilization. It also ensured that the permeate channel was completely filled with liquid when flux measurement was taken.

Prior to each MF run, the cleanliness of the membrane was determined by determining its water flux. , which was measured using reverse osmosis (RO) water at 20°C and an average TMP of 83 kPa (12psi).

The feed was cold, raw skim milk obtained from the Cornell Dairy Plant (Ithaca, NY). After draining out the water, 117 kg (113.6 L) of cold, raw skim milk were added in the feed tank and the pump was turned on to a low velocity for 20 s to flush out any water that remained in the system. Permeate flux data collection began after another 70 s, when a constant TMP and cross-flow velocity were reached. The processing conditions used were: 1) TMP of 83 kPa (12 psi) and a cross-flow velocity of 3.8 m/s, and 2) TMP of 159 kPa (23 psi) and a cross-flow velocity of 6.0 m/s. When CO_2 backpulsing was used, backpulsing began 5 s after the permeate collection started. The CO_2 pressure was set to 3-4 psi higher than the inlet pressure (P_1 in Figure 3.1). The MF process was conducted at a temperature of 6 ± 1°C, which was maintained by passing the milk through a countercurrent tubular heat exchanger using chilled water as the cooling medium. The duration of each MF experiment was either 45 min or 3 h.

Chemical Cleaning of the Membrane

After each MF run, a complete chemical cleaning cycle was carried out. The cleaning procedure consisted of a rinse with RO water for 10 min, followed by alkaline cleaning with Ultrasil-25 at a concentration of 16 mL/L at 80°C for 30 min, a second RO water rinse for 10 min or until neutrality (checked with pH strips), a acid cleaning with HNO_3 solution at a concentration of 5 mL/L at 50°C for 20 min, and a third RO water rinse for 10 min or until neutrality.

The effectiveness of cleaning and change in the membrane performance with time were monitored by determining the water flux under the same conditions as describes in the previous section.

Experimental Design

Four combinations of CO_2 backpulsing ports were tested: no CO_2 backpulsing (control), 1 port (adjacent to the feed inlet), 3 ports (equally spaced along the membrane) and 5 ports (the 4th port and 5th port were placed at the same distance, at an angle of 180 degree of the 2nd port and 3rd port), as depicted in Figure 3.1. Five combinations of CO_2 backpulsing frequencies and durations were tested: no backpulsing (control), 60 s frequency with 2 s duration, 60 s frequency with 1 s duration, 120 s frequency with 1 s duration, and 180 s frequency with 1 s duration. The duration of each milk MF run in the optimization stage was 45 min.

The optimum combination obtained, i.e. the combination that resulted in the maximum permeate flux, was then tested in 3 h long MF runs, both at cross-flow velocity of 3.8 m/s and at 6 m/s. Several 8 h runs were also performed.

Analyses

pH Measurement. The pH of milk was measured before and after processing (both permeate and retentate), using a Fisher Scientific accumet* Research AR10 pH/mV//°C Meter (Pittsburgh, PA). The pH measurements were conducted at 6°C.

Somatic Cell Analyses. Samples of feed, retentate and permeate from the 3 h MF runs were analyzed for somatic cell counts (SCC) by Flow Cytometry at the DairyOne laboratory (Ithaca, NY). The results are reported as thousands of somatic cells per mL of milk.

Infrared (IR) Milk Analyses. Samples of feed, retentate and permeate from the 3 h MF were analyzed for the percentage of fat, protein, lactose and total solids by IR analysis at the DairyOne laboratory (Ithaca, NY).

Microbiological Analyses. Microbiological analyses were carried out by the Milk Quality Improvement Program Laboratory, Cornell University.

Evaluation of vegetative bacteria. Samples of feed (raw skim milk) and permeate (MF skim milk) were spiral plated on Standard Plate Count (SPC) agar then incubated at 32°C for 48h prior to enumeration. The SPC method was used to quantify the colony-forming units (cfu/mL) present in the feed and permeate.

Evaluation of bacterial spores. Milk samples were Spore Pasteurized (SP) at 80°C for 12 min, then immediately cooled on ice (Wehr and Frank, 2004). This step was performed to inactivate all vegetative microflora and at the same time trigger spore germination. The SP samples were then spiral plated on SPC and incubated at 32°C for 48 h prior to enumeration.

All microbiological analyses were conducted in triplicate, and plating was conducted in duplicate.

Statistical Analyses

All experiments and analyses were performed in triplicate. Data was analyzed statistically using the analysis of variance (ANOVA), with the statistical software package JMP 8.0 (SAS Institute Inc., Cary, NC, USA). The Tukey-Kramer HSD test was used to determine significant differences between means at 5% level of probability.

RESULTS AND DISCUSSION

Before discussing the effectiveness of CO_2 backpulsing on permeate flux in cold MF of skim milk, it is important to discuss the effect of membrane cleanliness on the flux data. Although the same cleaning procedure was used for every experiment, the initial cleanliness of the membrane before each MF run, indicated by the water flux of the membrane, was somewhat variable throughout the optimization study.

Fritsch and Moraru (2008), in a study conducted using a similar setup and the same type of ceramic membrane, reported that for membrane water fluxes ranging from 300 to 1500 L/m^{2}h there was a significant dependence of the initial milk flux (measured at 5 min) on the water flux, with the initial milk flux decreasing with decreasing membrane water flux. This trend was also observed in the current study (see Figure 3.2). It must be noted though that the water flux seemed to affect the actual value of the flux (Figure 3.2a), but not necessarily the rate of fouling, as indicated by the relative flux (Figure 3.2b). Therefore, in order to clearly separate the effect of CO_2 injection on the flux from the effect of water flux, the comparison between controls and treatments with CO_2 backpulsing was done individually, under similar water flux conditions.

Effect of Multiple CO_2 Backpulsing Ports and CO_2 Backpulsing Parameters on MF

Backpulsing techniques have been used before for flux enhancement in membrane filtration. Typically, either permeate or clean water is forced back through the membrane into the cross-flow channel by reversing the transmembrane pressure. Backpulsing is commonly achieved either by using a series of solenoid valves that regulate the permeate flow, or a piston

that causes flow reversal through the membrane using a reciprocating action (Koh et al., 2008). In this study, backpulsing with CO_2 gas was achieved by injecting CO_2 gas at a pressure slightly higher than the feed inlet pressure through injection ports located on the permeate side of the membrane, as illustrated in Figure 3.3. As a result of backpulsing, the fouling deposits accumulated on the membrane surface are lifted and taken up by the cross-flow into the retentate. This technique minimizes the permeate loss that typically is incurred in conventional backpulsing, since only a limited amount of permeate is pushed back through the membrane.

All MF runs showed an initial rapid decrease of flux followed by a long and gradual flux decline, which is typical variation for any membrane filtration process. For all MF runs, a pseudo- steady state was reached after 30 min of processing.

The experimental results showed that at a similar water flux (1000 to 1054 L/m^2h), the combination of 3 CO_2 backpulsing ports at 120 s frequency with 1 s duration (120s/1s) gave the highest milk flux after 45 min (38.43 L/m^2h), followed by the control (37.01 L/m^2h) and backpulsing using 1 port at 60s/2s (34.64 L/m^2h). The combination of 3 ports at 120s/1s also produced the higher permeate flux (46.09 L/m^2h) than 3 ports at 60s/1s (average 39.62 L/m^2h) and 3 ports at 60s/2s (39.41 L/m^2h), at similar water flux conditions (1500 $\pm$ 50 L/m^2h). Meanwhile, even at a slightly lower water flux (1019 L/m^2h), the combination of 3 ports at 120s/1s still gave the higher permeate flux (38.43 L/m^2h) compared to 5 ports at 180s/1s at a water flux of 1175 L/m^2h (35.32 L/m^2h) and 5 ports at 120s/1s at a water flux of 1220 L/m^2h (29.73 L/m^2h). Overall, these results indicate that the backpulsing using 3 ports (120s/1s) yields the highest permeate flux of milk compared to the other combinations.

Interestingly, the drop in flux (J/J_0) in control (filled diamonds in Figure 3.2b) after 45 min was more pronounced than for all treatments involving CO_2 backpulsing, which suggests that

while the effect of CO_2 backpulsing on the initial flux varied depending on the backpulsing conditions, the use of this technique was able to minimize the flux drop in all cases.

The MF with the optimized backpulsing conditions (3 ports at 120s/1s) was compared with the control (without CO_2 backpulsing) and MF with 5 ports at 180s/1s for up to 3 h of processing time, both for "membrane 1" (Figure 3.4 and Figure 3.5) and membrane 2 (Figure 3.6 and Figure 3.7). As shown in Figure 3.4, both 3 ports at 120s/1s and 5 ports at 180s/1s resulted in significantly higher ($p < 0.05$) permeate flux after 3 h (30.02 ± 0.48 L/m^2h and 30.32 ± 0.99 L/m^2h, respectively) as compared to the control (25.85 ± 0.99 L/m^2h). However, there was no statistically significant difference between 3 ports at 120s/1s and 5 ports at 180s/1s. Three ports at 120s/1s also gave the highest relative permeate flux after 3 h (84.43 ± 6.09%), followed by 5 ports at 180s/1s (79.87 ± 4.31%) and control (70.17 ± 3.10%), as shown in Figure 3.5; the difference between 3 ports at 120s/1s and control was statistically significant whereas the difference between 5 ports at 180s/1s and control was not statistically significant. Similar results were obtained with membrane 2. Figure 3.6 depicts that 3 ports at 120s/1s using membrane 2 provided a significantly higher ($p < 0.1$) permeate flux after 3 h (33.03 ± 2.81 L/m^2h) as compared to the control (27.27 ± 2.86 L/m^2h). Three ports at 120s/1s using membrane 2 also maintained a higher ($p < 0.05$) relative permeate flux (85.77 ± 6.17%) as compared to the control (71.83 ± 1.78%) (Figure 3.7).

The amount of permeate collected from 3 ports at 120s/1s using membrane 2 was significantly higher ($p < 0.05$) (35.20 ± 2.99 kg) than the control for both membrane 1 and 2 (27.55 ± 1.06 kg and 29.06 ± 3.05 kg, respectively) (Figure 3.8). This again validates that the MF with 3 CO_2 backpulsing ports, at 120 s frequency with 1 s duration represents the optimum combination from those tested for improving flux in cold MF of skim milk.

The membrane separation process in the industry is always conducted for longer times. Predictions of the permeate flux after 12 h for the control and optimized MF have been done from the pseudo linear phase of the permeate flux (from 40 min to 180 min), using a linear regression. Using membrane 1, the predicted permeate flux after 12 h using 3 ports at 120s/1s was 22.90 ± 1.93 L/m^2h, while for the control was only 11.48 ± 2.08 L/m^2h (Figure 3.9a). For membrane 2, the predicted permeate flux after 12 h for 3 ports at 120s/1s was 20.97 ± 1.75 L/m^2h, whereas for the control was only 11.18 ± 3.69 L/m^2h (Figure 3.9b).

Effect of CO_2 Backpulsing on Milk pH

Table 3.1 shows the effect of CO_2 backpulsing on pH of milk for all 45 min MF experiments. After 45 min of milk processing, the average pH of permeate from all experiments that used CO_2 backpulsing was significantly lower (6.02 to 6.23) than the feed (6.73 to 6.78). The observed changes were most likely due to the dissolved CO_2 in the permeate at 6°C. The average pH of the permeate for CO_2 backpulsings with 3 ports at 120 s frequency with 1 s duration was 6.18 ±.0.00. The results indicated that the retentate pH was not affected by the CO_2 backpulsing. This was likely due to the fact that only very minute amount of permeate was backpulsed into the cross-flow channel. The same pH profiles were observed in the 3 h study as in the 45 min study (Table 3.2), which suggests that the longer processing time did not increase the amount of dissolved CO_2 in either the permeate or the retentate.

Effect of MF on Milk Composition

The composition of the MF milk after 3 h of cold microfiltration using membrane 1 is shown in Table 3.3. All permeate from the treatments showed very low Somatic Cell Count (SCC); most likely, the SCC in the permeate samples was just carryover of somatic cells in the instrument. Based on the pore size of the membrane, the permeate should have not contained any somatic cells. There was total transmission of lactose, which is soluble in milk (Table 3.3). The transmission of total solids and true protein into permeate was the highest for 3 ports at 120s/1s, followed by 5 ports at 180s/1s and control (Table 3.4). These results are in good agreement with the data reported by Fritsch and Moraru (2008), who showed a clear correlation between protein transmission and permeate flux, with protein transmission into permeate increasing with increasing permeate flux.

Verification Study of the Optimized CO_2 Backpulsing MF at a Different Cross-flow Velocity

The optimized CO_2 backpulsing system (3 backpulsing ports at 120 s frequency with 1 s duration) was also used in the skim milk MF at a higher cross-flow velocity (6 m/s). The permeate flux and relative permeate flux were also significantly greater than the control at this cross-flow velocity (Figure 3.10 and 3.11).

Figure 3.12 depicts the transmission of milk components in permeate between control and optimized MF with 3 ports at 120s/1s after 3 h, at 6 m/s. Both true protein and total solids transmission in permeate were higher in the optimized MF than in control (95.39 ± 0.44% and

98.31 ± 0.76% vs. 95.00 ± 0.44% and 96.88 ± 1.09%), although the differences are not statistically different.

In terms of somatic cells removal, permeate from both the control and the optimized MF had very low somatic cells, i.e. 0.50 x 1000/ mL and 0.67 x 1000/ mL, respectively, compared to their feed (39.67 x 1000/ mL and 26.33 x 1000/ mL, respectively) (Figure 3.13).

The efficiency of cold MF for the physical elimination of bacteria and spores from skim milk is depicted in Table 3.5. The raw skim milk used in this study had a microbial load ranging from 2.83 to 5.29 log cfu/ mL. After MF, no microorganisms were detected in the permeate in all trials. Spores were also fully eliminated from the feed by the cold microfiltration. These findings are consistent with the results reported by Fritsch and Moraru (2008), which found no bacteria and spores in the permeate of their study.

Lastly, 8 h runs of skim milk MF for both control and optimized MF were conducted. The results showed that the optimized CO2 backpulsing was able to consistently increase the permeate flux and reduce flux drop throughout the 8 h process (Figure 3.14 and 3.15).

CONCLUSIONS

This study found that the initial cleanliness of the membrane influences the performance of the membrane over a short period of time, but it does not have an influence on the flux at longer runs (at 3 h). CO_2 backpulsing using three ports at 120 s frequency with 1 s pulse duration was able to prevent a significant drop in the permeate flux at both low (3.8 m/s) and high (6.0 m/s) cross-flow velocity, indicating that backpulsing helped in reducing membrane fouling. The optimized conditions also gave the highest transmission of total solids and true protein into

permeate. However, the pH of the permeate was significantly decreased with the CO_2 backpulsing system. Nonetheless, this change in pH can be reversed by removing CO_2 from MF milk until a level similar to the levels found in untreated raw milk is reached, which can be achieved using vacuum.

The developed CO_2 backpulsing technique is a relatively simple and inexpensive method that can be used to significantly reduce membrane fouling and thus increase the permeate flux in cold MF of skim milk.

ACKNOWLEDGMENTS

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TABLES

Table 3.1. The effect of CO_2 backpulsing on pH of milk in 45 min of microfiltration

Stream	Treatment						
	Control	1 port 60 s/2 s	3 port 60 s/2 s	3 port 60 s/1 s	3 port 120 s/1 s	5 port 120 s/1 s	5 port 180 s/1 s
Feed	6.74 ± 0.02	6.75 ± 0.01	6.76 ± 0.03	6.73 ± 0.00	6.75 ± 0.03	6.78 ± 0.01	6.75 ± 0.00
Permeate	6.77 ± 0.02	6.02 ± 0.04	6.05 ± 0.07	6.02 ± 0.06	6.18 ± 0.00	6.13 ± 0.02	6.23 ± 0.05
Retentate	6.76 ± 0.03	6.76 ± 0.01	6.78 ± 0.05	6.75 ± 0.01	6.76 ± 0.03	6.81 ± 0.01	6.78 ± 0.01

Table 3.2. The effect of CO_2 backpulsing on pH of milk in 3 h runs

Membrane	Stream	Treatment		
		Control	3-port 120 s/ 1 s	5-port 180 s/ 1 s
1	Feed	6.73 ± 0.02	6.75 ± 0.03	6.74 ± 0.01
	Permeate	6.76 ± 0.02	6.10 ± 0.03	6.18 ± 0.01
	Retentate	6.76 ± 0.03	6.77 ± 0.04	6.77 ± 0.02
2	Feed	6.77 ± 0.02	6.78 ± 0.03	
	Permeate	6.80 ± 0.02	6.16 ± 0.05	
	Retentate	6.79 ± 0.02	6.79 ± 0.02	

Table 3.3. Milk composition after 3 h cold microfiltration of skim milk

Treatment	Stream	True Protein (%)	SCC x 1000	Lactose (%)	Total Solids (%)
Control	Feed	3.28	52	5.12	9.44
	Permeate	2.94	3	4.96	8.82
	Retentate	3.25	93	4.99	9.32
3 ports (120s/1s)	Feed	3.18	352	4.99	9.33
	Permeate	3.04	7	5.08	9.16
	Retentate	3.20	72	4.98	9.24
5 ports (180s/1s)	Feed	3.04	64	4.95	8.99
	Permeate	2.80	6	4.90	8.66
	Retentate	3.12	81	5.00	9.16

Table 3.4. The transmission of milk components in the permeate after 3 h cold microfiltration of skim milk

Treatment	Transmission of milk components in permeate (%)	
	Total Solids	True Protein
Control	93.43	89.63
3 ports (120s/1s)	98.18	95.60
5 ports (180s/1s)	96.33	92.11

Table 3.5. Reduction of microbiological load (vegetative bacteria and spores) in skim milk by cold microfiltration (cross-flow velocity = 6 m/s, samples were taken at the first hour of the 3 h MF processing)

Treatment	Replication	Stream	SPC (log cfu/mL)*	Spore Count (log cfu/mL)*
Control	1	Feed	3.42	<1
		Permeate	<1	<1
	2	Feed	3.00	1.60
		Permeate	<1	<1
	3	Feed	2.83	1.48
		Permeate	<1	<1
3 ports (120/1)	1	Feed	5.29	2.05
		Permeate	<1	<1
	2	Feed	3.41	1.48
		Permeate	<1	<1
	3	Feed	4.77	1.79
		Permeate	<1	<1

*Average value of two analytical replicates.

FIGURES

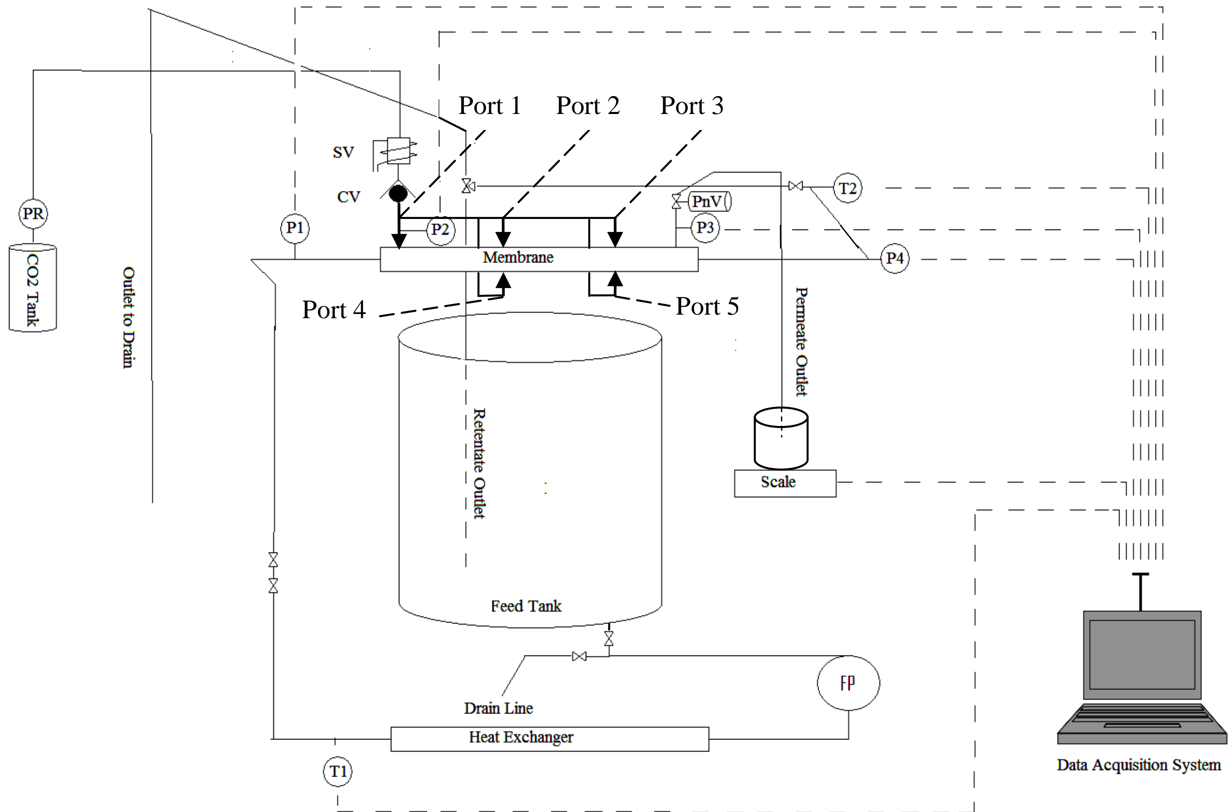
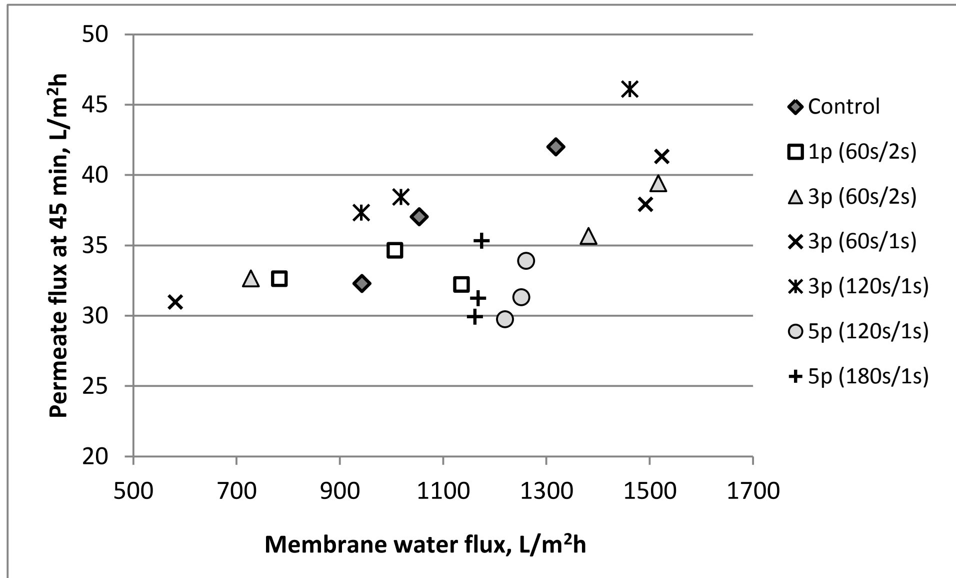


Figure 3.1. Schematic diagram of the microfiltration setup. (PR = pressure regulator; P1 = feed inlet pressure; P2 = CO_2 backpulsing pressure near inlet; P3 = CO_2 backpulsing pressure near outlet; P4 = retentate outlet pressure; T = thermocouple; FP = centrifugal pump; SV = solenoid valve; CV = control valve; PnV = pneumatics valve)

(a)



(b)

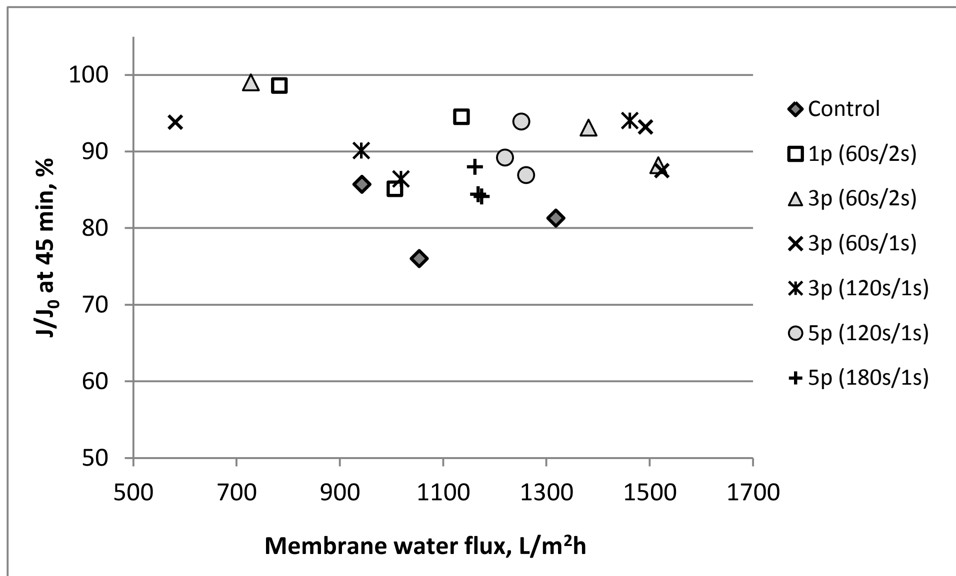


Figure 3.2. Effect of membrane water flux on (a) the permeate flux at 45 min and (b) the relative flux (J/J_0) after 45 min of MF in cold MF of skim milk, with and without CO_2 backpulsing. Data points represent individual MF experiments.

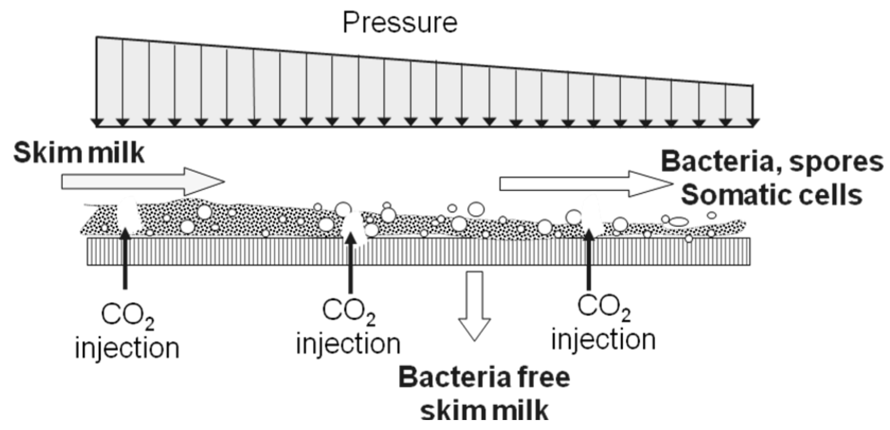


Figure 3.3. Principle of CO_2 backpulsing.

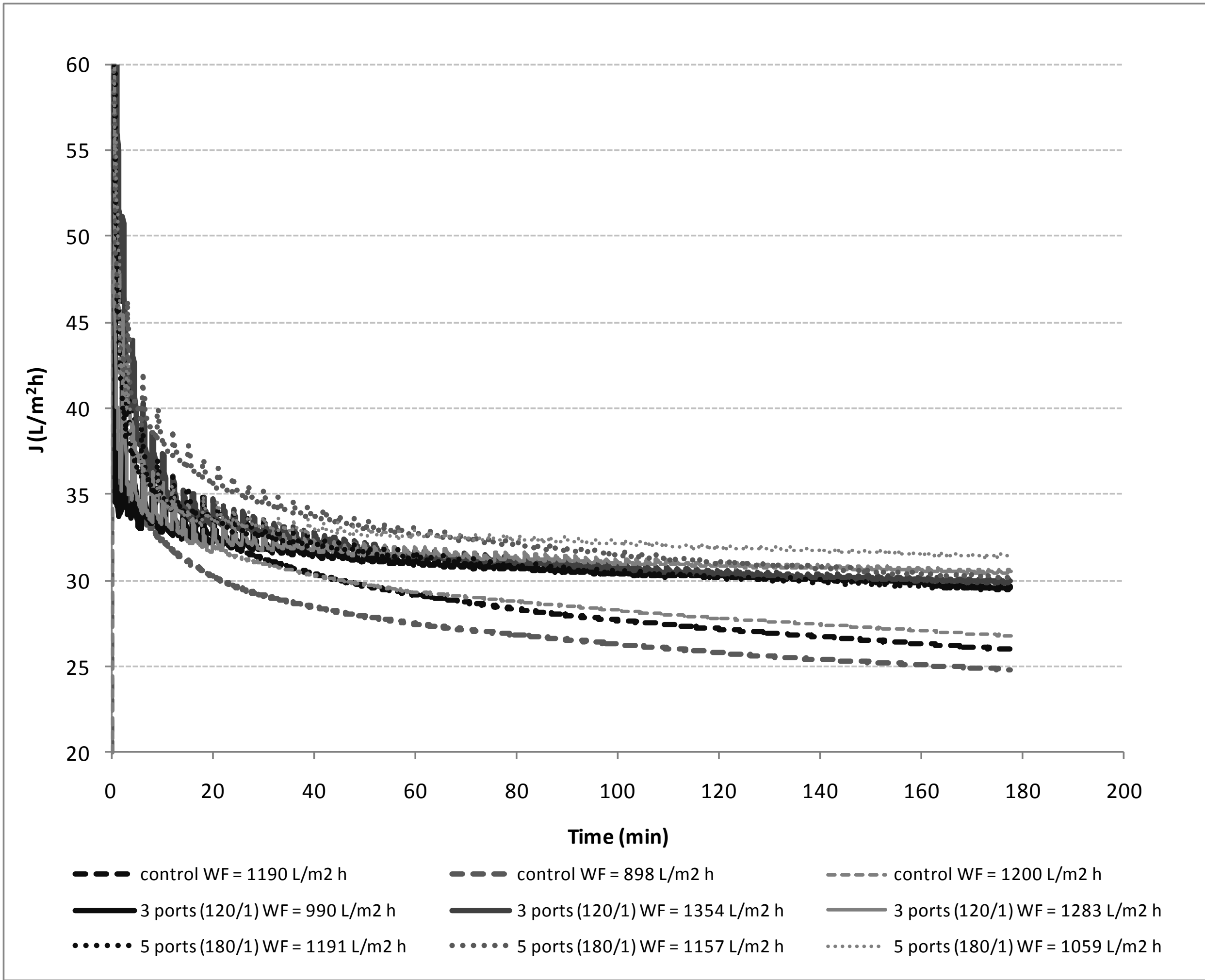


Figure 3.4. Comparison of permeate flux between control, optimized MF and 5 ports (180s/1s) in 3 h runs using membrane 1.

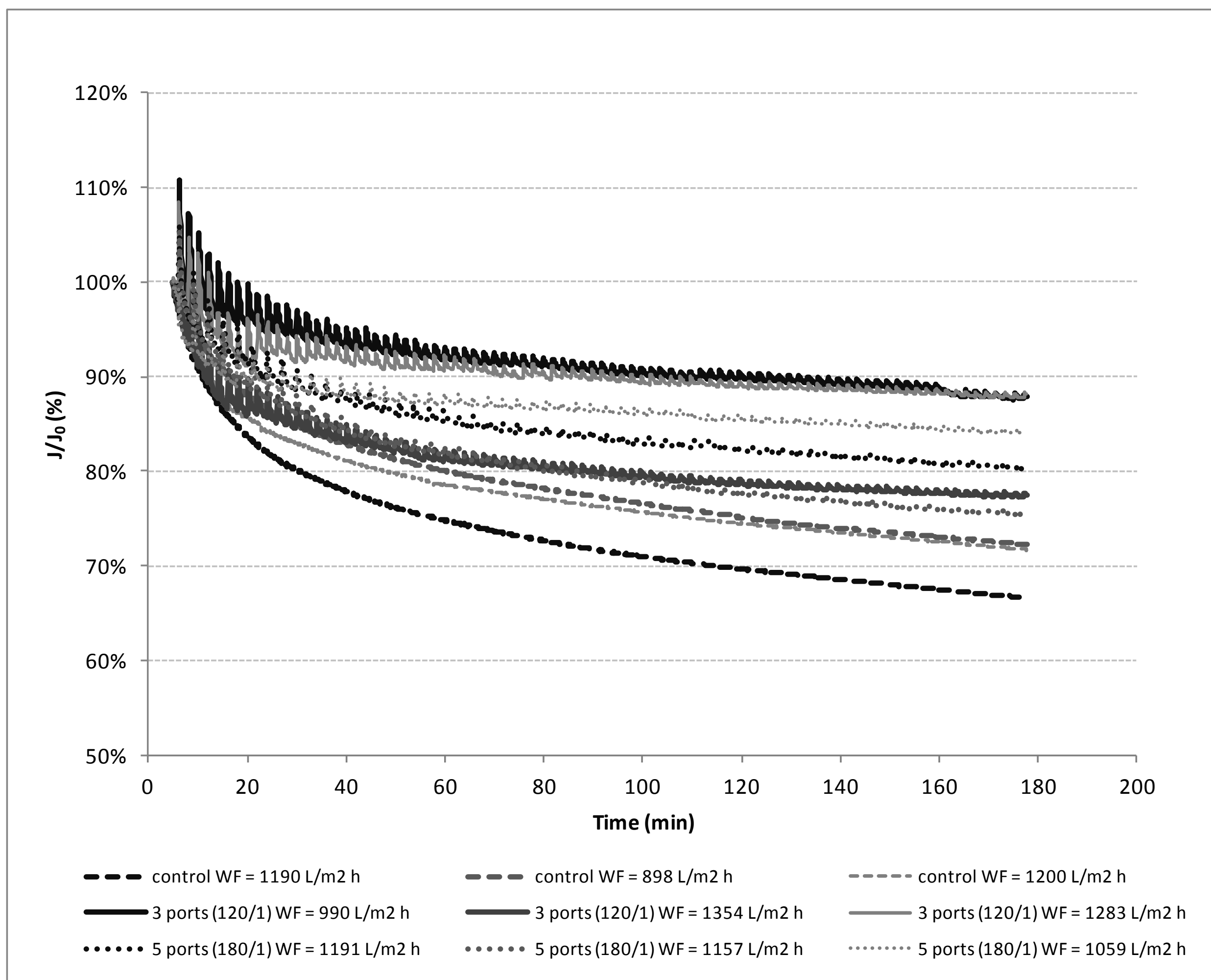


Figure 3.5. Comparison of relative permeate flux between control, optimized MF and 5 ports (180s/1s) in 3 h runs using membrane 1.

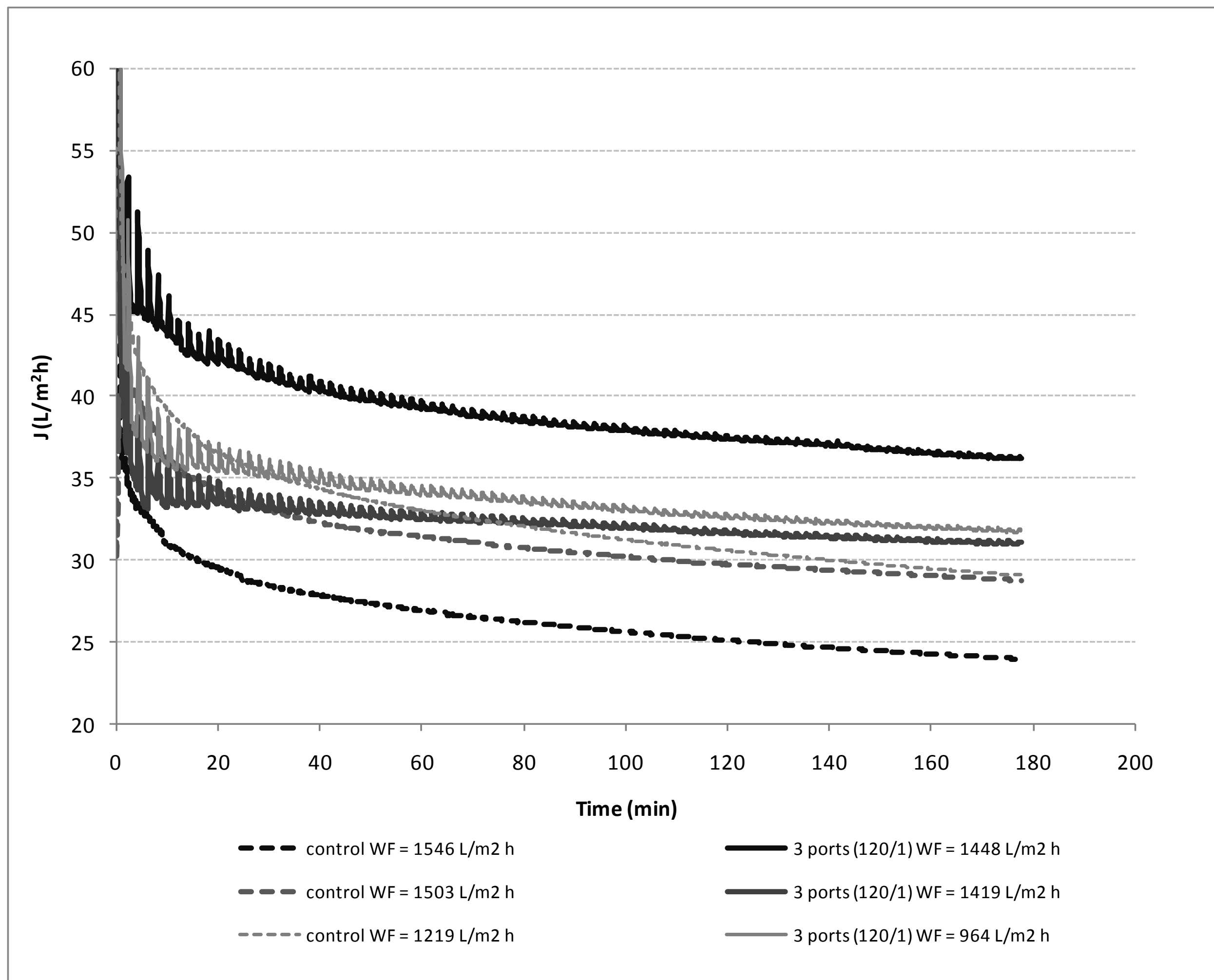


Figure 3.6. Comparison of permeate flux between control and optimized MF in 3 h runs using membrane 2.

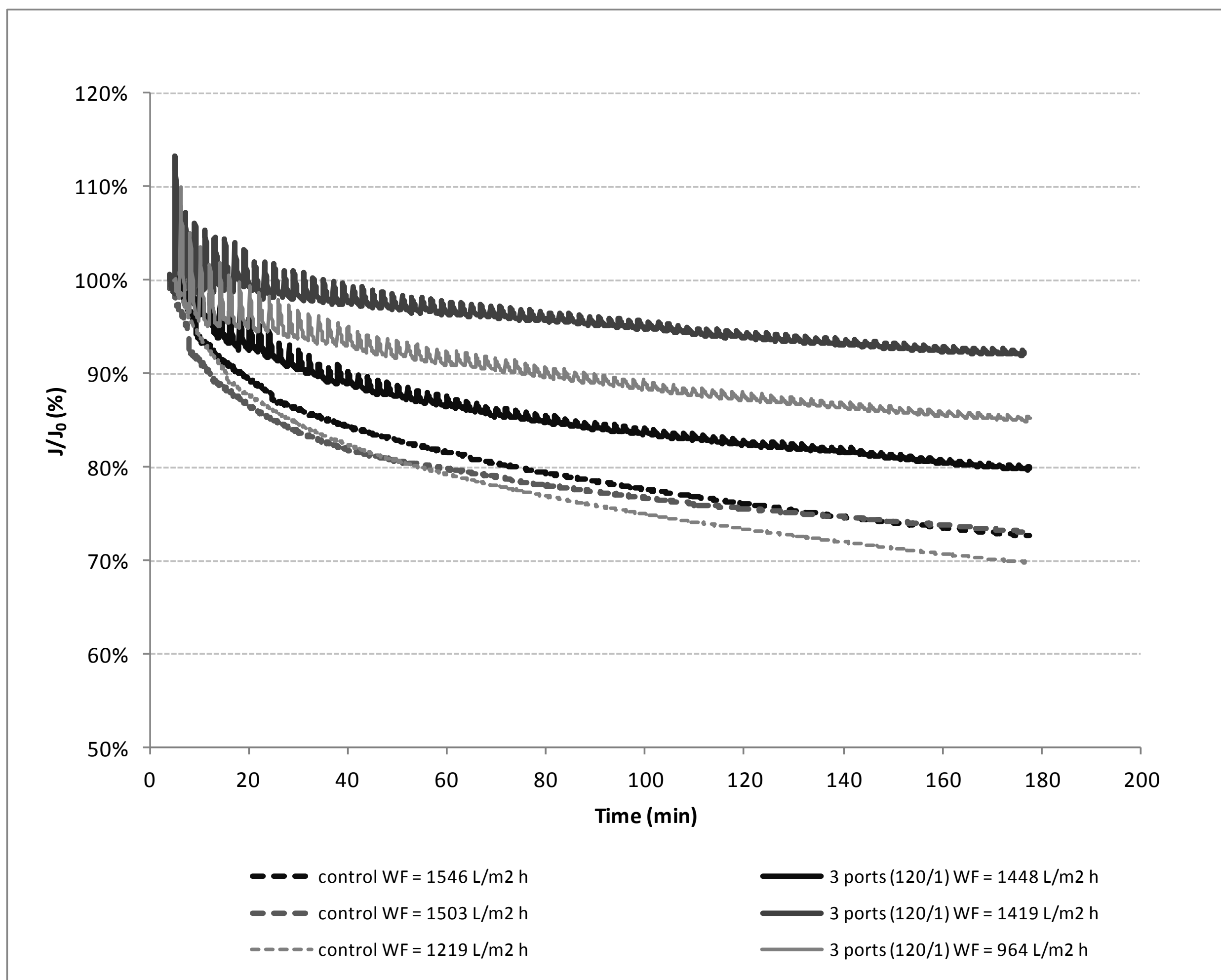


Figure 3.7. Comparison of relative permeate flux between control and optimized MF in 3 h runs using membrane 2.

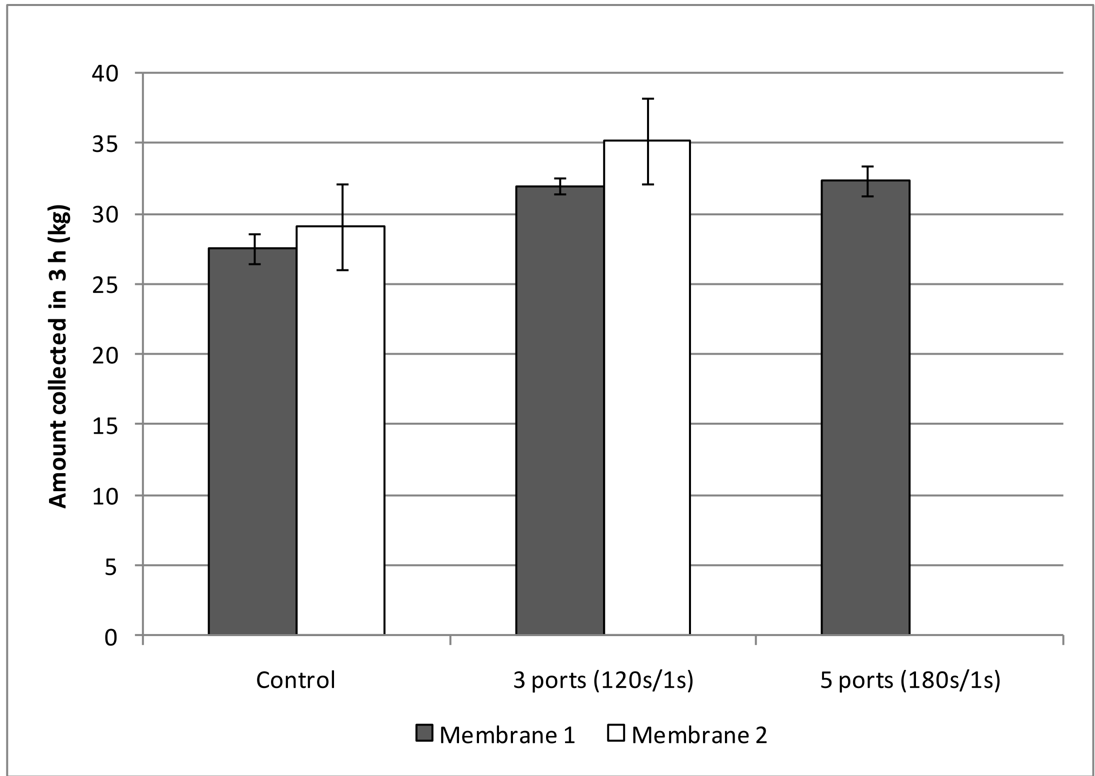
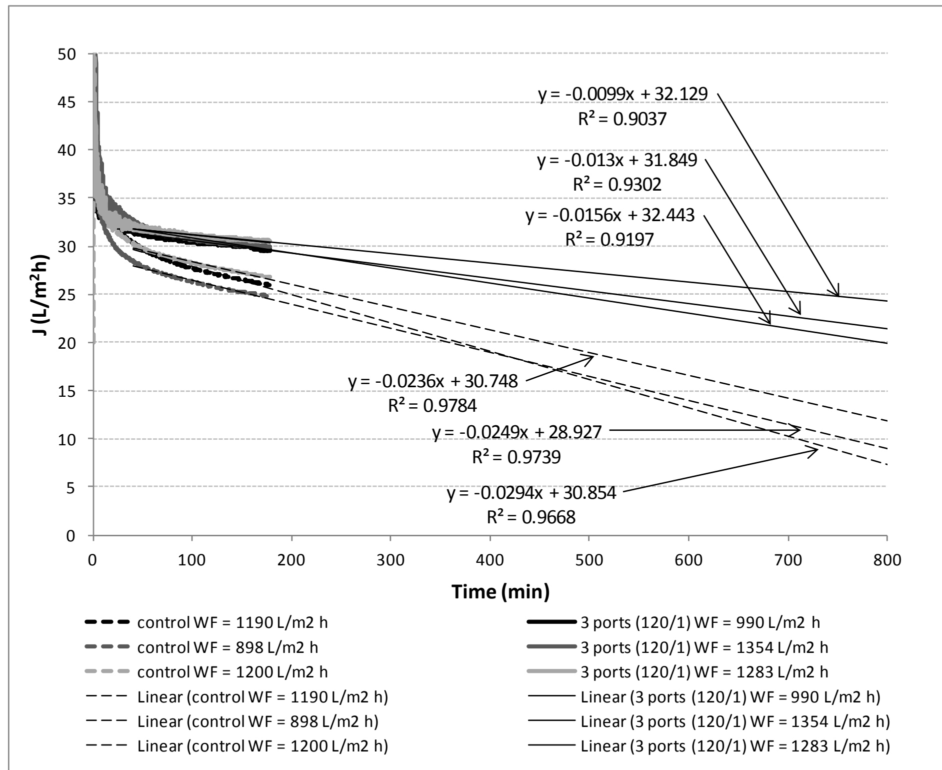


Figure 3.8. Comparison of amount of permeate collected in 3 h between control, optimized MF and 5 ports (180s/1s) using membrane 1 and 2.

(a)



(b)

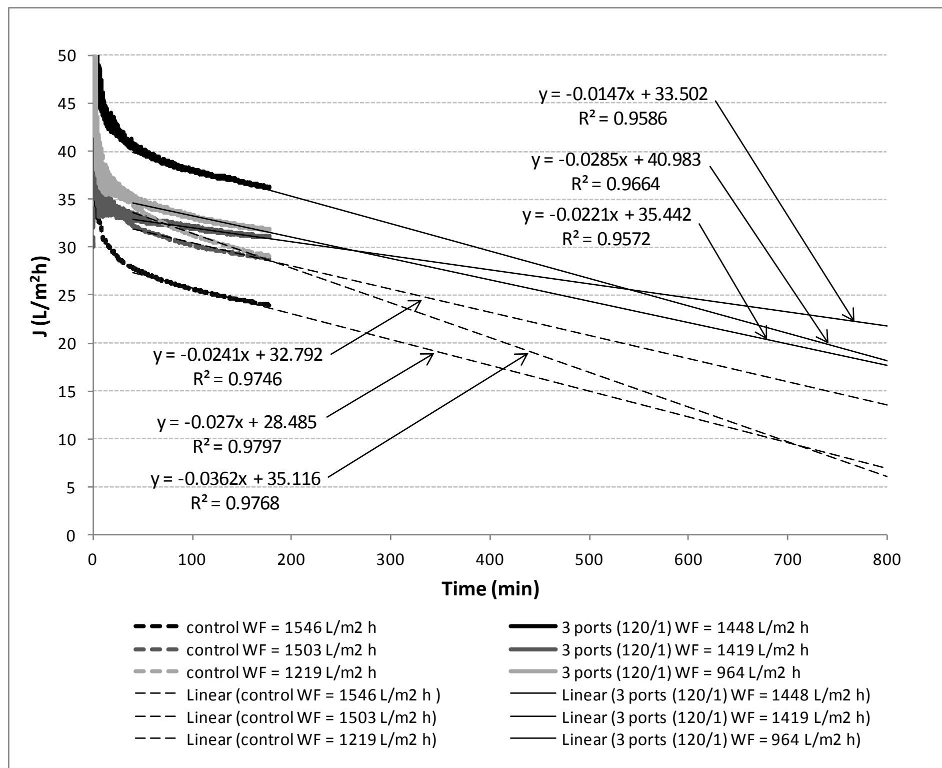


Figure 3.9. Prediction of permeate flux after 12 h in control and optimized MF runs using (a) membrane 1 and (b) membrane 2.

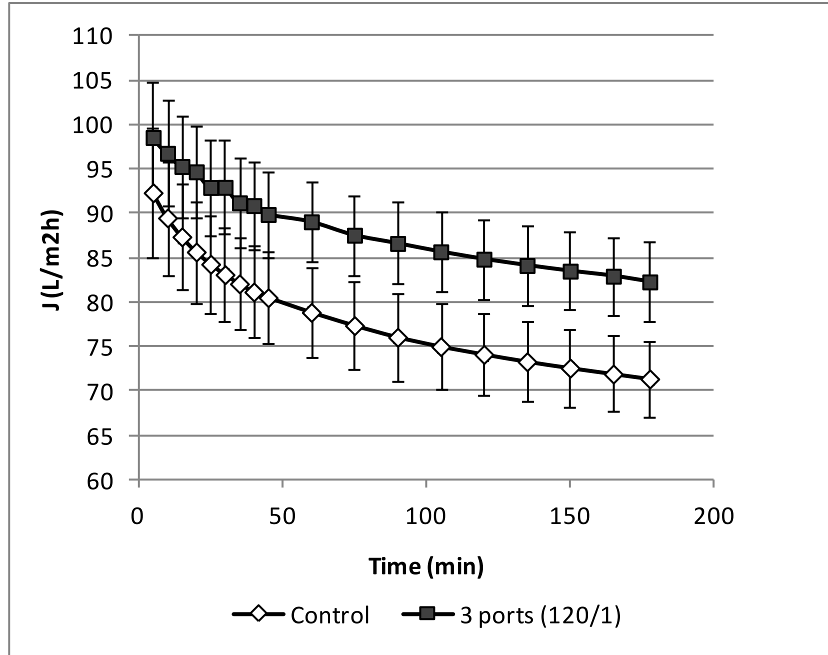


Figure 3.10. Comparison of permeate flux between control and optimized MF at cross-flow velocity of 6 m/s in 3 h runs.

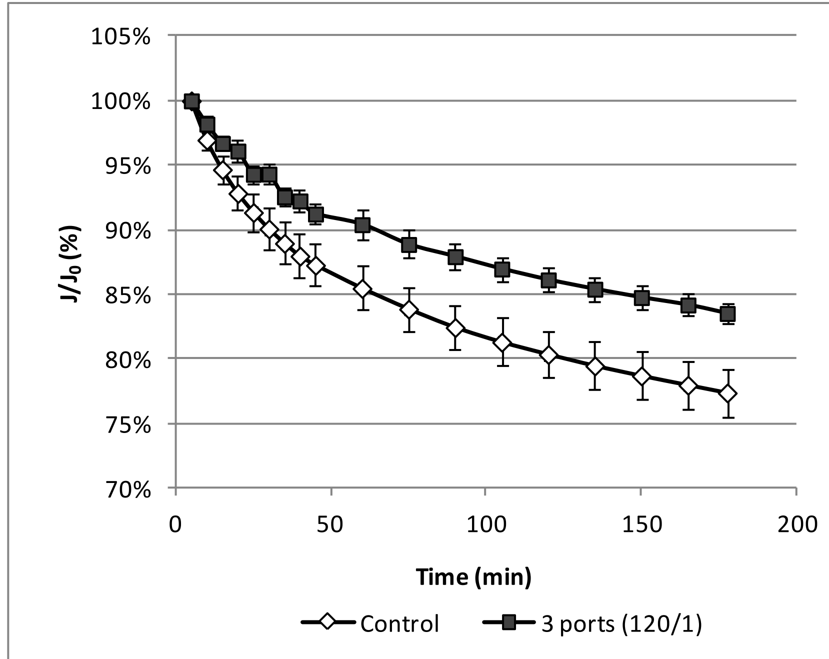


Figure 3.11. Comparison of relative permeate flux between control and optimized MF at cross-flow velocity of 6 m/s in 3 h runs.

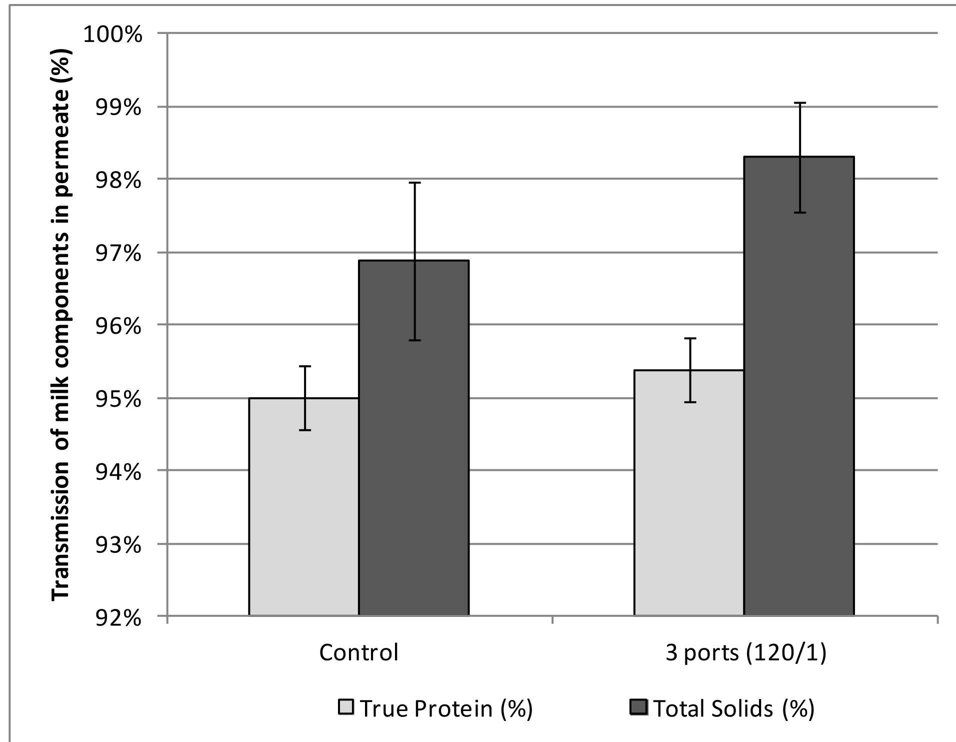


Figure 3.12. The transmission of milk components in the permeate between control and optimized MF after 3 h cold microfiltration of skim milk (cross-flow velocity = 6 m/s).

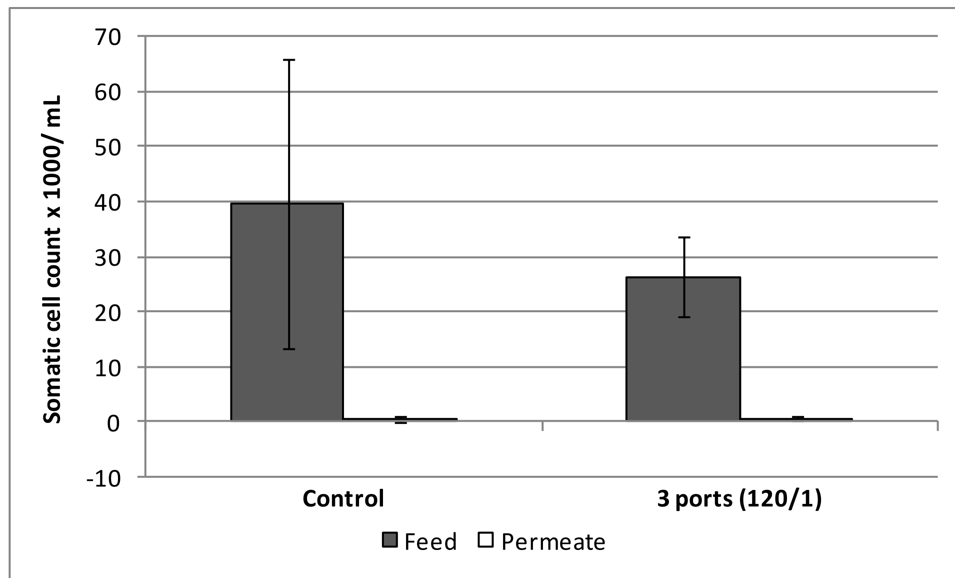


Figure 3.13. Effect of 3 h cold microfiltration between control and optimized MF on the SCC of skim milk.

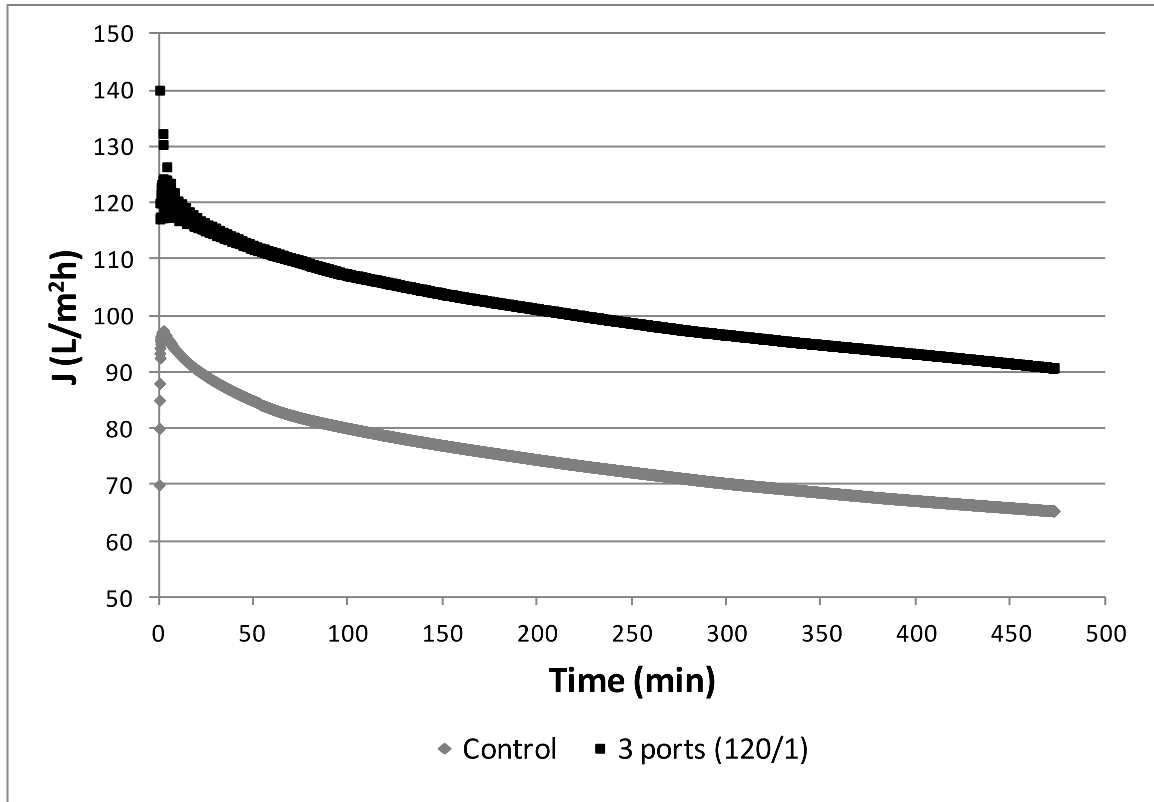


Figure 3.14. Permeate flux for control and optimized MF at cross-flow velocity of 6 m/s in 8 h runs.

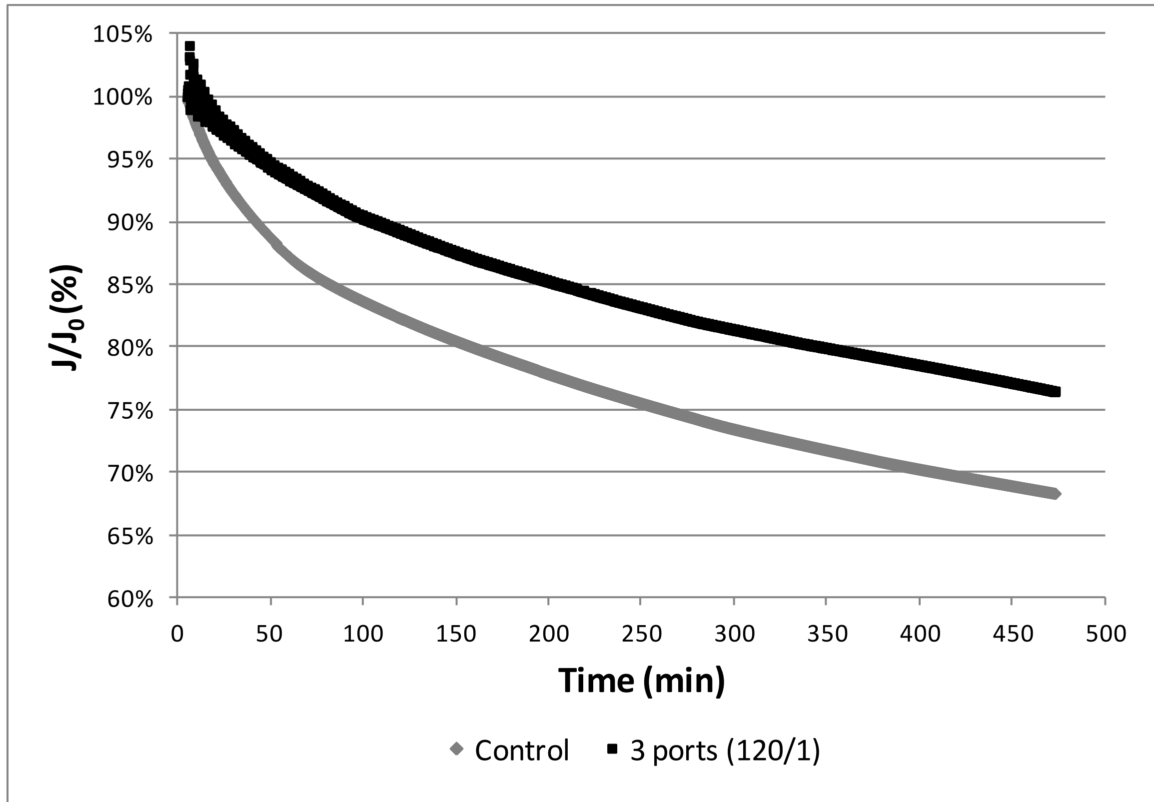


Figure 3.15. Relative permeate flux for control and optimized MF at cross-flow velocity of 6 m/s in 8 h runs.

CHAPTER 4

INVESTIGATION OF THE MECHANISMS OF MEMBRANE FOULING IN COLD MICROFILTRATION OF SKIM MILK

ABSTRACT

The main challenge in milk microfiltration (MF) is membrane fouling, which leads to a significant decline in permeate flux over time. This work aims to elucidate the mechanisms of membrane fouling in cold MF of skim milk, by identifying and quantifying the proteins and minerals involved in external and internal membrane fouling. The foulants were evaluated both after a brief contact between the membrane and milk, in order to evaluate instantaneous adsorption of foulants, and after MF – both without and with CO_2 backpulsing. . Skim milk was subjected to MF using a 1.4 μm ceramic membrane, at a temperature of 6 ± 1°C, cross-flow velocity of 6 m/s and transmembrane pressure of 159 kPa, for 90 min. Four foulant streams were collected: weakly attached external foulants (W_e), weakly attached internal foulants (W_i), strongly attached external foulants (S_e), and strongly attached internal foulants (S_i). Liquid chromatography coupled with tandem mass spectrometry analysis showed that all major milk proteins were present in all foulant streams. In the adsorption study, the α-lactalbumin level in W_e was higher than in milk, which indicates an affinity of this protein for the membrane material. The serum proteins α-lactalbumin and bovine serum albumin (BSA) were found in a higher proportion in the "weakly attached" fractions (W_e and W_i) from the adsorption study as compared to the control MF (without CO_2 backpulsing). This suggests that caseins were mostly

introduced into the fouling layer when transmembrane pressure was applied. Higher levels of κ-casein and lactotransferrin were found in S_i in CO_2 backpulsing MF as compared to control MF. BSA was significantly higher in W_i from CO_2 backpulsing MF as compared to control MF. Since these are minor proteins in milk, despite their higher ratio in the foulants they are not expected to have played a major role in fouling. More significantly, CO_2 backpulsing reduced the total protein concentration in W_e, with 52.98 ± 4.87 μg/mL for CO_2 backpulsing MF as compared to 62.20 ± 10.13 μg/mL for the control MF. It is also important to notice that casein concentration did not increase in the foulants from CO_2 backpulsing MF, despite the localized decrease in pH caused by the contact with CO_2. The concentration of minerals was very small in all foulant streams, below 2.5 ppm, and likely they do not contribute significantly to membrane fouling in cold MF. The knowledge generated in this study could be used to identify solutions to minimize membrane fouling and increase the efficiency of milk MF.

INTRODUCTION

Microfiltration (MF) has gained significant acceptance as a processing method for the removal of microorganisms from skim milk in recent years. Bacterial spores and somatic cells in milk are not affected by the standard heat treatment used in dairy processing plants, whereas they can be physically removed by MF. If not removed, bacterial spores can compromise the quality and shelf life of milk and other dairy products. In cheesemaking, thermophilic bacterial spores are of particular concern as they can cause late blowing defect in cheese (Gesan-Guiziou, 2010). High somatic cell counts can also lead to increased proteolytic and lipolytic activity in milk, thus compromising the flavor, texture, and shelf life of dairy foods (Azzara and Dimick, 1985; Verdi

and Barbano, 1988; Ma et al., 2000; Te Giffel and Van der Horst, 2004). In addition, MF reduces the use of heat treatment, which is beneficial in minimizing the heat-induced changes in milk, such as cooked flavor (cabbagy or boiled) (Lewis, 2003) and age gelation (Datta and Deeth, 2001) resulted from ultra high temperature (UHT) processing. Furthermore, UHT induces changes such as lactosylation and protein cross-linking between β-lactoglobulin and kappa-casein, with the extent of changes increase with elevated storage temperature. These changes can have adverse effects on the nutritional and functional properties of milk proteins (Holland et al., 2011b).

Microfiltration processes using 1.4 μm pores membranes has been shown to be very efficient in removal of bacteria, spores, and somatic cells from skim milk, while allowing almost complete permeation of other milk components (Saboya and Maubois, 2000; Te Giffel and Van der Horst, 2004; Fritsch and Moraru, 2008; Tan and Moraru, 2013). Shelf life of such MF milk stored under refrigerated conditions was reported to be 15 days (Saboya and Maubois, 2000).

The main problem in microfiltration is membrane fouling, which leads to a significant decline in the permeate flux. Membrane fouling is due to the specific physical or physicochemical interactions between the various solutes/ particles and the membrane. Fouling occurs because of the deposition of rejected particles on the membrane surface, referred to as external fouling, and to the constriction of pores by small particles, referred to as internal fouling. Membrane fouling consequently results in irreversible changes in membrane permeability and selectivity, which leads to a significant decline in both permeate flux and separation efficiency over time (Guerra et al., 1997).

The typical mechanisms of membrane fouling related to microfiltration include pore constriction, pore blocking and gel/ cake formation. Membrane fouling might be caused by a

combination of different mechanisms as a result of different size distribution of species in the feed. Particles with diameters much smaller than the membrane pore may cause pore constriction (internal fouling). Particles with a diameter equal to the membrane pore may cause pore blocking (internal/ external fouling). If the particles have diameters greater than the membrane pore, they will be retained on the membrane surface and cause cake formation (external fouling) (Fane and Chang, 2009).

In the membrane separation of dairy streams, milk proteins are typically involved in fouling. Using a polysulfone membrane with molecular weight cutoff of 10,000, Tong et al. (1988) found that flux decline is severe in the early stages of whole milk ultrafiltration (UF) and is associated with irreversible adsorption of milk proteins on the membrane surface. Their results showed that whey proteins (β-lactoglobulin and α-lactalbumin) constituted 95% of the membrane foulants and very little casein was identified as a membrane foulant. Membrane fouling during MF and UF of skim milk was also studied by James et al. (2003) using scanning electron microscopy (SEM), atomic force microscopy (AFM) and X-ray photoelectron spectroscopy. By examining the cross-sections of fouled polymeric membranes (MF membrane with pore size of 3 μm and UF membrane with molecular weight cutoff of 3500), James et al. (2003) found that the protein particles interacted with the pore walls of the membrane (caused by protein-polymer interactions), and formed agglomerates (as a result of protein-protein interactions) leading to narrowing and ultimately blocking of the pores. This mechanism tallies with the initial sharp decline in permeate flux. However, they did not identify the milk proteins that caused the fouling.

Using dynamic light scattering (DLS) measurements, Mourouzidis-Mourouzis and Karabelas (2006) reported that whey protein aggregates are responsible for the membrane

fouling in MF of whey protein isolate solution with ceramic membrane of pore size 0.8 µm. Wang (2008) also evaluated the external fouling in cold microfiltration of skim milk using DLS and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The particle size and SDS-PAGE analyses suggested that milk proteins, especially casein micelles, are the major components of the fouling layer on the membrane surface. However, κ-casein is difficult to be identified by SDS-PAGE due to its carbohydrate moiety prevents it from taking up stain (Wake and Baldwin, 1961). Thus the electrophoresis analyses might lead to an underestimation of κ-casein as a foulant, and hence the results were not conclusive. The precise protein compositions of both external and internal fouling need to be identified in order to fully explain the fouling mechanisms.

Microbiological fouling may also be one of the factors of flux decline in membrane processes in the dairy industry. Microfiltration of milk is usually operated under warm conditions, at temperatures ranging from 50 to 55°C. This favors the growth of thermophilic bacteria and germination of spores inside the membrane and the recirculation loop of the MF system.

Brans et al. (2004) proposed that one of the fouling mechanisms in the MF of skim milk for bacteria removal is complete pore blocking by bacteria and spores. However, Fritsch (2006) reported that bacteria and spores were not observed in the SEM images of the fouled ceramic membrane (pore size of 1.4 µm) after cold MF of skim milk. This might be due to the high cross-flow velocity (7 m/s) employed in the MF study that successfully prevented the deposition of microorganisms on the membrane. Nevertheless, in a study to investigate fouling mechanism relevant to adhesion of *Bacillus cereus* spores on a 0.45 µm tubular ceramic membrane, isolated or scattered spores and some clusters comprising dozens of cells were found adhered to the

membrane surface after filtering the spore suspension at a cross-flow velocity of 4 m/s (Blanpain-Avet et al., 2011).

In an attempt to mitigate the flux decline in MF of skim milk, Fritsch and Moraru (2008) developed and later Tan and Moraru (2013) optimized a CO_2 backpulsing method. The optimized system was comprised of multiple CO_2 injection ports that significantly increased the permeate flux and protein transmission in the cold microfiltration of skim milk (Tan and Moraru, 2013). However, the effect of CO_2 backpulsing on membrane fouling has yet to be evaluated.

To our knowledge, the fouling mechanisms in microfiltration for microbial removal of skim milk have not yet been fully elucidated. With regard to the important implication of membrane fouling in microfiltration, there is a definite need to understand the fouling mechanism, as this could provide insight to develop more effective solutions that will control fouling, thus improving the performance of the MF process both with respect to flux and separation efficiency.

This work aimed to understand the mechanism of membrane fouling in cold microfiltration for microbial removal of skim milk, and to evaluate the effect of a previously developed CO_2 backpulsing method on membrane fouling. The specific objectives were to: 1) identify and quantify the foulants involved in the external and internal fouling in adsorption study and cold milk MF, with a focus on milk proteins and minerals, and 2) evaluate the effect of CO_2 backpulsing on the external and internal fouling in cold MF of skim milk.

MATERIALS AND METHODS

MF Experiments: Control and CO_2 Backpulsing MF

The pilot-scale experimental MF unit consisted of a 189 L feed tank connected to a variable-speed centrifugal pump, a tubular heat exchanger, a flow meter and a tubular ceramic membrane of Tami design (GEA Fitration, WI) placed inside a stainless steel housing. The membrane had a nominal pore size of 1.4 μm, length of 1,200 mm, outside diameter of 25 mm, 23 internal channels each with a hydraulic diameter of 3.5 mm, and total membrane area of 0.35 m^2. A data acquisition port was used for collecting of the pressure, temperature, and flow rate data. The permeate flux data was obtained gravimetrically using an electronic scale that also connected to the data acquisition system. Three portable CO_2 injection ports (equally spaced along the membrane) were installed on the membrane housing for the MF with CO_2 backpulsing system. The CO_2 gas used was beverage grade.

The cold, raw skim milk was obtained from the Cornell Dairy Plant (Ithaca, NY). The cleanliness of the membrane was determined before each MF of milk, by measuring the water flux. The water flux was measured for 3 min using reverse osmosis (RO) water at the following conditions: 20°C, an average TMP of approximately 83 kPa (12psi).

A total of 117 kg of raw skim milk were added in the feed tank and the pump was turned on to a low velocity for 20 seconds to flush out any water that remained in the membrane system after water flux measurement. After another 70 seconds, permeate flux data collection began when the pump speed was adjusted to reach a cross-flow velocity (v) of 6.0 m/s and a constant transmembrane pressure of about 159 kPa (23psi). The MF process was conducted at a

temperature of 6 ± 1°C, by passing the milk through a countercurrent tubular heat exchanger using chilled water as the cooling medium. For every 20 kg of permeate collected, 20 kg of raw skim milk were added back into the feed tank to ensure a certain milk level in the tank. The duration of each microfiltration run was 90 min.

The control MF was carried out without the CO_2 backpulsing system. When CO_2 backpulsing was used, backpulsing began 5 seconds after permeate collection started. The CO_2 pressure was set to 3-4 psi higher than the pressure at gauge P_1.

Procedures for Collecting Membrane Foulants

Immediately after the MF experiment, the milk remaining in the feed tank was drained and the MF unit was flushed with RO water at 6 ± 1°C for 15 seconds to wash out the residue milk in the system. The membrane was then taken out of the MF unit and positioned vertically for 10 min to drain out the residual mixture of milk and RO water in the membrane. The MF unit was reassembled without the membrane, and it was cleaned with approximately 180 L of RO water for 2 min at 20°C, at a pump speed of 1710 rpm. After that, the membrane was installed back into the unit. Any foulants collected from the rinsing solution from this point were assumed to be attached to the membrane.

RO Water Rinsing. RO water rinsing was carried out to first collect the weakly attached external foulants (W_e) and then weakly attached internal foulants (W_i). This ensured that all the weakly attached foulants on the membrane surface had been removed and separately collected before collecting the weakly attached foulants within the internal pore structure. Twenty kg of RO water were added to the feed tank. RO water rinsing was conducted with the permeate outlet

fully closed and the retentate outlet fully opened to collect the external foulants that weakly attached on the membrane surface. The RO water rinsing was performed for 5 min at 20°C, at a pump speed of 1710 rpm that gave a cross-flow velocity of 3.1 m/s (flow rate of 10.8 gallons per minute [GPM]). The weakly attached external foulants were collected and the remaining rinsing solution was drained. Then the membrane was flushed with RO water again at 20°C for 15 seconds to wash out the residual rinsing solution. Afterward, the membrane was taken out from the MF unit and positioned vertically for 10 min to drain out the residual RO water in the membrane. The MF unit was reassembled without the membrane, and cleaned with RO water for 2 min at 20°C, at a pump speed of 1710 rpm. After that, the membrane was installed back into the unit.

Another 20 kg of RO water were added to the feed tank. RO water rinsing was conducted with the permeate outlet fully opened and the retentate outlet fully closed to collect the internal foulants that weakly attached in the membrane pores. The RO water rinsing was performed for 5 min at 20°C, at a pump speed of 1710 rpm, which gave a flow rate of 0.5 GPM (there was no cross-flow in this setting). The weakly attached internal foulants sample was collected and the remaining rinsing solution was drained. Next, the membrane was flushed with RO water again at 20°C for 15 seconds to wash out the residual rinsing solution. After that, the membrane was taken out from the MF unit and positioned vertically for 10 min to drain out the residual RO water in the membrane. The MF unit was reassembled without the membrane, and cleaned with RO water for 2 min at 20°C, at a pump speed of 1710 rpm. After that, the membrane was reinstalled into the unit.

Pressurized Hot Water Extraction. To extract the external and internal foulants that strongly attached to the membrane, the same procedures as described above were employed,

using 30 kg of pressurized hot water for strongly attached external foulants (S_e) collection and 20 kg of pressurized hot water for strongly attached internal foulants (S_i) collection. Each pressurized hot water extraction was carried out for 10 min at 70°C, at a pump speed of 3000 rpm, which gave a cross-flow velocity of 6.1 m/s (flow rate of 21.5 GPM) for collecting external foulants, and a flow rate of 2.6 GPM for collecting internal foulants. The schematic of the membrane foulants location is shown in Figure 4.1.

Adsorption Study

A study was also carried out to evaluate instantaneous adsorption of foulants to the membrane before transmembrane pressure (TMP) was applied. A tubular ceramic membrane of Tami design (GEA Fitration, WI) with a nominal pore size of 1.4 µm and total membrane area of 0.35 m^2 was wrapped with two layers of parafilm to seal the permeate side of the membrane. The sealed membrane was placed inside a heavy duty poly tubing (Uline, WI). The membrane was submersed in 1 L of raw skim milk at 6°C for 5 min. After 5 min, the membrane was taken out from the poly tubing and rinsed with 1 L of RO water vertically, from the membrane inlet to the outlet. A second rinse was performed from membrane outlet to inlet. After that, the parafilm was removed from the membrane. The membrane was positioned vertically for 5 min to drain out the residual rinsing solution.

The drained membrane was placed inside a new poly tubing bag and submersed in 1 L of RO water. After 10 min, the membrane was taken out from the poly tubing and positioned vertically for 5 min to drain out the rinsing solution. Any foulants that remained on the membrane at this point were assumed to be attached to membrane due to adsorption.

The procedures for collecting membrane foulants outlined above were also used to collect the adsorption foulants, starting from RO water rinsing.

Chemical Cleaning of the Membrane

After each use, a complete chemical cleaning cycle of the membrane was carried out. The cleaning procedure consisted of a RO water rinse for 10 min, followed by alkaline cleaning with Ultrasil-25 at a concentration of 16 mL/L at 80°C for 30 minutes, a second RO water rinse for 10 minutes or until neutrality (checked with pH strips), acid cleaning with HNO_3 solution at a concentration of 5 mL/L at 50°C for 20 minutes, and a third RO water rinse for 10 minutes or until neutrality.

The effectiveness of cleaning and change in the membrane performance with time was measured by determining the water flux as described above. At the end of each experiment, the membrane housing was disassembled and the membrane was taken out, air dried and then stored in a box.

Foulant Analyses

Quantification of Proteins using the Bradford Method. The collected external and internal foulants were quantified for protein content using micro assay of Bradford method. Bradford method is a rapid, reproducible, and sensitive method that able to measure microgram quantities of proteins. It was chosen because the amount of protein in the foulants was too low to be measured by Kjeldahl method. Bradford method is based on the principle that Coomassie

Brilliant Blue G-250 changes color from reddish to bluish when it binds to protein, and the absorption maximum of the dye is shifted from 465 to 595 nm. The change in the absorbance at 595 nm is proportional to the protein concentration in the sample.

One mL of samples containing foulants and 1 mL of protein standard of known concentration were mixed with 1 mL of the Bradford reagent. Absorbance at 595 nm was read against a reagent blank. The protein content in the sample was estimated from the standard curve.

In order to quantify the proteins correctly, the protein standard for Bradford method must be selected carefully. To determine the best protein to be used as standard, 3 different protein standards i.e. bovine serum albumin (BSA), α-casein and β-lactoglobulin (Sigma-Aldrich Co., St. Louis, MO) had been tested. The Bradford method was further calibrated using the Kjeldahl method to select the ideal standard.

Protein Identification and Relative Quantification by Proteomics. High performance liquid chromatography (HPLC) was used to separate the interferences from the sample and mass spectrometer was used to ionize molecules and subsequently separate and identify them according to their mass-to-charge ratio (*m/z*). Protein sequencing and identification was conducted by tandem mass spectrometry (MS/MS), using a four-step process: protein digestion, protein separation, MS analysis of the digest fragments (most commonly tryptic peptides) followed by matching the observed peptides to those in a protein database (O'Donnell et al., 2004). Prior to LC-MS/MS analysis, the collected foulant samples were concentrated by freeze drying (FreeZone 4.5 freeze dry system, Labconco, MO). The freeze dried foulants were dissolved in 1.0 mL of 3 M guanidine hydrochloride. The samples were used for protein identification and relative quantification of each individual protein using LC-MS/MS at the

Proteomics and Mass Spectrometry Core Facility at Cornell University (Ithaca, NY), using the procedure described by Sauer and Moraru (2012).

Identification and Quantification of Mineral Composition. The mineral composition of the foulants was analyzed at Dairy One Forage Analysis Laboratory (Ithaca, NY). Calcium, phosphorus, magnesium, potassium, and sodium were identified and quantified using Inductively Coupled Plasma (ICP) Radial Spectrophotometer while chlorine was identified and quantified by potentiometric titration. All mineral analyses for foulants from each MF experiment and adsorption study were performed in triplicate.

Statistical Analysis

All experiments and analyses were carried out in triplicate. In order to determine statistical differences between treatments, data was analyzed using analysis of variance (ANOVA) with the statistical software package JMP Pro 10.0.0 (SAS Institute Inc., Cary, NC). The Tukey-Kramer honestly significant difference (HSD) test was used to determine significant differences between means at 5% level of probability.

RESULTS AND DISCUSSION

Identificaiton of the Protein Standard for Bradford Method

The protein standard for Bradford method must be chosen carefully to quantify the protein concentration of a sample correctly. The Coomassie Brilliant Blue used by this method binds

specifically to the arginine residue. The amino acid composition of different proteins may lead to different absorbance-concentration curves, which gives significant protein-to-protein variation when using the Bradford method. The best protein to use as a standard is a purified preparation of the protein being assayed. In the absence of such an absolute reference protein, one must select another protein as a standard. The best standard to use is one which has similar properties to, and which gives a color yield similar or close to that of the protein(s) being assayed.

The absorbance-concentration curves of the 3 protein standards are shown in Figure 4.2. As expected, the 3 different protein standards have different linear equations in the absorbance-concentration curves with high R^2 values.

The Bradford method was calibrated with the Kjeldahl method using evaporated milk with different concentrations as samples. The calibration showed that β-lactoglobulin was the ideal standard as it provided the most accurate protein concentration among the three standards, with y = 1.13x (see Figure 4.3). Using BSA as standard may underestimate the protein concentration, while using α-casein as standard may overestimate the protein concentration in the sample. Hence, the protein in all foulant samples was quantified by Bradford method using β-lactoglobulin as a standard.

Protein Quantification in the Foulant Streams from Adsorption and Control MF

The results from protein quantification in the foulant streams from the adsorption study demonstrate that some foulants instantaneously attached to the membrane material, externally and internally, just by contact with milk, without MF (Figure 4.4). The protein concentration for weakly attached external foulants (W_e), weakly attached internal foulants (W_i), strongly attached

external foulants (S_e), and strongly attached internal foulants (S_i) from adsorption were 8.85 ± 1.95 μg/mL, 3.67 ± 1.48 μg/mL, 1.49 ± 0.21 μg/mL, and 1.15 ± 0.58 μg/mL. The amount of protein per membrane area for W_e, W_i, S_e, and S_i in the absence of concentration polarization were 0.51 ± 0.11 g/m^2, 0.21 ± 0.08 g/m^2, 0.09 ± 0.01 g/m^2, and 0.07 ± 0.03 g/m^2, respectively. Tong et al. (1988) reported that the amount of protein per membrane area from the adsorption foulants in whole milk ultrafiltration was approximately 0.6 g/m^2.

In general, the protein concentrations for all foulant streams increased after 90 min of MF, as more foulants had been deposited onto and inside the membrane pores due to the applied transmembrane pressure (Figure 4.4). The protein concentration for W_e, W_i, S_e, and S_i from control MF were 62.20 ± 10.13 μg/mL, 10.68 ± 0.95 μg/mL, 6.58 ± 0.69 μg/mL, 1.00 ± 0.10 μg/mL, corresponding to an amount of protein per membrane area of 3.55 ± 0.58 g/m^2, 0.61 ± 0.05 g/m^2, 0.38 ± 0.04 g/m^2, and 0.06 ± 0.01 g/m^2, respectively. It is important to notice that the protein concentration for S_i from the adsorption experiment did not increase after MF (Figure 4.4). This suggests that the strongly attached internal foulants were mostly due to adsorption fouling, before filtration occurred. However, the MF process did significantly increase the amount of protein foulants in W_i.

The results also show a higher protein concentration of external foulants than internal foulants accumulated during MF (Figure 4.4), likely due to the aggregation of foulants on the membrane surface. Meanwhile, the weakly attached foulants had a higher protein concentration compared to the strongly attached foulants, which suggests that strongly attached foulants form a thinner fouling layer compared to the weakly attached foulants. Although the procedures for collecting membrane foulants employed here might not be able to fully extract all of the foulants from the membrane, these findings indicate that any foulants that remained on the membrane

after foulants collection was minor compared to the foulants that had been collected. The protein in the alkaline cleaning solution after foulant collection was quantified. No protein was detected in the alkaline cleaning solution, which suggests that most foulants had been collected by RO water rinsing and pressurized hot water extraction.

Protein Identification and Relative Quantification

The proteins were identified and relatively quantified by LC-MS/MS using multiple reaction monitoring (MRM) approach. The MRM assays were performed on triple quadrupole mass spectrometer, with the sequential selection of a target peptide (peptide unique to the protein of interest) precursor ion in the Q1 mass analyzer while monitoring a characteristic fragment ion in the Q3 mass analyzer after fragmented in the collision cell (Q2) (Ebhardt et al., 2012; Zhang et al., 2012; Picotti et al., 2013). An example of bovine serum albumin identification is demonstrated here. The m/z spectrum from the Q1 mass analyzer together with amino acid sequence of a unique (non-redundant) peptide for BSA is shown in Figure 4.5. This peptide sequence is only found in BSA (Figure 4.6) and hence indicating BSA was in the analyzed sample.

All the milk proteins i.e. α_{s1}-casein, α_{s2}-casein, β-casein, κ-casein, α-lactalbumin, β-lactoglobulin, bovine serum albumin, and lactotransferrin were identified using LC-MS/MS. The name of the identified unique tryptic-digested peptides, designated with Pep1 or Pep2 or Pep3 after the name of the proteins where the peptides originate from, the peptide sequences, and their mass-to-charge ratios as well as retention times are listed in Table 4.1.

The relative quantification of the peptide, and therefore, the protein where it comes from, was performed by calculating the ratio of the peak area of the peptide to the peak area of the internal standard (apomyoglobin). The peptide with the highest response within each protein was selected and analyzed statistically for relative quantification.

Proteins Identified and Relatively Quantified in the Foulants from Adsorption and Control MF

The LC-MS/MS analyses showed that all major milk proteins were present in all foulants from both adsorption and control MF (Figure 4.7). In the adsorption study, α-lactalbumin content in W_e was significantly higher than in milk, which indicates a higher affinity of this proteins for the membrane material. β-lactoglobulin and BSA in W_e from adsorption were also higher than in milk, however the differences were not statistically significant. Overall, adsorption fouling was caused predominantly by serum proteins, which are more hydrophilic and less negatively charged than caseins at milk pH. The fouling might be attributed to the hydrophilic and electrostatic interaction between these serum proteins and the ceramic membrane, which is more hydrophilic than polymeric membrane and has a negative net surface charge.

The serum proteins α-lactalbumin and bovine serum albumin (BSA) were also found in a higher ratio in the "weakly attached" fractions (W_e and W_i) from the adsorption during the first contact between the milk and the membrane as compared to the control MF. This suggests that caseins were mostly introduced into the fouling layer when transmembrane pressure was applied. Thus, concentration/ particle polarization of casein micelles that account for approximately 80% of the milk protein could play a role in fouling during MF of milk.

It is known that casein micelle structure undergoes some changes at low temperature (below 10°C), such as an increase in micelle voluminosity as a result of weakened hydrophobic bonds within the micelles (Walstra, 1990). This subsequently allows some β-caseins to dissociate from the casein micelles and move into the serum phase of the milk (Hekken and Holsinger, 2000; Holland et al., 2011a). Since the microfiltration was conducted at cold temperature (6°C), it was expected that the fouling layer would have a higher amount of β-casein compared to the raw skim milk. However, the LC-MS/MS analyses revealed that β-casein content in all foulants did not increase in the cold MF. This is likely due to the fact that β-casein is the most hydrophobic casein and thus it would not be attracted to the hydrophilic membrane.

Contribution of Minerals to Fouling in Adsorption and Control MF

Minerals identified in the foulants were calcium (Ca), phosphorus (P), magnesium (Mg), potassium (K), sodium (Na), and chlorine (Cl). Chlorine data was not considered in the analysis, since the main source of Cl was from the extraction water, with a concentration of 1.33 ±.0.58 ppm (Table 4.2). Figure 4.8 shows the mineral profile of foulant streams from both the adsorption experiment and control MF. Overall, the mineral concentrations were very small in all foulant streams, below 2.5 ppm, and likely do not play a major role in the membrane fouling in cold MF. Nonetheless, the Ca and P concentrations in W_e, W_i, and S_e from control MF were significantly higher than from adsorption. This might be due to the increase of both the ratio and quantity of caseins in the foulants from MF, as much of the Ca and P in milk are associated with casein micelles and are present at low concentrations as free ions (Lucey and Horne, 2009). This

is in agreement with the finding from the relative quantification of protein in which caseins were mostly introduced into the foulants during MF.

Protein Fouling in MF with CO_2 Backpulsing

The results from the protein quantification showed that MF with optimized CO_2 backpulsing decreased the protein concentration in the weakly attached external foulants, with 52.98 ± 4.87 μg/mL for CO_2 backpulsing MF as compared to 62.20 ± 10.13 μg/mL for the control MF (Figure 4.9). This was probably due to the fact that some of the fouling layer was lifted by the CO_2 backpulse, and then taken up by the cross-flow to the retentate and thus reduced the external fouling. Although the reduction of external foulants by CO_2 backpulsing was insignificant, even a small change in the quantity of protein foulants on the membrane surface could have a huge impact on the permeability of the membrane. This justification is supported by the previous finding that showed both the permeate flux and relative permeate flux of CO_2 backpulsing MF were substantially higher than control MF (Tan and Moraru, 2013).

Meanwhile, it is very interesting to notice that weakly attached internal foulants from CO_2 backpulsing MF had a higher protein concentration (15.48 ± 2.59 μg/mL) than control MF (10.68 ± 0.95 μg/mL), as shown in Figure 4.9. The observed phenomenon might be caused by the a more compact external fouling layer in control MF prevented smaller foulants or feed components to enter the pores, thus less internal fouling in control vs. CO_2 backpulsing. Therefore, the lower protein concentration in internal foulants does not necessary imply better permeability, as the permeate flux for CO_2 backpulsing MF was significantly higher than for the control MF.

Proteins Identified and Relatively Quantified in the Foulants from CO_2 backpulsing MF

All major milk proteins were also present in all freeze dried foulants from CO_2 backpulsing MF (Figure 4.10). However, higher levels of κ-casein and lactotransferrin were found in S_i in CO_2 backpulsing MF as compared to control MF. BSA was significantly higher in W_i from CO_2 backpulsing MF as compared to control MF. The data also showed higher level of κ-casein in S_e from CO_2 than in milk. Since these are minor proteins in milk, despite their higher proportion (not total amount of protein) in the foulants, they are not expected to have played a major role in fouling. Meanwhile, β-lactoglobulin in S_i from CO_2 backpulsing MF was significantly lower than in milk. BSA in S_e and S_i from CO_2 backpulsing MF were also significantly lower than in milk. This suggests a decrease of these serum proteins in the strongly attached foulants from CO_2 backpulsing MF. It is also important to notice that casein concentration did not increase in the foulants from CO_2 backpulsing MF, despite the localized decrease in pH caused by the contact with CO_2.

Minerals in the Foulant Streams from CO_2 Backpulsing MF

The CO_2 backpulsing did not have a significant effect on the mineral profiles of MF foulants (Figure 4.11). The concentration of minerals was very small in the foulant streams, all below 2.5 ppm. The concentration and size of minerals are probably too small to be a major cause in fouling during cold MF with a 1.4 μm pore size membrane. Nevertheless, it is expected that minerals could have play a role in warm (50°C - 55°C) MF of milk due to the precipitation

of amorphous calcium phosphate at 50°C (Andritsos et al., 2002; Spanos et al., 2006; Beliciu and Moraru, 2009).

CONCLUSIONS

All milk proteins contributed to the external and internal fouling in cold MF of skim milk. Adsorption fouling was caused principally by serum proteins, with α-lactalbumin as the protein that had highest affinity to the membrane material. Caseins were mainly introduced into the fouling layer during microfiltration. Casein micelles probably contributed to fouling mainly due to concentration or particle polarization. CO_2 backpulsing diminished membrane fouling by physically removing the foulants, resulted in less weakly attached external foulants and possibly less loosely deposited materials on the membrane surface as compared to the control MF. Fouling in cold MF of milk using large pore membrane was mostly caused by proteins, and minerals did not play a major role in fouling.

The results of this study can be used to design MF processes able to further minimize membrane fouling and thus increase the yield in MF of milk, therefore enhancing the processing efficiency in the dairy industry.

ACKNOWLEDGMENTS

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TABLES

Table 4.1. Identified peptide names, peptide sequences, charge (*z*), mass-to-charge ratio values in Q1 and Q3 mass analyzer, and retention times (RT)

Identified peptide	Peptide sequence	*z*	Q1 mass (*m/z*)	Q3 mass (*m/z*)	RT (min)
k-casein Pep1y8	46-YIPIQYVLSR-55	2+	626.4	975.6	46.65
k-casein Pep1y6*			626.4	765.5	46.65
k-casein Pep2y13	90-SPAQILQWQVLSNTVPAK-107	2+	990.6	1483.8	49.65
k-casein Pep2y11			990.6	1242.7	49.65
k-casein Pep3y7	90-SPAQILQWQVLSNTVPAK-107	3+	660.8	716.5	49.63
k-casein Pep3b7			660.8	738.5	49.63
k-casein Pep3y8			660.8	829.6	49.63
aS1-casein Pep1y8	106-YLGYLEQLLR-115	2+	634.4	991.7	53.4
aS1-casein Pep1y6			634.4	771.6	53.4
aS1-casein Pep2y6*	38-FFVAPFPEVFGK-49	2+	692.9	920.6	56.07
aS1-casein Pep2y7			692.9	991.8	56.07
aS2-casein Pep1y9	40-NMAINPSKENLCSTFCK-56	3+	672.4	1158.5	32.6
aS2-casein Pep1y6			672.4	802.3	32.6
aS2-casein Pep1y7			672.4	915.4	32.6
aS2-casein Pep2y6*	96-ALNEINQFYQK-106	2+	684.4	827.5	34.4
aS2-casein Pep2y7			684.4	940.7	34.4
B-casein Pep1y6	121-HKEMPFPK-128	2+	507.3	748.4	18.25
B-casein Pep1b6			507.3	770.4	18.25
B-casein Pep2y7*	199-DMPIQAFLLYQEPVLGPVR-217	3+	729.5	994.7	62.95
B-casein Pep2y10			729.5	1157.6	62.95
B-casein Pep2y8			729.5	866.5	62.95
B-Lactoglobulin Pep1y5	141-TPEVDDEALEK-151	2+	623.3	819.6	23.75
B-Lactoglobulin Pep1y6			623.3	918.5	23.75
B-Lactoglobulin Pep2y5	165-LSFNPTQLEEQCHI-178	3+	572.6	686.3	43.85
B-Lactoglobulin Pep2y6			572.6	815.3	43.85
B-Lactoglobulin Pep2y7			572.6	928.4	43.85
B-Lactoglobulin Pep3y7	165-LSFNPTQLEEQCHI-178	2+	858.5	928.4	43.85
B-Lactoglobulin Pep3y10*			858.5	1254.6	43.85
a-Lactalbumin Pep1y6*	82-DDQNPHSSNICNISCDK-98	3+	668.9	736.3	20.2

Table 4.1
(Continued)

Identified peptide	Peptide sequence	z	Q1 mass (m/z)	Q3 mass (m/z)	RT (min)
a-Lactalbumin Pep1y7			668.9	896.5	20.2
a-Lactalbumin Pep1y8			668.9	995.6	20.2
a-Lactalbumin Pep2y11	114-IWCKDDQNPHSSNICNISCDK-127	3+	864.7	1297.8	23.87
a-Lactalbumin Pep2y10			864.7	1210.5	23.87
a-Lactalbumin Pep2y8			864.7	1009.4	23.87
Serum Albumin Pep1y10*	286-YICDNQDTISSK-297	2+	722.4	1167.5	20.35
Serum Albumin Pep1y9			722.4	1007.5	20.35
Serum Albumin Pep2y9	347-DAFLGSFLYEYSR-359	2+	784.9	1121.5	55.49
Serum Albumin Pep2y7			784.9	977.5	55.49
Lactotransferrin Pep1y7*	304-SFQLFGSPPGQR-315	3+	660.8	698.4	39.99
Lactotransferrin Pep1y8			660.8	845.4	39.99
Lactotransferrin Pep1y6			660.8	641.6	39.99
Lactotransferrin Pep2b10	670-LGGRPTYEEYLGTEYVTAIANLKK-693	3+	672.4	1166.5	48.68
Lactotransferrin Pep2b9			672.4	1003.8	48.68
Lactotransferrin Pep2y8			672.4	858.5	48.68

*Peptide with the highest response for the protein from which it was derived.

Table 4.2. Mineral concentrations of the RO water used for extraction of foulants

	Mineral concentration (ppm)				
Mineral	Rep 1	Rep 2	Rep 3	Average	StdDev
Ca	0.01	0.06	0.07	0.05	0.03
P	0.01	0.01	0.01	0.01	0.00
Mg	0.02	0.02	0.02	0.02	0.00
K	0.01	0.01	0.01	0.01	0.00
Na	0.20	0.15	0.16	0.17	0.03
Cl	1	2	1	1.33	0.58

FIGURES

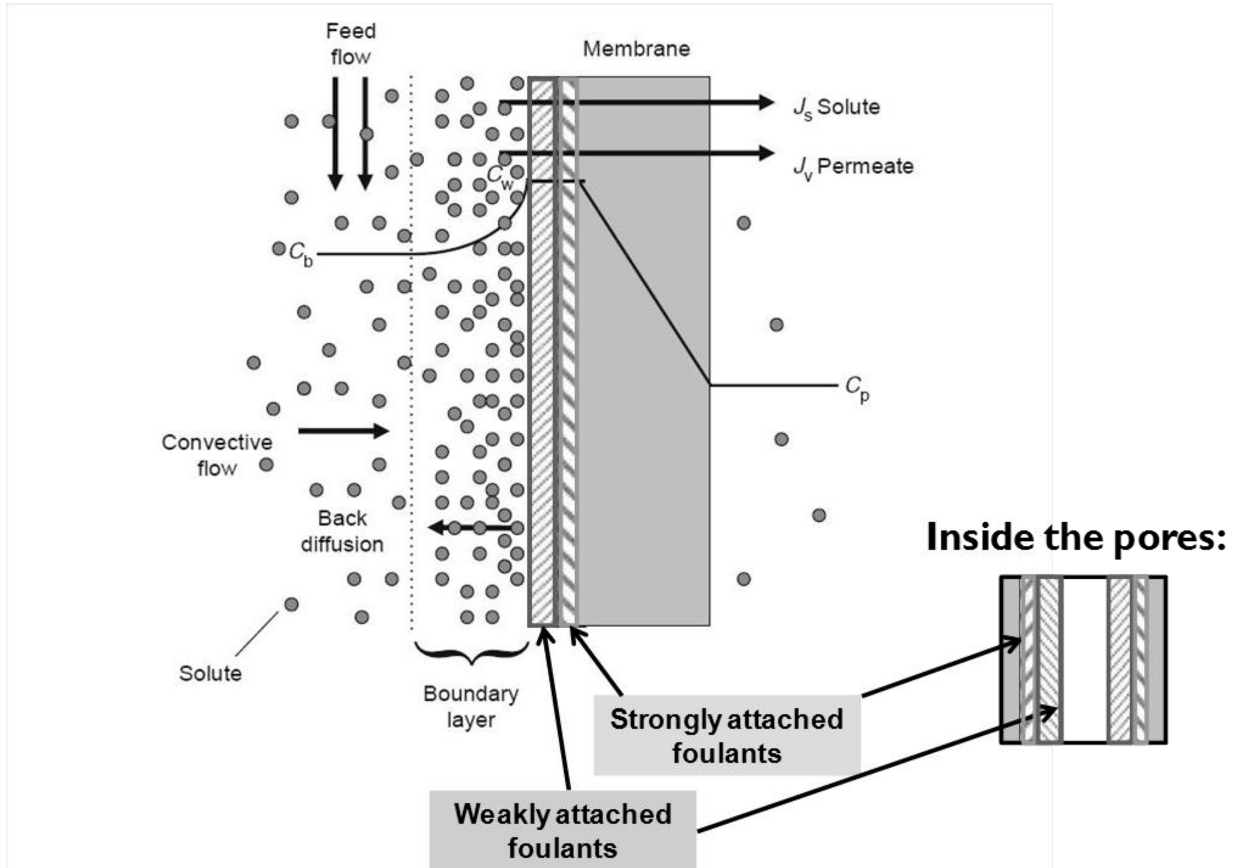

Figure 4.1. Diagram of membrane foulants location (*Modified from*: Goosen et al., 2004).

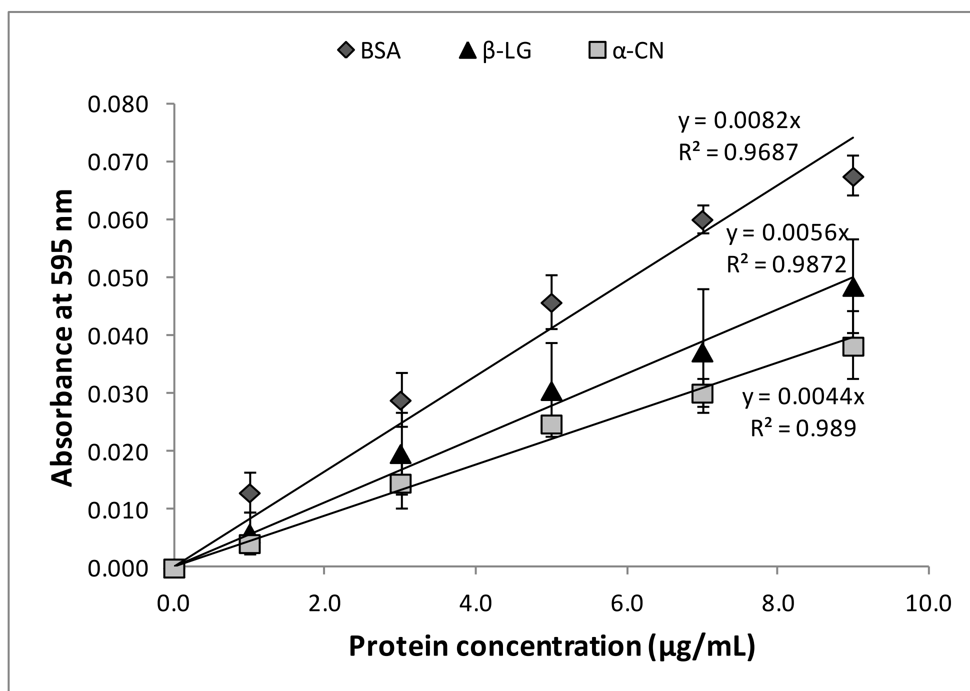

Figure 4.2. Standard curves of absorbance versus protein concentration of BSA, β-LG, and α-CN.

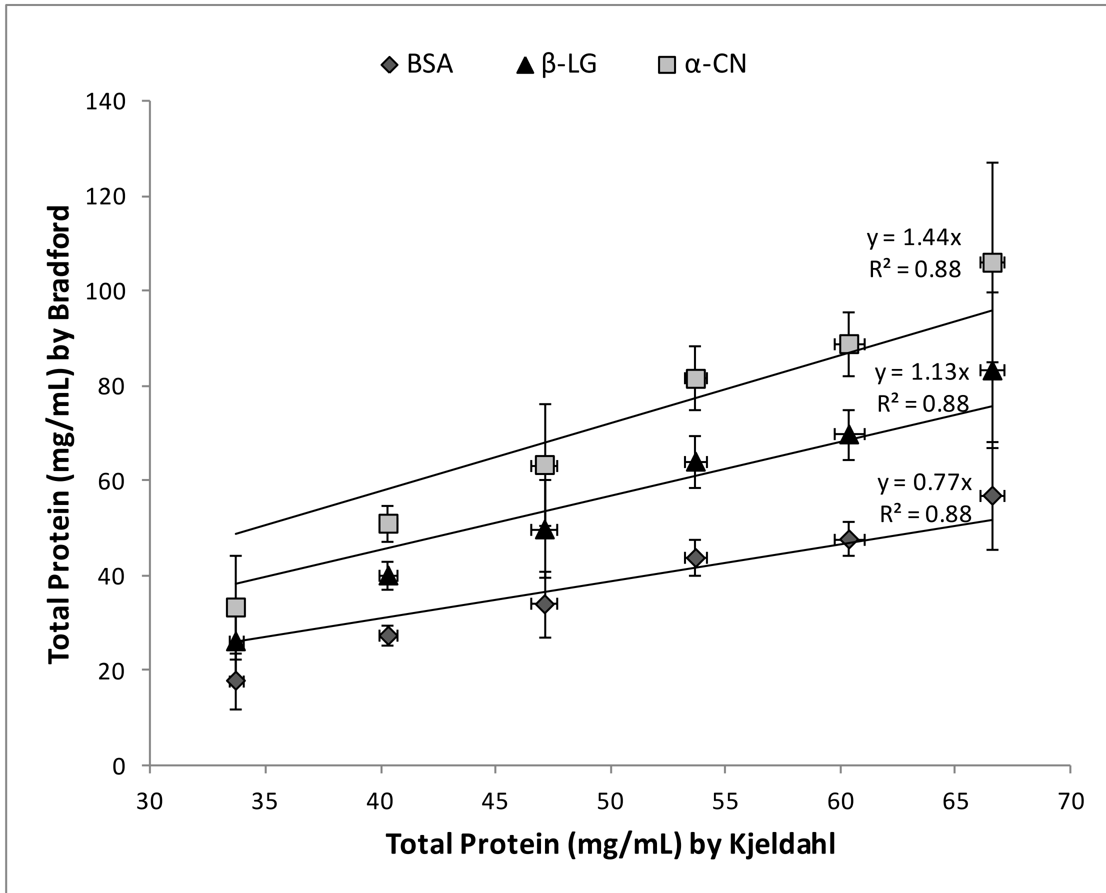

Figure 4.3. Calibration of Bradford method with Kjeldahl method.

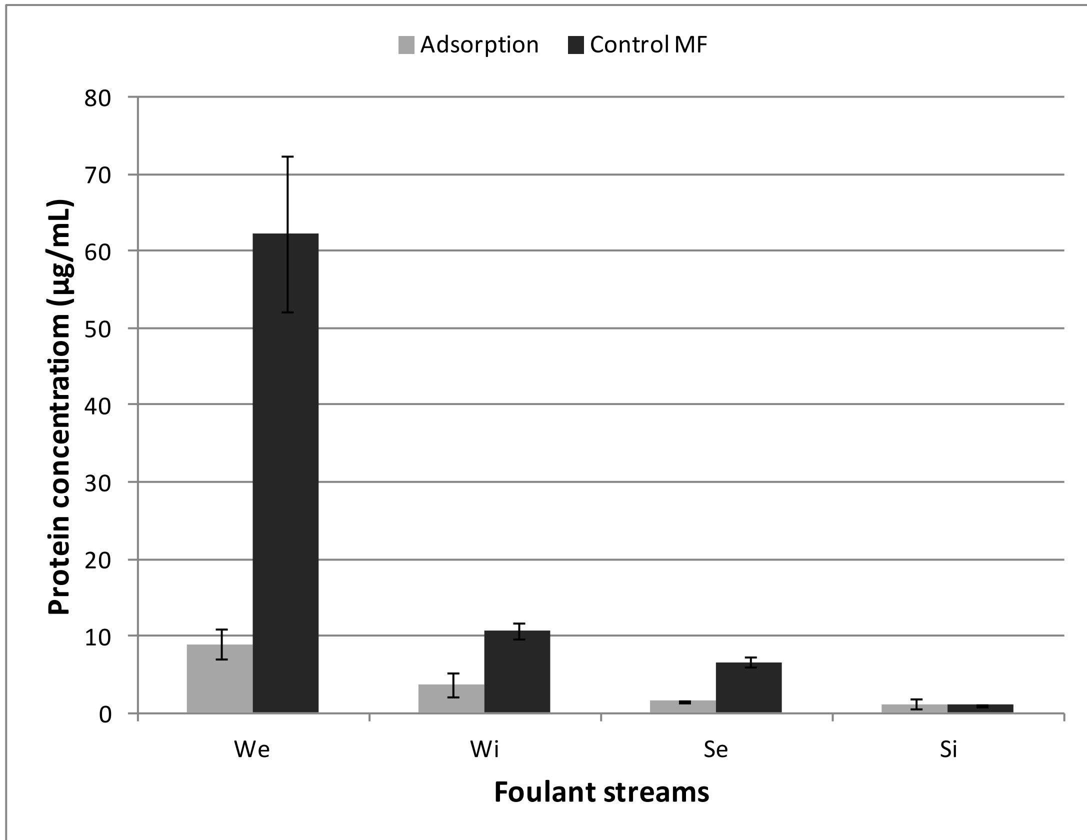

Figure 4.4. Protein concentrations of foulant streams from adsorption and control MF.

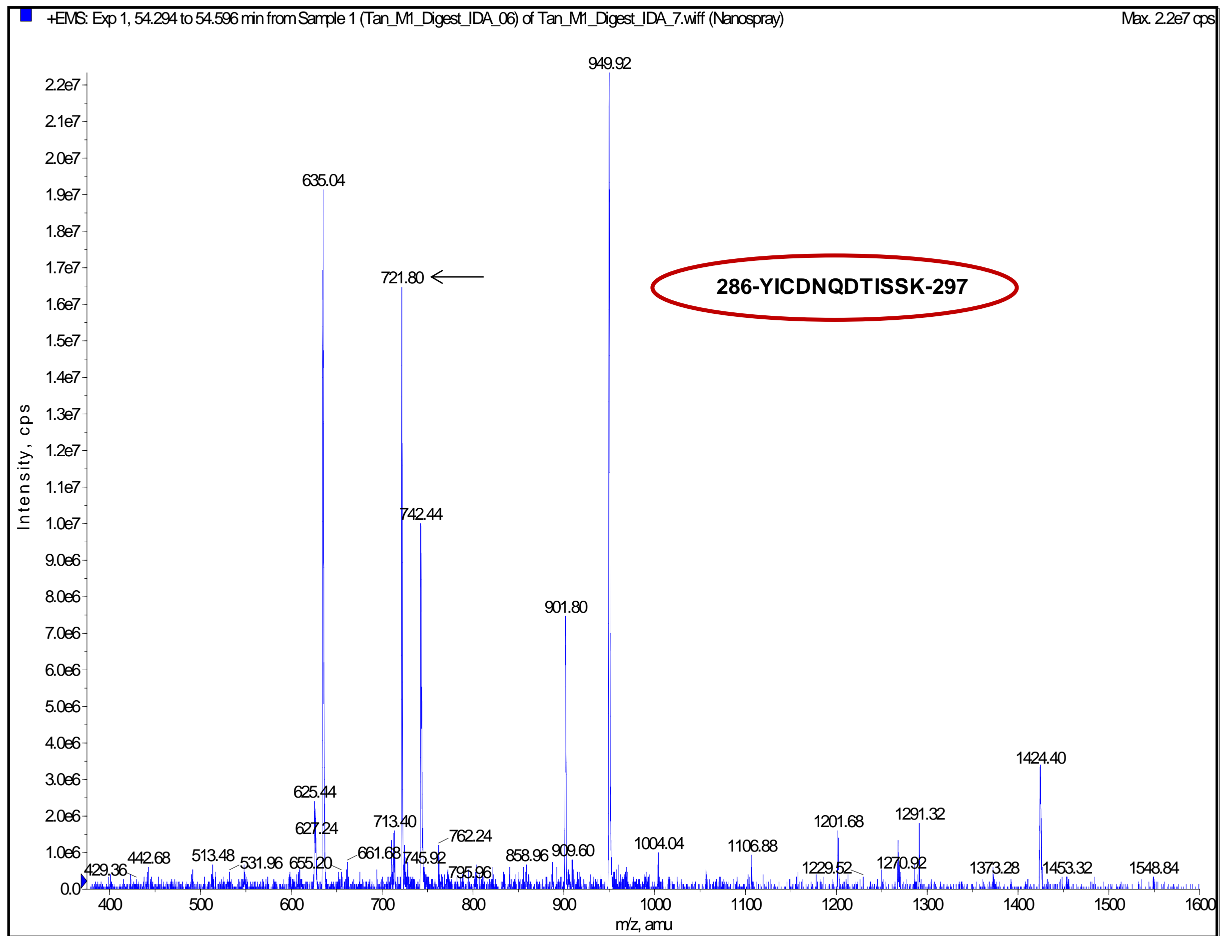

Figure 4.5. m/z spectrum with amino acid sequence for a tryptic-digested peptide unique to bovine serum albumin, i.e. serum albumin Pep1y10.

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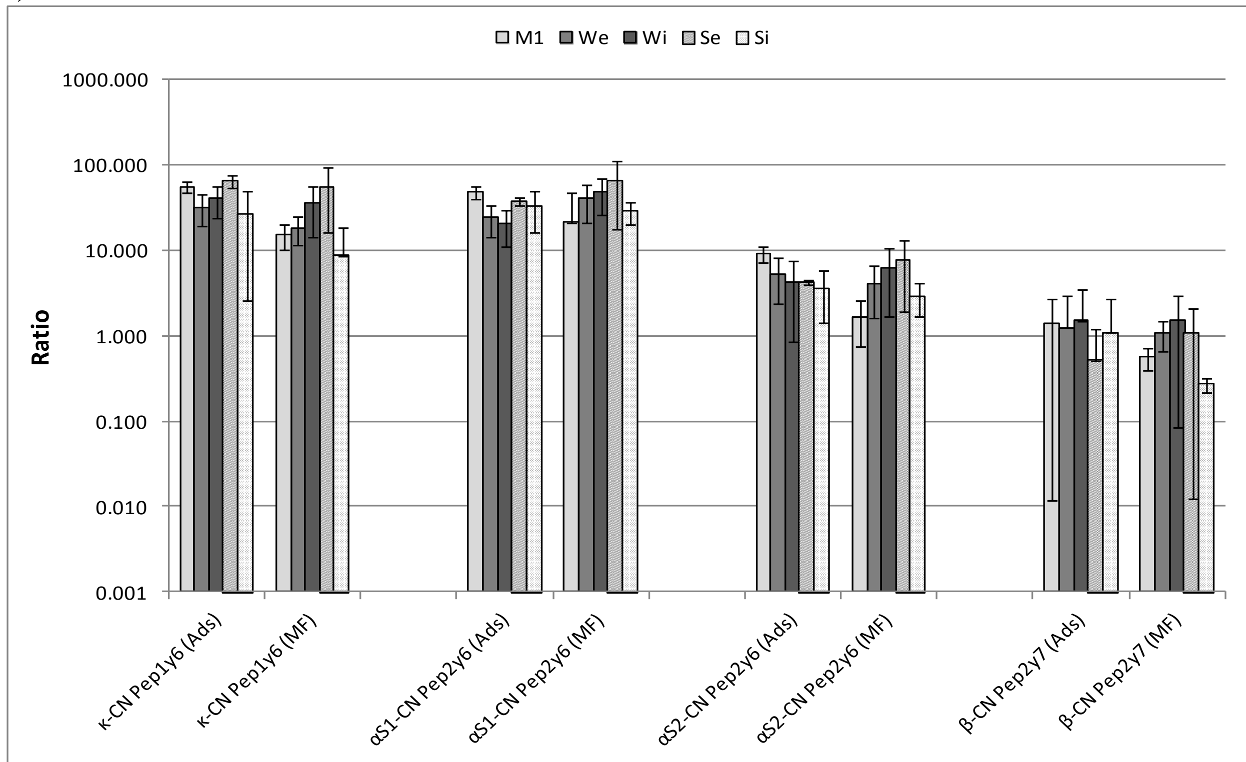
MKWVTFISLLLLFSSAYSRGVFRRDTHKSEIAHRFKDLGEEHFKGLVLIAFSQYLQQCPF
DEHVKLVNELTEFAKTCVADESHAGCEKSLHTLFGDELCKVASLRETYGDMADCCEKQEP
ERNECFLSHKDDSPDLPKLKPDPNTLCDEFKADEKKFWGKYLYEIARRHPYFYAPELLYY
ANKYNGVFQECCQAEDKGACLLPKIETMREKVLASSARQRLRCASIQKFGERALKAWSVA
RLSQKFPKAEFVEVTKLVTDLTKVHKECCHGDLLECADDRADLAKYICDNQDTISSKLKE
CCDKPLLEKSHCIAEVEKDAIPENLPPLTADFAEDKDVCKNYQEAKDAFLGSFLYEYSRR
HPEYAVSVLLRLAKEYEATLEECCAKDDPHACYSTVFDKLKHLVDEPQNLIKQNCDQFEK
LGEYGFQNALIVRYTRKVPQVSTPTLVEVSRSLGKVGTRCCTKPESERMPCTEDYLSLIL
NRLCVLHEKTPVSEKVTKCCTESLVNRRPCFSALTPDETYVPKAFDEKLFTFHADICTLP
DTEKQIKKQTALVELLKHKPKATEEQLKTVMENFVAFVDKCCAADDKEACFAVEGPKLVV
STQTALA
```

Figure 4.6. Bovine serum albumin sequence data; the underlined amino acid sequence is serum albumin Pep1y10 (Source: http://www.uniprot.org/uniprot/P02769).

a)



b)

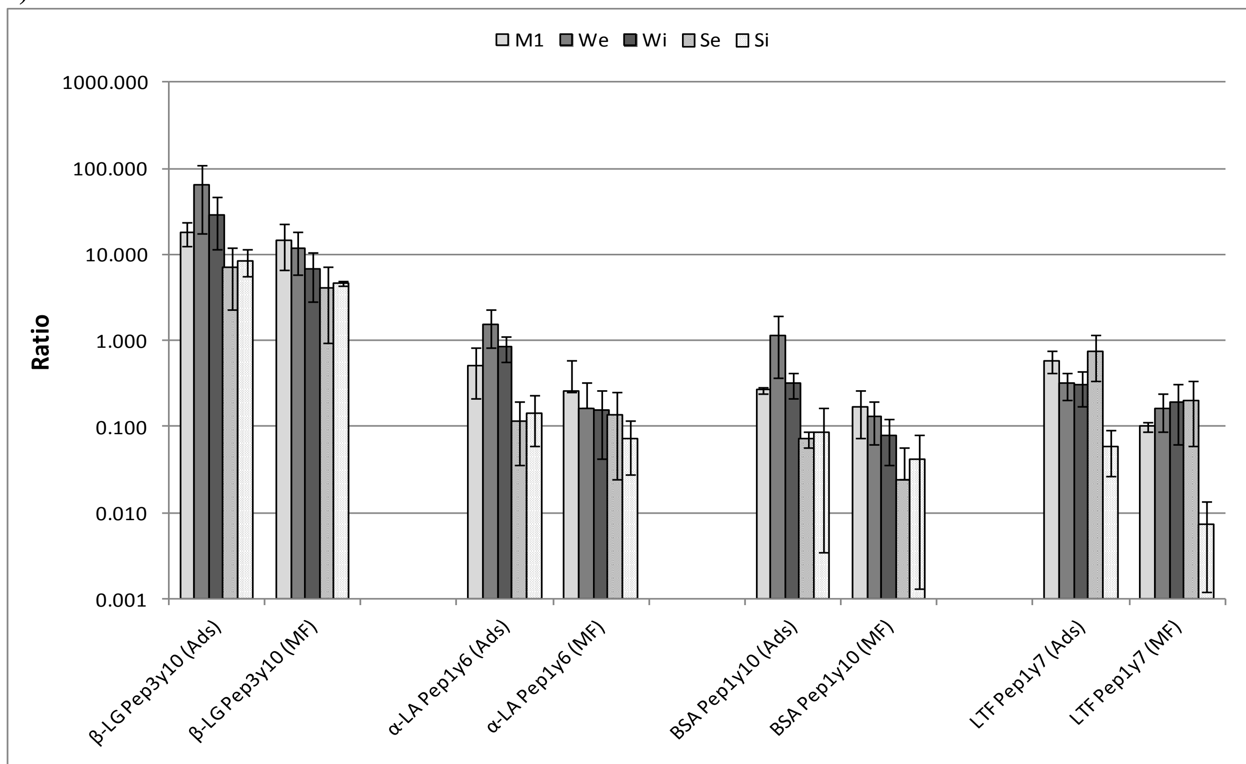


Figure 4.7. Comparison of ratio of identified milk proteins in raw skim milk (M1) and foulants between adsorption (Ads) and control MF (MF): a) caseins; b) serum proteins.

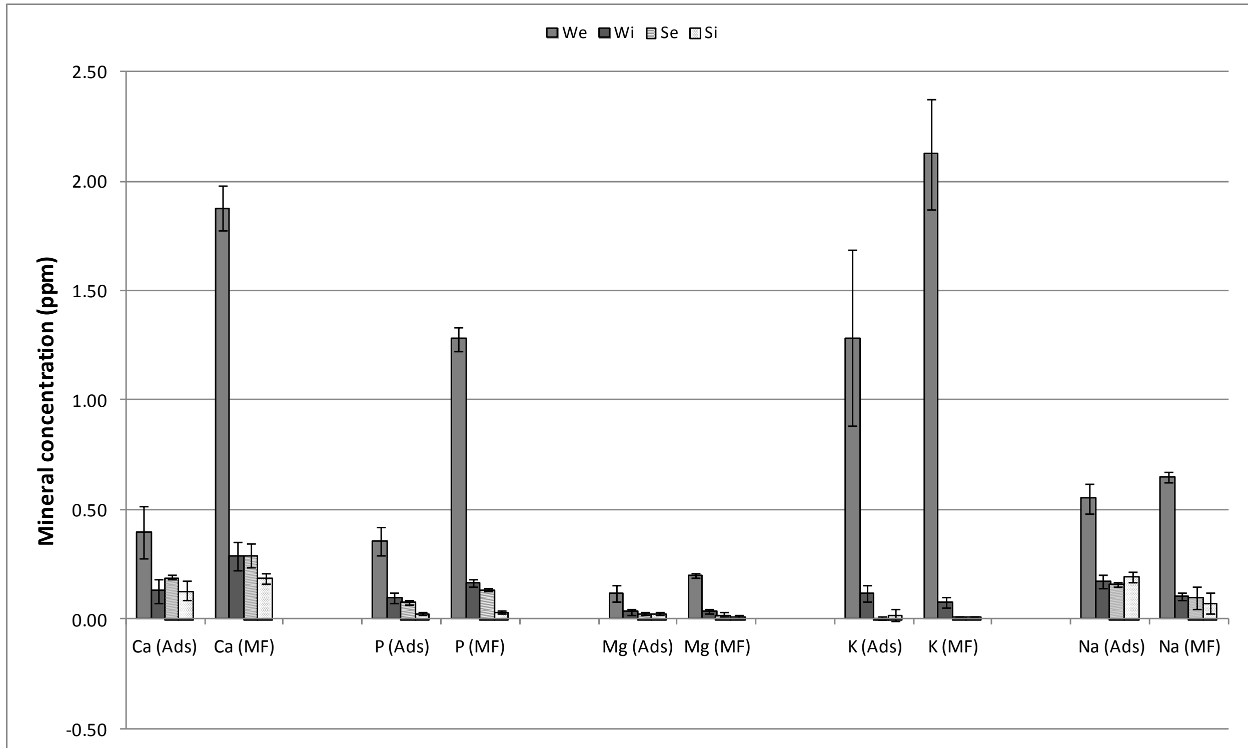


Figure 4.8. Mineral concentrations in foulant streams from adsorption (Ads) and control MF (MF).

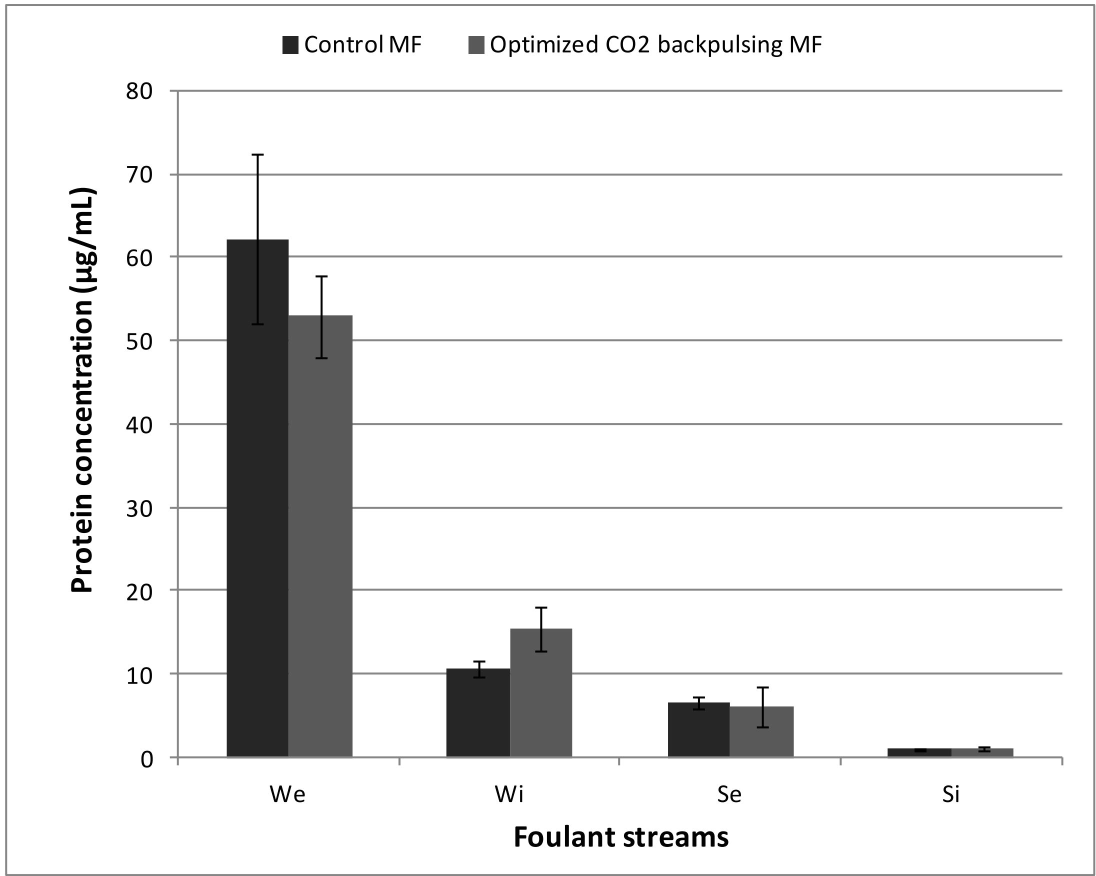
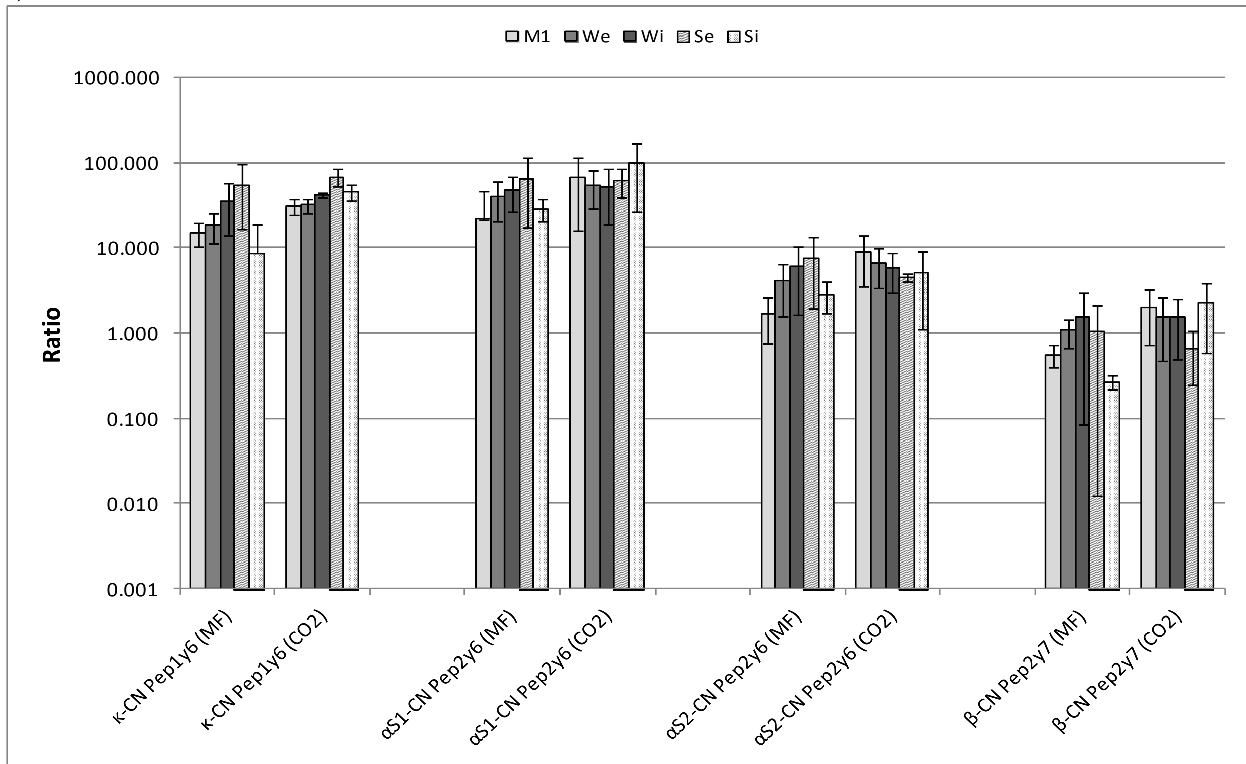


Figure 4.9. Protein concentrations of foulant streams from control MF and MF with optimized CO_2 backpulsing.

a)



b)

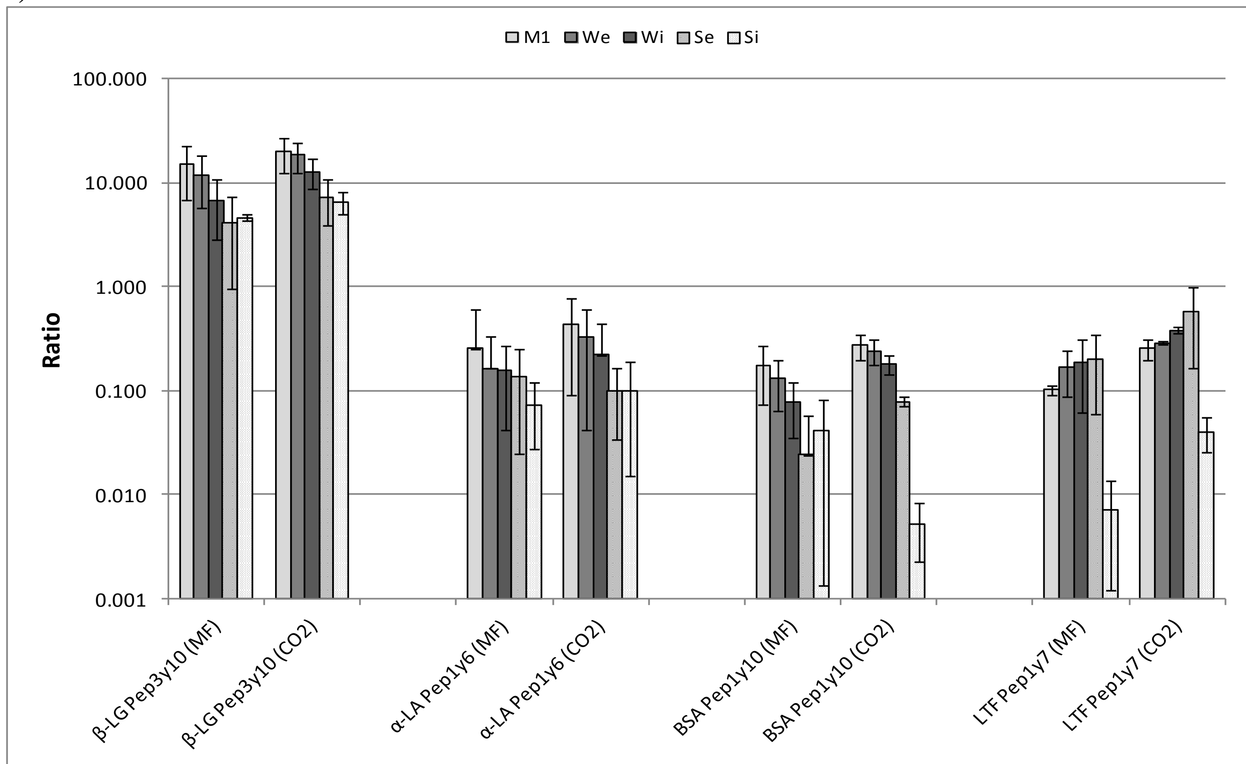


Figure 4.10. Comparison of ratio of identified milk proteins in raw skim milk (M1) and foulants between control MF (MF) and CO_2 backpulsing MF (CO_2): a) caseins; b) serum proteins.

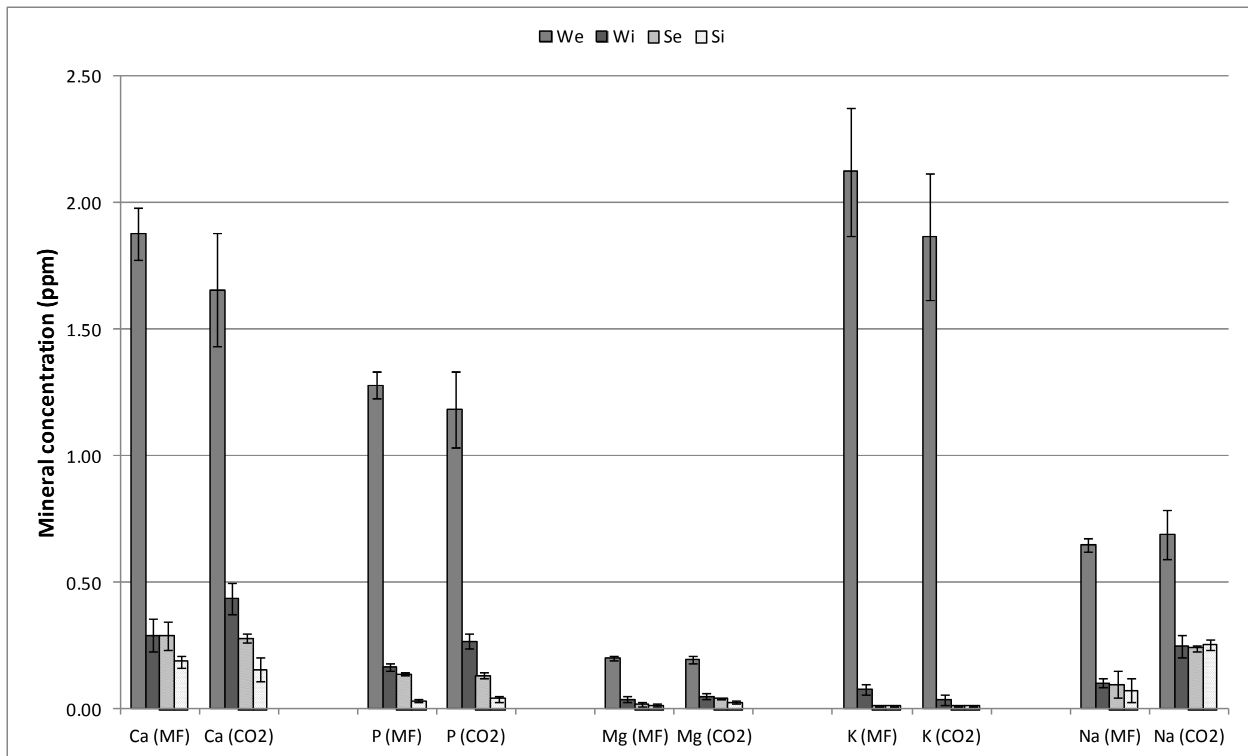


Figure 4.11. Mineral concentrations in foulant streams from control MF (MF) and CO_2 backpulsing MF (CO_2).

CHAPTER 5

CONCLUSIONS AND FUTURE DIRECTIONS

The processing of raw skim milk using cold microfiltration (MF) could produce fresh-like milk with un-altered quality and nutritional value, safe to be consumed, and with extended shelf life. The lack of a systematic investigation of the mechanisms of membrane fouling and enhancement of permeate flux in large pore cold microfiltration of skim milk had created the need for this study.

The MF process, with or without CO_2 backpulsing, was very effective in removal of somatic cells, bacteria and spores from skim milk. The optimized CO_2 backpulsing MF resulted in a significant enhancement in permeate flux and higher transmission of total solids and protein as compared to control MF. The slight decrease in pH of the permeate from CO_2 backpulsing MF was not deemed problematic, and could in fact be beneficial for preventing microbial growth in milk. CO_2 could also be removed by vacuum post-processing, if necessary.

The investigation of fouling mechanisms showed that fouling in cold MF of milk was almost exclusively caused by proteins; minerals did not play a major role in fouling. All major milk proteins were present in all foulant streams. Adsorption fouling on ceramic membrane was caused predominantly by serum proteins, which are more hydrophilic and less negatively charged than caseins at milk pH. Caseins were mostly introduced into the fouling layer when transmembrane pressure was applied; concentration or particle polarization of casein micelles is likely to contribute to membrane fouling. Casein precipitation did not seem to occur in the foulants from CO_2 backpulsing MF, despite the localized decrease in pH. More notably, the CO_2

backpulsing reduced the membrane fouling by physically removing the foulants from membrane surface, resulted in less weakly attached external foulants and possibly less loosely deposited materials on the membrane surface as compared to MF without CO_2 backpulsing.

The findings from this work will contribute to a better understanding of the mechanisms of membrane fouling and can help increase the yield for fluid milk MF, as well as improve the quality and shelf life of milk and dairy products. The CO_2 backpulsing method could also be applied to other cold microfiltration processes, including filtration of juice, beer, or heat sensitive fluids that require cold processing.

Future work needs to be carried out to elucidate the mechanisms of membrane fouling in warm microfiltration (50°C) of skim milk. The membrane separation process in the dairy industry is always conducted at longer hour (> 8 h) to reduce down time. Due to the favorable environment for the growth of thermophilic bacteria and germination of spores as well as precipitation of calcium phosphate, microorganisms and minerals could contribute profoundly to membrane fouling in warm MF of skim milk.